

## pTurboYFP-PRL-dest1 vector

### Table of restriction sites

| Enzyme name | Sequence                  | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|---------------------------|-------|--|
| AanI        | TTA!TAA                   | 2     | 1112; 1317   |
| AarI        | CACCTGCNNNN!              | 0     |  |
| AarI        | !NNNNNNNNGCAGGTG          | 0     |  |
| AasI        | GACNNNN!NNGTC             | 3     | 1400; 2356; 4145   |
| AatI        | AGG!CCT                   | 1     | 2150   |
| AatII       | GACGT!C                   | 0     |  |
| AbsI        | CC!TCGAGG                 | 0     |  |
| AccI        | GT!MKAC                   | 1     | 58   |
| AccII       | CG!CG                     | 20    | 69; 185; 416; 455; 740; 770; 978; 1253; 1629; 1649; 1673; 1704; 2395; 2696; 3134; 3218; 3281; 3350; 3623; 4204   |
| AccIII      | T!CCGGA                   | 1     | 811  |
| Acc16I      | TGC!GCA                   | 1     | 2431   |
| Acc36I      | ACCTGCNNNN!               | 2     | 2597; 3047   |
| Acc36I      | !NNNNNNNNGCAGGT           | 1     | 2216   |
| Acc65I      | G!GTACC                   | 1     | 63   |
| AccB1I      | G!GYRCC                   | 6     | 63; 106; 295; 1482; 2328; 2363   |
| AccB7I      | CCANNNN!NTGG              | 1     | 498  |
| AccBSI      | CCG!CTC                   | 2     | 1753; 2942   |
| AccBSI      | GAG!CGG                   | 3     | 917; 1589; 2996  |
| AceIII      | CAGCTCNNNNNNN!            | 1     | 1285   |
| AceIII      | !NNNNNNNNNNGAGCTG         | 2     | 313; 333   |
| AciI        | C!CGC                     | 31    | 67; 183; 414; 738; 783; 859; 976; 1644; 1647; 1661; 1751; 1999; 2011; 2020; 2032; 2042; 2053; 2099; 2238; 2301; 2803; 2848; 2914; 2940; 3065; 3068; 3216; 3348; 3655; 3799; 4295 |
| AciI        | G!CGG                     | 28    | 69; 104; 185; 662; 665; 865; 871; 917; 1589; 1616; 1704; 1814; 2395; 2459; 2560; 2563; 2843; 2898; 2996; 3134; 3281; 3421; 3664; 3909; 4030; 4049; 4176; 4204                    |
| AclI        | AA!CGTT                   | 0     |  |
| AclWI       | GGATCNNNN!                | 10    | 86; 964; 977; 2197; 2508; 2754; 3146; 3505; 3603; 3689   |
| AclWI       | !NNNNNGATCC               | 6     | 73; 123; 2573; 3117; 3504; 3601  |
| AcoI        | Y!GGCCR                   | 9     | 165; 468; 489; 629; 973; 2235; 2409; 2800; 2827  |
| AcsI        | R!AATY                    | 4     | 47; 1143; 1247; 1258   |
| AcuI        | CTGAAGNNNNNNNNNNNNNNNN!   | 5     | 349; 592; 775; 2475; 2907  |
| AcuI        | !NNNNNNNNNNNNNCTTCAG      | 2     | 159; 3705  |
| AcvI        | CAC!GTG                   | 3     | 495; 678; 708  |
| AcyI        | GR!CGYC                   | 6     | 107; 296; 803; 2329; 3031; 3110  |
| AdeI        | CACNNN!GTG                | 1     | 1445   |
| AfaI        | GT!AC                     | 6     | 65; 316; 448; 541; 688; 2635   |
| AfeI        | AGC!GCT                   | 1     | 14   |
| AfiI        | CCNNNNN!NNGG              | 22    | 138; 189; 263; 420; 498; 661; 864; 1341; 1667; 2059; 2310; 2854; 3135; 3167; 3299; 3334; 3384; 3416; 3769; 4048; 4214; 4232  |
| AflII       | C!TTAAG                   | 1     | 1211   |
| AflIII      | A!CRYGT                   | 1     | 4247   |
| AgeI        | A!CCGGT                   | 1     | 84   |
| AgsI        | TTS!AA                    | 9     | 408; 480; 519; 585; 1740; 1784; 1794; 2207; 3783   |
| AhdI        | GACNNN!NNGTC              | 0     |  |
| AhlI        | A!CTAGT                   | 0     |  |
| AjiI        | CAC!GTC                   | 0     |  |
| AjiI        | GAC!GTG                   | 0     |  |
| AjnI        | !CCWGG                    | 13    | 262; 272; 470; 908; 1863; 1918; 1935; 2714; 3293; 3396; 4086; 4099; 4220   |
| AjuI        | GAANNNNNNNTTGGNNNNNNNNNN! | 0     |  |
| AjuI        | !NNNNNNCCAANNNNNNNTTC     | 0     |  |
| AjuI        | CCAANNNNNNNTTCNNNNNNNNNN! | 0     |  |
| AjuI        | !NNNNNNNGAANNNNNNNTTGG    | 0     |  |

| Enzyme name | Sequence                     | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|------------------------------|-------|--|
| AleI        | CACNN!NNGTG                  | 1     | 274  |
| AlfI        | GCANNNNNNTGCNNNNNNNNNNNN!    | 2     | 1903; 1975   |
| AlfI        | !NNNNNNNNNNGCANNNNNNNTGC     | 2     | 1869; 1941   |
| AloI        | GAACNNNNNNNTCCNNNNNNNNNNNN!  | 1     | 1411   |
| AloI        | !NNNNNNNNGGANNNNNNGTTC       | 1     | 214  |
| AloI        | GGANNNNNNGTTCNNNNNNNNNNNN!   | 1     | 246  |
| AloI        | !NNNNNNNGAACNNNNNNNTCC       | 1     | 1379   |
| AluI        | AG!CT                        | 19    | 36; 42; 327; 347; 462; 701; 714; 824; 1108; 1275; 1532; 1827; 2115; 2435; 2893; 3690; 3947; 3993; 4083   |
| AluBI       | AG!CT                        | 19    | 36; 42; 327; 347; 462; 701; 714; 824; 1108; 1275; 1532; 1827; 2115; 2435; 2893; 3690; 3947; 3993; 4083   |
| AlwI        | GGATCNNNN!                   | 10    | 86; 964; 977; 2197; 2508; 2754; 3146; 3505; 3603; 3689   |
| AlwI        | !NNNNNGATCC                  | 6     | 73; 123; 2573; 3117; 3504; 3601  |
| Alw21I      | GWGCW!C                      | 6     | 38; 271; 677; 2442; 2632; 3937   |
| Alw26I      | GTCTCN!                      | 1     | 835  |
| Alw26I      | !NNNNNGAGAC                  | 5     | 754; 1753; 2171; 3190; 3318  |
| Alw44I      | G!TGCAC                      | 2     | 673; 3933  |
| AlwNI       | CAGNNN!CTG                   | 2     | 3430; 3838   |
| Ama87I      | C!YCGRG                      | 4     | 31; 74; 322; 682   |
| Aor13HI     | T!CCGGA                      | 1     | 811  |
| Aor51HI     | AG!GCT                       | 1     | 14   |
| ApaI        | GGGCC!C                      | 1     | 75   |
| ApaBI       | GCANNNNN!TGC                 | 4     | 944; 1897; 1969; 3431  |
| ApaLI       | G!TGCAC                      | 2     | 673; 3933  |
| ApeKI       | G!CWGC                       | 19    | 101; 347; 440; 608; 891; 937; 1105; 1624; 2289; 2390; 2432; 2473; 2566; 2953; 3406; 3632; 3838; 3841; 3906   |
| ApoI        | R!AATTY                      | 4     | 47; 1143; 1247; 1258   |
| ApyPI       | ATCGACNNNNNNNNNNNNNNNNNNNN!  | 1     | 2844   |
| ApyPI       | !NNNNNNNNNNNNNNNNNNNGTCGAT   | 3     | 2638; 3291; 4129   |
| AquII       | GCCGNACNNNNNNNNNNNNNNNNNNNN! | 1     | 2728   |
| AquII       | !NNNNNNNNNNNNNNNNNNNGTNCGGC  | 1     | 296  |
| AquIII      | GAGGAGNNNNNNNNNNNNNNNNNNNN!  | 1     | 2157   |
| AquIII      | !NNNNNNNNNNNNNNNNNNNCTCCTC   | 0     |  |
| AquIV       | GRGGAAGNNNNNNNNNNNNNNNNNNNN! | 0     |  |
| AquIV       | !NNNNNNNNNNNNNNNNNNCTCCYC    | 1     | 2899   |
| ArsI        | GACNNNNNTTYGNNNNNNNNNNNN!    | 0     |  |
| ArsI        | !NNNNNNCRAANNNNNNGTC         | 0     |  |
| ArsI        | CRAANNNNNNGTCNNNNNNNNNNNN!   | 0     |  |
| ArsI        | !NNNNNNNNGACNNNNNNNTTYG      | 0     |  |
| AscI        | GG!CGGCC                     | 0     |  |
| AseI        | AT!TAAT                      | 0     |  |
| Asi256I     | G!ATC                        | 27    | 28; 79; 129; 553; 600; 634; 733; 836; 957; 970; 987; 2167; 2171; 2190; 2501; 2579; 2660; 2669; 2747; 3123; 3139; 3498; 3510; 3588; 3596; 3607; 3682                |
| AsiGI       | A!CCGGT                      | 1     | 84   |
| AsiSI       | GCGAT!CGC                    | 0     |  |
| AspI        | GACN!NNGTC                   | 1     | 2447   |
| Asp700I     | GAANN!NNTTC                  | 1     | 334  |
| Asp718I     | G!GTACC                      | 1     | 63   |
| AspA2I      | C!CTAGG                      | 2     | 2151; 3166   |
| AspEI       | GACNNN!NNGTC                 | 0     |  |
| AspLEI      | GCG!C                        | 29    | 15; 109; 298; 377; 418; 580; 770; 772; 876; 1595; 1603; 1629; 1651; 1660; 1673; 1704; 2323; 2331; 2395; 2432; 2698; 2958; 3134; 3220; 3623; 3732; 3906; 4006; 4073 |
| AspS9I      | G!GNCC                       | 11    | 71; 72; 319; 667; 924; 1436; 2845; 3290; 3336; 3394; 3427  |
| AssI        | AGT!ACT                      | 0     |  |
| AsuII       | TT!CGAA                      | 2     | 45; 3011   |
| AsuC2I      | CC!SGG                       | 6     | 75; 76; 2333; 2493; 3106; 3870   |
| AsuHPI      | GGTGANNNNNNNN!               | 4     | 288; 435; 3400; 3516   |
| AsuHPI      | !NNNNNNNTCACC                | 7     | 375; 516; 594; 727; 922; 1446; 2507  |
| AsuNHI      | G!CTAGC                      | 0     |  |
| AvaI        | C!YCGRG                      | 4     | 31; 74; 322; 682   |
| AvaII       | G!GWCC                       | 3     | 924; 2845; 3290  |
| AviII       | TGC!GCA                      | 1     | 2431   |
| AvrII       | C!CTAGG                      | 2     | 2151; 3166   |
| AxyI        | CC!TNAGG                     | 2     | 1809; 3441   |

| Enzyme name | Sequence                 | Count | Cutting Position (the base to the left of the cut site)   |
|-------------|--------------------------|-------|---|
| BaeI        | ACNNNNGTAYCNNNNNNNNNNNN! | 0     |   |
| BaeI        | !NNNNNNNGRTACNNNNNGT     | 0     |   |
| BaeI        | GRTACNNNNGTNNNNNNNNNNNN! | 0     |   |
| BaeI        | !NNNNNNNNNACNNNNNGTAYC   | 0     |   |
| BaeGI       | GKGC!C                   | 9     | 75; 257; 263; 677; 683; 909; 2275; 2368; 3937   |
| BalI        | TGG!CCA                  | 4     | 167; 470; 491; 2411   |
| BamHI       | G!GATCC                  | 1     | 78  |
| BanI        | G!GYRCC                  | 6     | 63; 106; 295; 1482; 2328; 2363  |
| BanII       | GRGCY!C                  | 5     | 38; 75; 1520; 2694; 3404  |
| BanIII      | AT!CGAT                  | 1     | 2169  |
| BarI        | GAAGNNNNNTACNNNNNNNNNN!  | 1     | 354   |
| BarI        | !NNNNNNNGTANNNNNNCTTC    | 0     |   |
| BarI        | GTANNNNNCTTCNNNNNNNNNN!  | 0     |   |
| BarI        | !NNNNNNNGAAGNNNNNTAC     | 1     | 322   |
| BasI        | CCANNNN!NTGG             | 1     | 498   |
| BauI        | C!ACGAG                  | 3     | 595; 3047; 4074   |
| BauI        | C!TCGTG                  | 1     | 2921  |
| BbeI        | GGCGC!C                  | 3     | 110; 299; 2332  |
| Bbr7I       | GAAGACNNNNNN!            | 0     |   |
| Bbr7I       | !NNNNNNNNNNNGTCTTC       | 0     |   |
| BbrPI       | CAC!GTG                  | 3     | 495; 678; 708   |
| BbsI        | GAAGACNN!                | 0     |   |
| BbsI        | !NNNNNNGTCTTC            | 0     |   |
| BbuI        | GCATG!C                  | 4     | 354; 1899; 1971; 2734   |
| BbvI        | GCAGCNNNNNN!             | 10    | 113; 452; 620; 949; 1117; 2402; 2444; 2965; 3418; 3918  |
| BbvI        | !NNNNNNNNNNNGCTGC        | 8     | 334; 878; 1611; 2276; 2460; 2553; 3619; 3825  |
| Bbv12I      | GWGCW!C                  | 6     | 38; 271; 677; 2442; 2632; 3937  |
| BbvCI       | CC!TCAGC                 | 0     |   |
| BbvCI       | GC!TGAGG                 | 0     |   |
| BccI        | CCATCNNNN!               | 6     | 388; 664; 1459; 2023; 2549; 3051  |
| BccI        | !NNNNNGATGG              | 10    | 141; 157; 352; 391; 481; 877; 915; 1427; 2207; 2635   |
| BceAI       | ACGGCNNNNNNNNNN!         | 7     | 138; 213; 309; 333; 582; 2755; 3242   |
| BceAI       | !NNNNNNNNNNNGCCGT        | 4     | 757; 1470; 2288; 3747   |
| BceFI       | ACGGCNNNNNNNNNN!         | 7     | 138; 213; 309; 333; 582; 2755; 3242   |
| BceFI       | !NNNNNNNNNNNGCCGT        | 4     | 758; 1471; 2289; 3748   |
| BcgI        | CGANNNNNNTGCNNNNNNNNNN!  | 0     |   |
| BcgI        | !NNNNNNNNNGCANNNNNTCG    | 0     |   |
| BcgI        | GCANNNNNNTCGNNNNNNNNNN!  | 0     |   |
| BcgI        | !NNNNNNNNNCGANNNNNNTGC   | 0     |   |
| BciVI       | GTATCCNNNNN!             | 3     | 1758; 2548; 4049  |
| BciVI       | !NNNNNGGATAC             | 0     |   |
| BclI        | T!GATCA                  | 0     |   |
| BcnI        | CC!SGG                   | 6     | 75; 76; 2333; 2493; 3106; 3870  |
| BcuI        | A!CTAGT                  | 0     |   |
| BdaI        | TGANNNNNNTCANNNNNNNNNN!  | 1     | 4182  |
| BdaI        | !NNNNNNNNNNTGANNNNNNTCA  | 1     | 4148  |
| BfaI        | C!TAG                    | 9     | 10; 953; 984; 1178; 1596; 2152; 3167; 3501; 3754  |
| BfiI        | ACTGGGNNNN!              | 1     | 2278  |
| BfiI        | !NNNCCCAGT               | 3     | 222; 294; 2030  |
| BfmI        | C!TRYAG                  | 5     | 52; 935; 1664; 3791; 3982   |
| BfoI        | RGCGC!Y                  | 8     | 16; 110; 299; 581; 1596; 1604; 2332; 4007   |
| BfrI        | C!TTAAG                  | 1     | 1211  |
| BfuI        | GTATCCNNNNN!             | 3     | 1758; 2548; 4049  |
| BfuI        | !NNNNNGGATAC             | 0     |   |
| BfuAI       | ACCTGCNNNN!              | 2     | 2597; 3047  |
| BfuAI       | !NNNNNNNGCAGGT           | 1     | 2216  |
| BfuCI       | !GATC                    | 27    | 27; 78; 128; 552; 599; 633; 732; 835; 956; 969; 986; 2166; 2170; 2189; 2500; 2578; 2659; 2668; 2746; 3122; 3138; 3497; 3509; 3587; 3595; 3606; 3681   |
| BglI        | GCCNNNN!NGGC             | 1     | 2104  |
| BglII       | A!GATCT                  | 2     | 27; 835   |
| BisI        | GC!NGC                   | 41    | 102; 105; 186; 348; 441; 609; 663; 666; 783; 872; 892; 938; 976; 1106; 1625; 1647; 1661; 2099; 2238; 2290; 2301; 2391; 2396; 2433; 2474; 2561; 2564; 2567; 2803; 2899; 2940; 2954; 3068; 3407; 3422; 3633; 3839; 3842; 3907; 4050; 4205 |

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|-------------|---------------------------|-------|---|
| BlnI        | C!CTAGG                   | 2     | 2151; 3166  |
| BlpI        | GC!TNAGC                  | 1     | 715   |
| BlsI        | GCN!GC                    | 41    | 103; 106; 187; 349; 442; 610; 664; 667; 784; 873; 893; 939; 977; 1107; 1626; 1648; 1662; 2100; 2239; 2291; 2302; 2392; 2397; 2434; 2475; 2562; 2565; 2568; 2804; 2900; 2941; 2955; 3069; 3408; 3423; 3634; 3840; 3843; 3908; 4051; 4206 |
| BmcAI       | AGT!ACT                   | 0     |   |
| Bme18I      | G!GWCC                    | 3     | 924; 2845; 3290   |
| Bme1390I    | CC!NGG                    | 19    | 75; 76; 264; 274; 472; 910; 1865; 1920; 1937; 2333; 2493; 2716; 3106; 3295; 3398; 3870; 4088; 4101; 4222  |
| BmeRI       | GACNNN!NNGTC              | 0     |   |
| BmeT110I    | C!YCGRG                   | 4     | 31; 74; 322; 682  |
| BmgBI       | CAC!GTC                   | 0     |   |
| BmgBI       | GAC!GTG                   | 0     |   |
| BmgT120I    | GG!NCC                    | 11    | 72; 73; 320; 668; 925; 1437; 2846; 3291; 3337; 3395; 3428   |
| BmiI        | GGN!NCC                   | 21    | 65; 73; 80; 108; 297; 321; 669; 810; 1484; 1505; 1517; 1708; 1869; 1941; 2330; 2365; 3213; 3292; 3337; 4180; 4219   |
| BmrI        | ACTGGGNNNNN!              | 1     | 2278  |
| BmrI        | !NNNNCCCAGT               | 3     | 222; 294; 2030  |
| BmrFI       | CC!NGG                    | 19    | 75; 76; 264; 274; 472; 910; 1865; 1920; 1937; 2333; 2493; 2716; 3106; 3295; 3398; 3870; 4088; 4101; 4222  |
| BmsI        | GCATCNNNNN!               | 7     | 188; 1145; 1908; 1980; 2628; 2692; 2967   |
| BmsI        | !NNNNNNNNNGATGC           | 6     | 192; 213; 2287; 2542; 2758; 4150  |
| BmtI        | GCTAG!C                   | 0     |   |
| BmuI        | ACTGGGNNNNN!              | 1     | 2278  |
| BmuI        | !NNNNCCCAGT               | 3     | 222; 294; 2030  |
| BoxI        | GACNN!NNGTC               | 0     |   |
| BpiI        | GAAGACNN!                 | 0     |   |
| BpiI        | !NNNNNNGTCTTC             | 0     |   |
| BpI         | GAGNNNNNCTCNNNNNNNNNNNN!  | 2     | 2118; 3017  |
| BpI         | !NNNNNNNNGAGNNNNNCTC      | 2     | 2086; 2985  |
| BpmI        | CTGGAGNNNNNNNNNNNNNN!     | 3     | 285; 532; 3169  |
| BpmI        | !NNNNNNNNNNNNNCTCCAG      | 1     | 3112  |
| Bpu10I      | CC!TNAGC                  | 0     |   |
| Bpu10I      | GC!TNAGG                  | 0     |   |
| Bpu14I      | TT!CGAA                   | 2     | 45; 3011  |
| Bpu1102I    | GC!TNAGC                  | 1     | 715   |
| BpuAI       | GAAGACNN!                 | 0     |   |
| BpuAI       | !NNNNNNGTCTTC             | 0     |   |
| BpuEI       | CTTGAGNNNNNNNNNNNNNN!     | 2     | 3623; 4162  |
| BpuEI       | !NNNNNNNNNNNNNCTCAAG      | 3     | 22; 2705; 3864  |
| BpuMI       | CC!SGG                    | 6     | 75; 76; 2333; 2493; 3106; 3870  |
| BpvUI       | CGAT!CG                   | 0     |   |
| BsaI        | GGTCTCN!                  | 1     | 835   |
| BsaI        | !NNNNNGAGACC              | 2     | 754; 3318   |
| Bsa29I      | AT!CGAT                   | 1     | 2169  |
| BsaAI       | YAC!GTR                   | 7     | 138; 366; 495; 678; 708; 1445; 2633   |
| BsaBI       | GATNN!NNATC               | 2     | 991; 2188   |
| BsaHI       | GR!CGYC                   | 6     | 107; 296; 803; 2329; 3031; 3110   |
| BsaJI       | C!CNNGG                   | 23    | 67; 74; 120; 183; 262; 272; 419; 471; 773; 849; 908; 1863; 1935; 2058; 2093; 2102; 2151; 2492; 2761; 3166; 3294; 3397; 4087   |
| BsaMI       | GAATGCN!                  | 1     | 1080  |
| BsaMI       | G!CATTC                   | 1     | 1173  |
| BsaWI       | W!CCGGW                   | 8     | 18; 84; 811; 826; 2360; 3204; 3894; 4041  |
| BsaXI       | ACNNNNNCTCCNNNNNNNNNN!    | 1     | 1409  |
| BsaXI       | !NNNNNNNGGAGNNNNNNGT      | 0     |   |
| BsaXI       | GGAGNNNNNNGTNNNNNNNNNN!   | 0     |   |
| BsaXI       | !NNNNNNNNNACNNNNNCTCC     | 1     | 1379  |
| BsbI        | CAACACNNNNNNNNNNNNNNNNNN! | 0     |   |
| BsbI        | !NNNNNNNNNNNNNNNNNNGTGTG  | 2     | 1330; 3237  |
| Bsc4I       | CCNNNNN!NNGG              | 22    | 138; 189; 263; 420; 498; 661; 864; 1341; 1667; 2059; 2310; 2854; 3135; 3167; 3299; 3334; 3384; 3416; 3769; 4048; 4214; 4232   |
| BscAI       | GCATCNNNN!                | 7     | 187; 1144; 1907; 1979; 2627; 2691; 2966   |
| BscAI       | !NNNNNNGATGC              | 6     | 195; 216; 2290; 2545; 2761; 4153  |

| Enzyme name | Sequence                | Count | Cutting Position (the base to the left of the cut site)   |
|-------------|-------------------------|-------|---|
| BseI        | ACTGGN!                 | 3     | 2273; 2474; 3720  |
| BseI        | C!CAGT                  | 6     | 228; 300; 1358; 2036; 3832; 3845  |
| Bse8I       | GATNN!NNATC             | 2     | 991; 2188   |
| Bse21I      | CC!TNAGG                | 2     | 1809; 3441  |
| Bse118I     | R!CCGGY                 | 9     | 84; 244; 604; 805; 861; 1546; 2648; 2829; 3112  |
| BseAI       | T!CCGGA                 | 1     | 811   |
| BseBI       | CC!WGG                  | 13    | 264; 274; 472; 910; 1865; 1920; 1937; 2716; 3295; 3398; 4088; 4101; 4222  |
| BseCI       | AT!CGAT                 | 1     | 2169  |
| BseDI       | C!CNNGG                 | 23    | 67; 74; 120; 183; 262; 272; 419; 471; 773; 849; 908; 1863; 1935; 2058; 2093; 2102; 2151; 2492; 2761; 3166; 3294; 3397; 4087                           |
| Bse3DI      | GCAATGNN!               | 2     | 162; 2562   |
| Bse3DI      | !CATTGC                 | 0     |   |
| BseGI       | GGATGNN!                | 10    | 168; 228; 492; 729; 885; 926; 2189; 2646; 2671; 3125  |
| BseGI       | !CATCC                  | 2     | 179; 2015   |
| BseJI       | GATNN!NNATC             | 2     | 991; 2188   |
| BseLI       | CCNNNNN!NNGG            | 22    | 138; 189; 263; 420; 498; 661; 864; 1341; 1667; 2059; 2310; 2854; 3135; 3167; 3299; 3334; 3384; 3416; 3769; 4048; 4214; 4232                           |
| BseMI       | GCAATGNN!               | 2     | 162; 2562   |
| BseMI       | !CATTGC                 | 0     |   |
| BseMII      | CTCAGNNNNNNNNNN!        | 3     | 38; 846; 3455   |
| BseMII      | !NNNNNNNNNCTGAG         | 6     | 706; 1800; 2102; 2983; 3555; 3964   |
| BseNI       | ACTGGN!                 | 3     | 2273; 2474; 3720  |
| BseNI       | C!CAGT                  | 6     | 228; 300; 1358; 2036; 3832; 3845  |
| BsePI       | G!CGCGC                 | 1     | 768   |
| BseRI       | GAGGAGNNNNNNNNNN!       | 1     | 2147  |
| BseRI       | !NNNNNNNNNCTCCTC        | 0     |   |
| BseSI       | GKGC!C                  | 9     | 75; 257; 263; 677; 683; 909; 2275; 2368; 3937   |
| BseXI       | GCAGC!NNNNNNNN!         | 10    | 113; 452; 620; 949; 1117; 2402; 2444; 2965; 3418; 3918  |
| BseXI       | !NNNNNNNNNNNGTGC        | 8     | 334; 878; 1611; 2276; 2460; 2553; 3619; 3825  |
| BseX3I      | C!GGCCG                 | 2     | 973; 2235   |
| BseYI       | C!CCAGC                 | 3     | 1872; 1944; 3943  |
| BseYI       | G!CTGGG                 | 2     | 498; 2833   |
| BsgI        | GTGCAGNNNNNNNNNNNNNNNN! | 1     | 388   |
| BsgI        | !NNNNNNNNNNNNNCTGCAC    | 1     | 222   |
| Bsh1236I    | CG!CG                   | 20    | 69; 185; 416; 455; 740; 770; 978; 1253; 1629; 1649; 1673; 1704; 2395; 2696; 3134; 3218; 3281; 3350; 3623; 4204  |
| Bsh1285I    | CGRY!CG                 | 4     | 89; 976; 2238; 3913   |
| BshFI       | GG!CC                   | 27    | 73; 167; 320; 470; 491; 559; 569; 631; 668; 975; 1295; 1437; 2092; 2098; 2107; 2150; 2237; 2411; 2802; 2829; 3338; 3395; 3428; 3773; 4207; 4225; 4236 |
| BshNI       | G!GYRCC                 | 6     | 63; 106; 295; 1482; 2328; 2363  |
| BshTI       | A!CCGGT                 | 1     | 84  |
| BshVI       | AT!CGAT                 | 1     | 2169  |
| BsiEI       | CGRY!CG                 | 4     | 89; 976; 2238; 3913   |
| BsiHKAI     | GWGCW!C                 | 6     | 38; 271; 677; 2442; 2632; 3937  |
| BsiHKCI     | C!YCGRG                 | 4     | 31; 74; 322; 682  |
| BsiSI       | C!CGG                   | 25    | 19; 75; 85; 245; 605; 806; 812; 827; 862; 1547; 2234; 2311; 2333; 2361; 2492; 2582; 2649; 2830; 3105; 3113; 3205; 3679; 3869; 3895; 4042              |
| BsiWI       | C!GTACG                 | 0     |   |
| BsII        | CCNNNNN!NNGG            | 22    | 138; 189; 263; 420; 498; 661; 864; 1341; 1667; 2059; 2310; 2854; 3135; 3167; 3299; 3334; 3384; 3416; 3769; 4048; 4214; 4232                           |
| BsIFI       | GGGACNNNNNNNNNN!        | 3     | 2480; 3012; 3121  |
| BsIFI       | !NNNNNNNNNNNNNGTCCC     | 4     | 1845; 1917; 1981; 3276  |
| BsmI        | GAATGCN!                | 1     | 1080  |
| BsmI        | G!CATTC                 | 1     | 1173  |
| BsmAI       | GTCTCN!                 | 1     | 835   |
| BsmAI       | !NNNNNGAGAC             | 5     | 754; 1753; 2171; 3190; 3318   |
| BsmBI       | CGTCTCN!                | 0     |   |
| BsmBI       | !NNNNNGAGACG            | 0     |   |
| BsmFI       | GGGACNNNNNNNNNN!        | 3     | 2480; 3012; 3121  |
| BsmFI       | !NNNNNNNNNNNNNGTCCC     | 4     | 1845; 1917; 1981; 3276  |

| Enzyme name | Sequence               | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|------------------------|-------|--|
| BsnI        | GG!CC                  | 27    | 73; 167; 320; 470; 491; 559; 569; 631; 668; 975; 1295; 1437; 2092; 2098; 2107; 2150; 2237; 2411; 2802; 2829; 3338; 3395; 3428; 3773; 4207; 4225; 4236                            |
| Bso31I      | GGTCTCN!               | 1     | 835  |
| Bso31I      | !NNNNNGAGACC           | 2     | 754; 3318  |
| BsoBI       | C!YCGRG                | 4     | 31; 74; 322; 682   |
| Bsp13I      | T!CCGGA                | 1     | 811  |
| Bsp19I      | C!CATGG                | 3     | 849; 2058; 2761  |
| Bsp24I      | GACNNNNNTGGNNNNNNNNNN! | 1     | 3132   |
| Bsp24I      | !NNNNNNCCANNNNNGTC     | 2     | 3553; 3731   |
| Bsp24I      | CCANNNNNGTCNNNNNNNNNN! | 2     | 3585; 3763   |
| Bsp24I      | !NNNNNNNGACNNNNNTGG    | 1     | 3100   |
| Bsp68I      | TCG!CGA                | 0     |  |
| Bsp119I     | TT!CGAA                | 2     | 45; 3011   |
| Bsp120I     | G!GGCCC                | 1     | 71   |
| Bsp143I     | !GATC                  | 27    | 27; 78; 128; 552; 599; 633; 732; 835; 956; 969; 986; 2166; 2170; 2189; 2500; 2578; 2659; 2668; 2746; 3122; 3138; 3497; 3509; 3587; 3595; 3606; 3681                              |
| Bsp1286I    | GDGCH!C                | 16    | 38; 75; 257; 263; 271; 677; 683; 909; 1520; 2275; 2368; 2442; 2632; 2694; 3404; 3937   |
| Bsp1407I    | T!GTACA                | 2     | 446; 539   |
| Bsp1720I    | GC!TNAGC               | 1     | 715  |
| BspACI      | C!CGC                  | 31    | 67; 183; 414; 738; 783; 859; 976; 1644; 1647; 1661; 1751; 1999; 2011; 2020; 2032; 2042; 2053; 2099; 2238; 2301; 2803; 2848; 2914; 2940; 3065; 3068; 3216; 3348; 3655; 3799; 4295 |
| BspACI      | G!CGG                  | 28    | 69; 104; 185; 662; 665; 865; 871; 917; 1589; 1616; 1704; 1814; 2395; 2459; 2560; 2563; 2843; 2898; 2996; 3134; 3281; 3421; 3664; 3909; 4030; 4049; 4176; 4204                    |
| BspCNI      | CTCAGNNNNNNNN!         | 3     | 37; 845; 3454  |
| BspCNI      | !NNNNNNCTGAG           | 6     | 707; 1801; 2103; 2984; 3556; 3965  |
| BspDI       | AT!CGAT                | 1     | 2169   |
| BspD6I      | GACTCNNNN!             | 5     | 30; 988; 1402; 3008; 3885  |
| BspD6I      | !NNNNNGAGTC            | 2     | 1365; 1797   |
| BspEI       | T!CCGGA                | 1     | 811  |
| BspFNI      | CG!CG                  | 20    | 69; 185; 416; 455; 740; 770; 978; 1253; 1629; 1649; 1673; 1704; 2395; 2696; 3134; 3218; 3281; 3350; 3623; 4204   |
| BspHI       | T!CATGA                | 2     | 1755; 3527   |
| BspLI       | GGN!NCC                | 21    | 65; 73; 80; 108; 297; 321; 669; 810; 1484; 1505; 1517; 1708; 1869; 1941; 2330; 2365; 3213; 3292; 3337; 4180; 4219  |
| BspMI       | ACCTGCNNNN!            | 2     | 2597; 3047   |
| BspMI       | !NNNNNNNGCAGGT         | 1     | 2216   |
| BspOI       | GCTAG!C                | 0     |  |
| BspPI       | GGATCNNNN!             | 10    | 86; 964; 977; 2197; 2508; 2754; 3146; 3505; 3603; 3689   |
| BspPI       | !NNNNNGATCC            | 6     | 73; 123; 2573; 3117; 3504; 3601  |
| BspQI       | GCTCTCN!               | 0     |  |
| BspQI       | !NNNNGAAGAGC           | 3     | 568; 2673; 2883  |
| BspTI       | C!TTAAG                | 1     | 1211   |
| BspT104I    | TT!CGAA                | 2     | 45; 3011   |
| BspT107I    | G!GYRCC                | 6     | 63; 106; 295; 1482; 2328; 2363   |
| BspTNI      | GGTCTCN!               | 1     | 835  |
| BspTNI      | !NNNNNGAGACC           | 2     | 754; 3318  |
| BsrI        | ACTGGN!                | 3     | 2273; 2474; 3720   |
| BsrI        | C!CAGT                 | 6     | 228; 300; 1358; 2036; 3832; 3845   |
| BsrBI       | CCG!CTC                | 2     | 1753; 2942   |
| BsrBI       | GAG!CGG                | 3     | 917; 1589; 2996  |
| BsrDI       | GCAATGNN!              | 2     | 162; 2562  |
| BsrDI       | !CATTGC                | 0     |  |
| BsrFI       | R!CCGGY                | 9     | 84; 244; 604; 805; 861; 1546; 2648; 2829; 3112   |
| BsrGI       | T!GTACA                | 2     | 446; 539   |
| BsrSI       | ACTGGN!                | 3     | 2273; 2474; 3720   |
| BsrSI       | C!CAGT                 | 6     | 228; 300; 1358; 2036; 3832; 3845   |
| BssAI       | R!CCGGY                | 9     | 84; 244; 604; 805; 861; 1546; 2648; 2829; 3112   |
| BssECI      | C!CNNGG                | 23    | 67; 74; 120; 183; 262; 272; 419; 471; 773; 849; 908; 1863; 1935; 2058; 2093; 2102; 2151; 2492; 2761; 3166; 3294; 3397; 4087  |
| BssHII      | G!CGCGC                | 1     | 768  |

| Enzyme name | Sequence      | Count | Cutting Position (the base to the left of the cut site)   |
|-------------|---------------|-------|---|
| BssKI       | !CCNGG        | 19    | 73; 74; 262; 272; 470; 908; 1863; 1918; 1935; 2331; 2491; 2714; 3104; 3293; 3396; 3868; 4086; 4099; 4220  |
| BssMI       | !GATC         | 27    | 27; 78; 128; 552; 599; 633; 732; 835; 956; 969; 986; 2166; 2170; 2189; 2500; 2578; 2659; 2668; 2746; 3122; 3138; 3497; 3509; 3587; 3595; 3606; 3681                         |
| BssNI       | GR!CGYC       | 6     | 107; 296; 803; 2329; 3031; 3110   |
| BssNAI      | GTA!TAC       | 0     |   |
| BssSI       | C!ACGAG       | 3     | 595; 3047; 4074   |
| BssSI       | C!TCGTG       | 1     | 2921  |
| BssT1I      | C!CWWGG       | 5     | 849; 2058; 2151; 2761; 3166   |
| Bst6I       | CTCTTCN!      | 0     |   |
| Bst6I       | !NNNNGAAGAG   | 4     | 568; 1796; 2673; 2883   |
| Bst98I      | C!TTAAG       | 1     | 1211  |
| Bst1107I    | GTA!TAC       | 0     |   |
| BstACI      | GR!CGYC       | 6     | 107; 296; 803; 2329; 3031; 3110   |
| BstAFI      | C!TTAAG       | 1     | 1211  |
| BstAPI      | GCANNNN!NTGC  | 4     | 943; 1896; 1968; 3430   |
| BstAUI      | T!GTACA       | 2     | 446; 539  |
| BstBI       | TT!CGAA       | 2     | 45; 3011  |
| Bst2BI      | C!ACGAG       | 3     | 595; 3047; 4074   |
| Bst2BI      | C!TCGTG       | 1     | 2921  |
| BstBAI      | YAC!GTR       | 7     | 138; 366; 495; 678; 708; 1445; 2633   |
| Bst4CI      | ACN!GT        | 11    | 63; 765; 928; 1420; 2709; 2825; 2933; 3256; 3747; 4217; 4288  |
| BstC8I      | GCN!NGC       | 30    | 71; 207; 352; 770; 863; 889; 1548; 1591; 1605; 1878; 1897; 1950; 1969; 2223; 2409; 2628; 2694; 2700; 2732; 2773; 2777; 2831; 3114; 3301; 3348; 3404; 3426; 3637; 4197; 4234 |
| BstDEI      | C!TNAG        | 9     | 24; 715; 832; 1809; 2111; 2992; 3441; 3564; 3973  |
| BstDSI      | C!CRYGG       | 7     | 67; 120; 183; 773; 849; 2058; 2761  |
| BstEII      | G!GTNACC      | 2     | 276; 423  |
| BstENI      | CCTNN!NNNAGG  | 0     |   |
| BstF5I      | GGATGNN!      | 10    | 168; 228; 492; 729; 885; 926; 2189; 2646; 2671; 3125  |
| BstF5I      | !CATCC        | 2     | 179; 2015   |
| BstFNI      | CG!CG         | 20    | 69; 185; 416; 455; 740; 770; 978; 1253; 1629; 1649; 1673; 1704; 2395; 2696; 3134; 3218; 3281; 3350; 3623; 4204  |
| BstH2I      | RGCGC!Y       | 8     | 16; 110; 299; 581; 1596; 1604; 2332; 4007   |
| BstHHI      | GCG!C         | 29    | 15; 109; 298; 377; 418; 580; 770; 772; 876; 1595; 1603; 1629; 1651; 1660; 1673; 1704; 2323; 2331; 2395; 2432; 2698; 2958; 3134; 3220; 3623; 3732; 3906; 4006; 4073          |
| BstKTI      | GAT!C         | 27    | 30; 81; 131; 555; 602; 636; 735; 838; 959; 972; 989; 2169; 2173; 2192; 2503; 2581; 2662; 2671; 2749; 3125; 3141; 3500; 3512; 3590; 3598; 3609; 3684                         |
| BstMAI      | GTCTCN!       | 1     | 835   |
| BstMAI      | !NNNNGAGAC    | 5     | 754; 1753; 2171; 3190; 3318   |
| BstMBI      | !GATC         | 27    | 27; 78; 128; 552; 599; 633; 732; 835; 956; 969; 986; 2166; 2170; 2189; 2500; 2578; 2659; 2668; 2746; 3122; 3138; 3497; 3509; 3587; 3595; 3606; 3681                         |
| BstMCI      | CGRY!CG       | 4     | 89; 976; 2238; 3913   |
| BstMWI      | GCNNNNN!NNGC  | 27    | 185; 206; 245; 302; 575; 614; 871; 943; 1556; 1586; 1613; 1655; 1896; 1968; 2019; 2098; 2320; 2404; 2427; 2566; 2689; 2772; 3039; 3226; 3430; 3629; 4201                    |
| BstNI       | CC!WGG        | 13    | 264; 274; 472; 910; 1865; 1920; 1937; 2716; 3295; 3398; 4088; 4101; 4222  |
| BstNSI      | RCATG!Y       | 5     | 354; 1899; 1971; 2734; 4251   |
| BstOI       | CC!WGG        | 13    | 264; 274; 472; 910; 1865; 1920; 1937; 2716; 3295; 3398; 4088; 4101; 4222  |
| BstPI       | G!GTNACC      | 2     | 276; 423  |
| BstPAI      | GACNN!NNGTC   | 0     |   |
| BstSCI      | !CCNGG        | 19    | 73; 74; 262; 272; 470; 908; 1863; 1918; 1935; 2331; 2491; 2714; 3104; 3293; 3396; 3868; 4086; 4099; 4220  |
| BstSFI      | C!TRYAG       | 5     | 52; 935; 1664; 3791; 3982   |
| BstSLI      | GKGCM!C       | 9     | 75; 257; 263; 677; 683; 909; 2275; 2368; 3937   |
| BstSNI      | TAC!GTA       | 0     |   |
| BstUI       | CG!CG         | 20    | 69; 185; 416; 455; 740; 770; 978; 1253; 1629; 1649; 1673; 1704; 2395; 2696; 3134; 3218; 3281; 3350; 3623; 4204  |
| Bst2UI      | CC!WGG        | 13    | 264; 274; 472; 910; 1865; 1920; 1937; 2716; 3295; 3398; 4088; 4101; 4222  |
| BstVII      | GCAGCNNNNNNN! | 10    | 113; 452; 620; 949; 1117; 2402; 2444; 2965; 3418; 3918  |

| Enzyme name | Sequence                     | Count | Cutting Position (the base to the left of the cut site)   |
|-------------|------------------------------|-------|---|
| BstV1I      | !NNNNNNNNNNNGCTGC            | 8     | 334; 878; 1611; 2276; 2460; 2553; 3619; 3825  |
| BstV2I      | GAAGACNN!                    | 0     |   |
| BstV2I      | !NNNNNNGTCTTC                | 0     |   |
| BstXI       | CCANNNNN!NTGG                | 0     |   |
| BstX2I      | R!GATCY                      | 11    | 27; 78; 128; 835; 2500; 2746; 3138; 3497; 3509; 3595; 3606  |
| BstYI       | R!GATCY                      | 11    | 27; 78; 128; 835; 2500; 2746; 3138; 3497; 3509; 3595; 3606  |
| BstZI       | C!GGCCG                      | 2     | 973; 2235   |
| BstZ17I     | GTA!TAC                      | 0     |   |
| BsuI        | GTATCCNNNNNN!                | 3     | 1758; 2548; 4049  |
| BsuI        | !NNNNNGGATAC                 | 0     |   |
| Bsu15I      | AT!CGAT                      | 1     | 2169  |
| Bsu36I      | CC!TNAGG                     | 2     | 1809; 3441  |
| BsuRI       | GG!CC                        | 27    | 73; 167; 320; 470; 491; 559; 569; 631; 668; 975; 1295; 1437; 2092; 2098; 2107; 2150; 2237; 2411; 2802; 2829; 3338; 3395; 3428; 3773; 4207; 4225; 4236   |
| BsuTUI      | AT!CGAT                      | 1     | 2169  |
| BtgI        | C!CRYGG                      | 7     | 67; 120; 183; 773; 849; 2058; 2761  |
| BtgZI       | GCGATGNNNNNNNNNN!            | 5     | 215; 263; 410; 1446; 2781   |
| BtgZI       | !NNNNNNNNNNNNNNNCATCGC       | 2     | 2600; 2944  |
| BthCI       | GCNG!C                       | 41    | 104; 107; 188; 350; 443; 611; 665; 668; 785; 874; 894; 940; 978; 1108; 1627; 1649; 1663; 2101; 2240; 2292; 2303; 2393; 2398; 2435; 2476; 2563; 2566; 2569; 2805; 2901; 2942; 2956; 3070; 3409; 3424; 3635; 3841; 3844; 3909; 4052; 4207 |
| BtrI        | CAC!GTC                      | 0     |   |
| BtrI        | GAC!GTG                      | 0     |   |
| BtsI        | GCAGTGNN!                    | 0     |   |
| BtsI        | !CACTGC                      | 2     | 531; 1168   |
| BtsCI       | GGATGNN!                     | 10    | 168; 228; 492; 729; 885; 926; 2189; 2646; 2671; 3125  |
| BtsCI       | !CATCC                       | 2     | 179; 2015   |
| BtuMI       | TCG!CGA                      | 0     |   |
| BveI        | ACCTGCNNNN!                  | 2     | 2597; 3047  |
| BveI        | !NNNNNNNNNGCAGGT             | 1     | 2216  |
| Cac8I       | GCN!NGC                      | 30    | 71; 207; 352; 770; 863; 889; 1548; 1591; 1605; 1878; 1897; 1950; 1969; 2223; 2409; 2628; 2694; 2700; 2732; 2773; 2777; 2831; 3114; 3301; 3348; 3404; 3426; 3637; 4197; 4234   |
| CaiI        | CAGNNN!CTG                   | 2     | 3430; 3838  |
| CciI        | T!CATGA                      | 2     | 1755; 3527  |
| CciNI       | GC!GGCCCG                    | 0     |   |
| CdiI        | CATC!G                       | 6     | 627; 660; 2618; 2623; 2821; 2962  |
| CdiI        | C!GATG                       | 6     | 201; 249; 357; 396; 1432; 2767  |
| CdpI        | GCGGAGNNNNNNNNNNNNNNNNNNNN!  | 1     | 4201  |
| CdpI        | !NNNNNNNNNNNNNNNNNNNNCTCCGC  | 2     | 1990; 2032  |
| CelII       | GC!TNAGC                     | 1     | 715   |
| CfoI        | GCG!C                        | 29    | 15; 109; 298; 377; 418; 580; 770; 772; 876; 1595; 1603; 1629; 1651; 1660; 1673; 1704; 2323; 2331; 2395; 2432; 2698; 2958; 3134; 3220; 3623; 3732; 3906; 4006; 4073  |
| CfrI        | Y!GGCCR                      | 9     | 165; 468; 489; 629; 973; 2235; 2409; 2800; 2827   |
| Cfr9I       | C!CCGGG                      | 1     | 74  |
| Cfr10I      | R!CCGGY                      | 9     | 84; 244; 604; 805; 861; 1546; 2648; 2829; 3112  |
| Cfr13I      | G!GNCC                       | 11    | 71; 72; 319; 667; 924; 1436; 2845; 3290; 3336; 3394; 3427   |
| Cfr42I      | CCGC!GG                      | 2     | 70; 186   |
| ChaI        | GATC!                        | 27    | 31; 82; 132; 556; 603; 637; 736; 839; 960; 973; 990; 2170; 2174; 2193; 2504; 2582; 2663; 2672; 2750; 3126; 3142; 3501; 3513; 3591; 3599; 3610; 3685   |
| CjeI        | CCANNNNNNGTNNNNNNNNNNNNNNNN! | 6     | 556; 921; 2146; 3586; 3686; 3764  |
| CjeI        | !NNNNNNNNNACNNNNNNNTGG       | 5     | 1813; 1908; 3100; 3650; 4205  |
| CjeI        | ACNNNNNNNTGGNNNNNNNNNNNNNN!  | 5     | 1847; 1942; 3134; 3684; 4239  |
| CjeI        | !NNNNNNNNCCANNNNNNGT         | 6     | 522; 887; 2112; 3552; 3652; 3730  |
| CjePI       | CCANNNNNNNTCNNNNNNNNNNNNNN!  | 2     | 3586; 3764  |
| CjePI       | !NNNNNNNNGANNNNNNNTGG        | 3     | 450; 3100; 3699   |
| CjePI       | GANNNNNNNTGGNNNNNNNNNNNNNN!  | 3     | 483; 3133; 3732   |
| CjePI       | !NNNNNNNNCCANNNNNNNTC        | 2     | 3553; 3731  |
| ClaI        | AT!CGAT                      | 1     | 2169  |
| CpoI        | CG!GWCCG                     | 1     | 2845  |
| CseI        | GACGCNNNNN!                  | 3     | 811; 3039; 3118   |
| CseI        | !NNNNNNNNNNNGCGTC            | 3     | 1662; 3557; 4135  |



| Enzyme name | Sequence                   | Count | Cutting Position (the base to the left of the cut site)   |
|-------------|----------------------------|-------|---|
| CsiI        | A!CCWGGT                   | 1     | 1918  |
| CspI        | CG!GWCCG                   | 1     | 2845  |
| Csp6I       | G!TAC                      | 6     | 64; 315; 447; 540; 687; 2634  |
| Csp45I      | TT!CGAA                    | 2     | 45; 3011  |
| CspAI       | A!CCGGT                    | 1     | 84  |
| CspCI       | CAANNNNNGTGGNNNNNNNNNNNN!  | 0     |   |
| CspCI       | !NNNNNNNNNNCCACNNNNNTTG    | 0     |   |
| CspCI       | CCACNNNNNTTGNNNNNNNNNNNN!  | 0     |   |
| CspCI       | !NNNNNNNNNNCAANNNNNGTGG    | 0     |   |
| CstMI       | AAGGAGNNNNNNNNNNNNNNNNNN!  | 3     | 782; 1609; 3218   |
| CstMI       | !NNNNNNNNNNNNNNNNNNCTCCTT  | 0     |   |
| CviAII      | C!ATG                      | 18    | 96; 351; 696; 850; 897; 1756; 1896; 1968; 2059; 2200; 2545; 2731; 2762; 2788; 3144; 3528; 4248; 4299  |
| CviJI       | RG!CY                      | 91    | 36; 42; 73; 167; 194; 320; 327; 347; 362; 462; 470; 476; 491; 559; 569; 631; 668; 701; 714; 752; 824; 848; 854; 940; 975; 998; 1108; 1275; 1295; 1437; 1518; 1532; 1546; 1827; 1868; 1940; 2063; 2092; 2098; 2107; 2115; 2138; 2150; 2156; 2237; 2254; 2262; 2289; 2314; 2398; 2407; 2411; 2435; 2473; 2549; 2566; 2585; 2648; 2692; 2702; 2719; 2802; 2829; 2833; 2870; 2893; 2909; 3089; 3116; 3177; 3299; 3338; 3395; 3402; 3409; 3428; 3439; 3690; 3719; 3762; 3773; 3838; 3917; 3942; 3947; 3993; 4083; 4181; 4207; 4225; 4236 |
| CviKI-1     | RG!CY                      | 91    | 36; 42; 73; 167; 194; 320; 327; 347; 362; 462; 470; 476; 491; 559; 569; 631; 668; 701; 714; 752; 824; 848; 854; 940; 975; 998; 1108; 1275; 1295; 1437; 1518; 1532; 1546; 1827; 1868; 1940; 2063; 2092; 2098; 2107; 2115; 2138; 2150; 2156; 2237; 2254; 2262; 2289; 2314; 2398; 2407; 2411; 2435; 2473; 2549; 2566; 2585; 2648; 2692; 2702; 2719; 2802; 2829; 2833; 2870; 2893; 2909; 3089; 3116; 3177; 3299; 3338; 3395; 3402; 3409; 3428; 3439; 3690; 3719; 3762; 3773; 3838; 3917; 3942; 3947; 3993; 4083; 4181; 4207; 4225; 4236 |
| CviQI       | G!TAC                      | 6     | 64; 315; 447; 540; 687; 2634  |
| DdeI        | C!TNAG                     | 9     | 24; 715; 832; 1809; 2111; 2992; 3441; 3564; 3973  |
| DinI        | GGC!GCC                    | 3     | 108; 297; 2330  |
| DpnI        | GA!TC                      | 27    | 29; 80; 130; 554; 601; 635; 734; 837; 958; 971; 988; 2168; 2172; 2191; 2502; 2580; 2661; 2670; 2748; 3124; 3140; 3499; 3511; 3589; 3597; 3608; 3683   |
| DpnII       | !GATC                      | 27    | 27; 78; 128; 552; 599; 633; 732; 835; 956; 969; 986; 2166; 2170; 2189; 2500; 2578; 2659; 2668; 2746; 3122; 3138; 3497; 3509; 3587; 3595; 3606; 3681   |
| DraI        | TTT!AAA                    | 3     | 1031; 3473; 3492  |
| DraII       | RG!GNCCY                   | 1     | 3427  |
| DraIII      | CACNNN!GTG                 | 1     | 1445  |
| DraRI       | CAAGNACNNNNNNNNNNNNNNNNNN! | 2     | 338; 3809   |
| DraRI       | !NNNNNNNNNNNNNNNNNGTNCCTG  | 0     |   |
| DrdI        | GACNNN!NNGTC               | 3     | 1400; 2356; 4145  |
| DrdIV       | TACGACNNNNNNNNNNNNNNNNNN!  | 1     | 818   |
| DrdIV       | !NNNNNNNNNNNNNNNNNGTCGTA   | 0     |   |
| DriI        | GACNNN!NNGTC               | 0     |   |
| DseDI       | GACNNN!NNGTC               | 3     | 1400; 2356; 4145  |
| EaeI        | Y!GGCCR                    | 9     | 165; 468; 489; 629; 973; 2235; 2409; 2800; 2827   |
| EagI        | C!GGCCG                    | 2     | 973; 2235   |
| Eam1104I    | CTCTTCN!                   | 0     |   |
| Eam1104I    | !NNNNGAAGAG                | 4     | 568; 1796; 2673; 2883   |
| Eam1105I    | GACNNN!NNGTC               | 0     |   |
| EarI        | CTCTTCN!                   | 0     |   |
| EarI        | !NNNNGAAGAG                | 4     | 568; 1796; 2673; 2883   |
| EciI        | GGCGANNNNNNNNNNNN!         | 4     | 1829; 2858; 4045; 4191  |
| EciI        | !NNNNNNNNNTCCGCC           | 3     | 2000; 2021; 2042  |
| Ecl136II    | GAG!CTC                    | 1     | 36  |
| EclXI       | C!GGCCG                    | 2     | 973; 2235   |
| Eco24I      | GRGCY!C                    | 5     | 38; 75; 1520; 2694; 3404  |
| Eco31I      | GGTCTCN!                   | 1     | 835   |
| Eco31I      | !NNNNNGAGACC               | 2     | 754; 3318   |
| Eco32I      | GAT!ATC                    | 0     |   |
| Eco47I      | G!GWCC                     | 3     | 924; 2845; 3290   |
| Eco47III    | AGC!GCT                    | 1     | 14  |

| Enzyme name | Sequence                | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|-------------------------|-------|--|
| Eco52I      | C!GGCCG                 | 2     | 973; 2235  |
| Eco57I      | CTGAAGNNNNNNNNNNNNNNNN! | 5     | 349; 592; 775; 2475; 2907  |
| Eco57I      | !NNNNNNNNNNNNNNCTTCAG   | 2     | 159; 3705  |
| Eco72I      | CAC!GTG                 | 3     | 495; 678; 708  |
| Eco81I      | CC!TNAGG                | 2     | 1809; 3441   |
| Eco88I      | C!YCGRG                 | 4     | 31; 74; 322; 682   |
| Eco91I      | G!GTNACC                | 2     | 276; 423   |
| Eco105I     | TAC!GTA                 | 0     |  |
| Eco130I     | C!CWWGG                 | 5     | 849; 2058; 2151; 2761; 3166  |
| Eco147I     | AGG!CCT                 | 1     | 2150   |
| EcoHI       | !CCSGG                  | 6     | 73; 74; 2331; 2491; 3104; 3868   |
| EcoICRI     | GAG!CTC                 | 1     | 36   |
| Eco57MI     | CTGRAGNNNNNNNNNNNNNNNN! | 8     | 285; 349; 532; 592; 775; 2475; 2907; 3169  |
| Eco57MI     | !NNNNNNNNNNNNNNCTYCAG   | 3     | 159; 3112; 3705  |
| EcoNI       | CCTNN!NNNAGG            | 0     |  |
| Eco065I     | G!GTNACC                | 2     | 276; 423   |
| Eco0109I    | RG!GNCCY                | 1     | 3427   |
| EcoRI       | G!AATTC                 | 1     | 47   |
| EcoRII      | !CCWGG                  | 13    | 262; 272; 470; 908; 1863; 1918; 1935; 2714; 3293; 3396; 4086; 4099; 4220   |
| EcoRV       | GAT!ATC                 | 0     |  |
| EcoT14I     | C!CWWGG                 | 5     | 849; 2058; 2151; 2761; 3166  |
| EcoT22I     | ATGCA!T                 | 3     | 1901; 1973; 4304   |
| EcoT38I     | GRGCY!C                 | 5     | 38; 75; 1520; 2694; 3404   |
| Eco53kI     | GAG!CTC                 | 1     | 36   |
| EgeI        | GGC!GCC                 | 3     | 108; 297; 2330   |
| EheI        | GGC!GCC                 | 3     | 108; 297; 2330   |
| ErhI        | C!CWWGG                 | 5     | 849; 2058; 2151; 2761; 3166  |
| EsaBC3I     | TC!GA                   | 18    | 33; 46; 59; 390; 432; 801; 841; 1479; 2170; 2443; 2599; 2623; 2659; 2821; 3012; 3057; 3312; 4150   |
| Esp3I       | CGTCTCN!                | 0     |  |
| Esp3I       | !NNNNNGAGACG            | 0     |  |
| FaeI        | CATG!                   | 18    | 99; 354; 699; 853; 900; 1759; 1899; 1971; 2062; 2203; 2548; 2734; 2765; 2791; 3147; 3531; 4251; 4302   |
| Fail        | YA!TR                   | 43    | 97; 352; 697; 748; 851; 898; 991; 1001; 1067; 1112; 1317; 1745; 1757; 1888; 1897; 1960; 1969; 1993; 2060; 2084; 2201; 2265; 2546; 2571; 2732; 2763; 2789; 2861; 3077; 3145; 3223; 3275; 3436; 3453; 3455; 3457; 3529; 3807; 3996; 4112; 4185; 4249; 4300 |
| FalI        | AAGNNNNNCTNNNNNNNNNNNN! | 2     | 597; 2669  |
| FalI        | !NNNNNNNNAAGNNNNNNCTT   | 2     | 565; 2637  |
| FaqI        | GGGACNNNNNNNNNN!        | 3     | 2480; 3012; 3121   |
| FaqI        | !NNNNNNNNNNNNNGTCCC     | 4     | 1845; 1917; 1981; 3276   |
| FatI        | !CATG                   | 18    | 95; 350; 695; 849; 896; 1755; 1895; 1967; 2058; 2199; 2544; 2730; 2761; 2787; 3143; 3527; 4247; 4298   |
| FauI        | CCCGCNNNN!              | 7     | 421; 866; 1651; 2006; 2027; 3223; 3355   |
| FauI        | !NNNNNGCGGG             | 7     | 62; 910; 1582; 2452; 2989; 3127; 3274  |
| FauNDI      | CA!TATG                 | 0     |  |
| FbaI        | T!GATCA                 | 0     |  |
| FblI        | GT!MKAC                 | 1     | 58   |
| FmuI        | GGNC!C                  | 11    | 74; 75; 322; 670; 927; 1439; 2848; 3293; 3339; 3397; 3430  |
| Fnu4HI      | GC!NGC                  | 41    | 102; 105; 186; 348; 441; 609; 663; 666; 783; 872; 892; 938; 976; 1106; 1625; 1647; 1661; 2099; 2238; 2290; 2301; 2391; 2396; 2433; 2474; 2561; 2564; 2567; 2803; 2899; 2940; 2954; 3068; 3407; 3422; 3633; 3839; 3842; 3907; 4050; 4205                  |
| FokI        | GGATGNNNNNNNN!          | 10    | 175; 235; 499; 736; 892; 933; 2196; 2653; 2678; 3132   |
| FokI        | !NNNNNNNNNNNNNCATCC     | 2     | 166; 2002  |
| FriOI       | GRGCY!C                 | 5     | 38; 75; 1520; 2694; 3404   |
| FseI        | GGCCGG!CC               | 0     |  |
| FspI        | TGC!GCA                 | 1     | 2431   |
| FspAI       | RTGC!GCAY               | 0     |  |
| FspBI       | C!TAG                   | 9     | 10; 953; 984; 1178; 1596; 2152; 3167; 3501; 3754   |

| Enzyme name | Sequence                   | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|----------------------------|-------|--|
| FspEI       | CCNNNNNNNNNNNN!            | 147   | 32; 80; 95; 123; 145; 181; 196; 219; 240; 255; 269; 285; 303; 324; 368; 393; 426; 441; 484; 505; 540; 573; 594; 618; 645; 666; 682; 696; 718; 744; 766; 786; 804; 819; 840; 862; 908; 922; 940; 954; 989; 1012; 1051; 1065; 1206; 1301; 1325; 1348; 1371; 1389; 1411; 1432; 1451; 1470; 1499; 1520; 1534; 1560; 1648; 1674; 1723; 1764; 1782; 1821; 1837; 1875; 1932; 1947; 2004; 2024; 2038; 2055; 2069; 2106; 2121; 2164; 2247; 2314; 2345; 2365; 2380; 2425; 2439; 2505; 2519; 2538; 2554; 2595; 2615; 2662; 2712; 2728; 2748; 2773; 2793; 2816; 2843; 2861; 2887; 2927; 2953; 2977; 3033; 3047; 3075; 3118; 3139; 3174; 3218; 3306; 3330; 3352; 3372; 3388; 3409; 3423; 3442; 3526; 3546; 3574; 3589; 3623; 3665; 3692; 3707; 3752; 3776; 3790; 3812; 3838; 3858; 3882; 3908; 3956; 3975; 3994; 4020; 4055; 4100; 4130; 4144; 4195; 4209; 4234; 4250; 4271; 4300 |
| FspEI       | !NNNNNNNNNNNNNNNGG         | 121   | 4; 46; 70; 89; 107; 127; 145; 170; 196; 230; 249; 278; 302; 344; 376; 406; 452; 473; 496; 531; 551; 590; 613; 644; 706; 742; 760; 791; 812; 836; 856; 895; 939; 957; 1000; 1100; 1169; 1199; 1277; 1326; 1348; 1376; 1412; 1457; 1486; 1522; 1541; 1569; 1589; 1652; 1675; 1783; 1815; 1833; 1862; 1905; 1934; 2045; 2074; 2117; 2138; 2166; 2199; 2219; 2244; 2271; 2296; 2318; 2346; 2372; 2393; 2444; 2465; 2483; 2531; 2567; 2623; 2648; 2672; 2701; 2724; 2748; 2774; 2796; 2815; 2840; 2880; 2916; 2981; 3066; 3090; 3119; 3153; 3174; 3194; 3239; 3266; 3285; 3318; 3370; 3401; 3428; 3480; 3578; 3649; 3694; 3755; 3820; 3854; 3880; 3898; 3934; 4004; 4027; 4049; 4073; 4106; 4154; 4189; 4207; 4264  |
| Fsp4HI      | GC!NGC                     | 41    | 102; 105; 186; 348; 441; 609; 663; 666; 783; 872; 892; 938; 976; 1106; 1625; 1647; 1661; 2099; 2238; 2290; 2301; 2391; 2396; 2433; 2474; 2561; 2564; 2567; 2803; 2899; 2940; 2954; 3068; 3407; 3422; 3633; 3839; 3842; 3907; 4050; 4205  |
| GdiII       | C!GGCCR                    | 2     | 973; 2235  |
| GdiII       | Y!GGCCG                    | 5     | 629; 973; 2235; 2800; 2827   |
| GlaI        | GC!GC                      | 29    | 14; 108; 297; 376; 417; 579; 769; 771; 875; 1594; 1602; 1628; 1650; 1659; 1672; 1703; 2322; 2330; 2394; 2431; 2697; 2957; 3133; 3219; 3622; 3731; 3905; 4005; 4072   |
| GluI        | GC!NGC                     | 41    | 102; 105; 186; 348; 441; 609; 663; 666; 783; 872; 892; 938; 976; 1106; 1625; 1647; 1661; 2099; 2238; 2290; 2301; 2391; 2396; 2433; 2474; 2561; 2564; 2567; 2803; 2899; 2940; 2954; 3068; 3407; 3422; 3633; 3839; 3842; 3907; 4050; 4205  |
| GsaI        | CCCAG!C                    | 3     | 1876; 1948; 3947   |
| GsaI        | GCTGG!G                    | 2     | 502; 2837  |
| GsuI        | CTGGAGNNNNNNNNNNNNNNNN!    | 3     | 285; 532; 3169   |
| GsuI        | !NNNNNNNNNNNNNNCTCCAG      | 1     | 3112   |
| HaeI        | WGG!CCW                    | 9     | 167; 470; 491; 559; 2150; 2411; 3773; 4225; 4236   |
| HaeII       | RGCG!Y                     | 8     | 16; 110; 299; 581; 1596; 1604; 2332; 4007  |
| HaeIII      | GG!CC                      | 27    | 73; 167; 320; 470; 491; 559; 569; 631; 668; 975; 1295; 1437; 2092; 2098; 2107; 2150; 2237; 2411; 2802; 2829; 3338; 3395; 3428; 3773; 4207; 4225; 4236  |
| HaeIV       | GAYNNNNNRTCNNNNNNNNNNNNNN! | 4     | 860; 945; 1004; 2525   |
| HaeIV       | !NNNNNNNNNGAYNNNNNNRTC     | 4     | 826; 911; 970; 2491  |
| HaeIV       | GAYNNNNNRTCNNNNNNNNNNNNNN! | 4     | 859; 944; 1003; 2524   |
| HaeIV       | !NNNNNNNGAYNNNNNNRTC       | 4     | 828; 913; 972; 2493  |
| HapII       | C!CGG                      | 25    | 19; 75; 85; 245; 605; 806; 812; 827; 862; 1547; 2234; 2311; 2333; 2361; 2492; 2582; 2649; 2830; 3105; 3113; 3205; 3679; 3869; 3895; 4042   |
| HgaI        | GACGCNNNNN!                | 3     | 811; 3039; 3118  |
| HgaI        | !NNNNNNNNNNNGCGTC          | 3     | 1662; 3557; 4135   |
| HhaI        | GCG!C                      | 29    | 15; 109; 298; 377; 418; 580; 770; 772; 876; 1595; 1603; 1629; 1651; 1660; 1673; 1704; 2323; 2331; 2395; 2432; 2698; 2958; 3134; 3220; 3623; 3732; 3906; 4006; 4073   |
| Hin1I       | GR!CGYC                    | 6     | 107; 296; 803; 2329; 3031; 3110  |
| Hin1II      | CATG!                      | 18    | 99; 354; 699; 853; 900; 1759; 1899; 1971; 2062; 2203; 2548; 2734; 2765; 2791; 3147; 3531; 4251; 4302   |
| Hin4I       | GAYNNNNNVTCNNNNNNNNNNNNNN! | 4     | 840; 944; 1003; 2524   |
| Hin4I       | !NNNNNNNNGABNNNNNNRTC      | 5     | 827; 912; 971; 2492; 2522  |
| Hin4I       | GABNNNNNRTCNNNNNNNNNNNNNN! | 5     | 859; 944; 1003; 2524; 2554   |
| Hin4I       | !NNNNNNNNGAYNNNNNVTC       | 5     | 808; 827; 912; 971; 2492   |
| Hin6I       | G!CGC                      | 29    | 13; 107; 296; 375; 416; 578; 768; 770; 874; 1593; 1601; 1627; 1649; 1658; 1671; 1702; 2321; 2329; 2393; 2430; 2696; 2956; 3132; 3218; 3621; 3730; 3904; 4004; 4071   |

| Enzyme name | Sequence           | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|--------------------|-------|--|
| HinP1I      | G!CGC              | 29    | 13; 107; 296; 375; 416; 578; 768; 770; 874; 1593; 1601; 1627; 1649; 1658; 1671; 1702; 2321; 2329; 2393; 2430; 2696; 2956; 3132; 3218; 3621; 3730; 3904; 4004; 4071   |
| HincII      | GTY!RAC            | 2     | 59; 1092   |
| HindII      | GTY!RAC            | 2     | 59; 1092   |
| HindIII     | A!AGCTT            | 1     | 40   |
| HinfI       | G!ANTC             | 14    | 22; 507; 842; 980; 1372; 1394; 1804; 2814; 2948; 3000; 3058; 3095; 3877; 4273  |
| HpaI        | GTT!AAC            | 1     | 1092   |
| HpaII       | C!CGG              | 25    | 19; 75; 85; 245; 605; 806; 812; 827; 862; 1547; 2234; 2311; 2333; 2361; 2492; 2582; 2649; 2830; 3105; 3113; 3205; 3679; 3869; 3895; 4042   |
| HphI        | GGTGANNNNNNNN!     | 4     | 288; 435; 3400; 3516   |
| HphI        | !NNNNNNNTCACC      | 7     | 375; 516; 594; 727; 922; 1446; 2507  |
| Hpy8I       | GTN!NAC            | 8     | 59; 447; 675; 1092; 1376; 1393; 1448; 3935   |
| Hpy99I      | CGWCG!             | 9     | 63; 805; 2419; 2447; 2741; 2756; 3033; 3418; 4151  |
| Hpy166II    | GTN!NAC            | 8     | 59; 447; 675; 1092; 1376; 1393; 1448; 3935   |
| Hpy188I     | TCN!GA             | 12    | 27; 835; 1503; 2112; 2296; 2640; 2993; 3094; 3573; 3707; 4060; 4138  |
| Hpy188III   | TC!NNGA            | 27    | 31; 408; 432; 480; 511; 812; 839; 984; 1756; 1808; 2121; 2174; 2347; 2663; 2672; 2755; 2811; 2855; 2945; 2978; 3047; 3528; 3602; 3685; 3783; 3881; 4015  |
| HpyAV       | CCTTCNNNNNN!       | 7     | 183; 396; 438; 591; 2974; 3081; 3760   |
| HpyAV       | !NNNNNGAAGG        | 10    | 324; 477; 750; 1562; 2457; 3186; 3202; 3385; 3702; 4012  |
| HpyCH4III   | ACN!GT             | 11    | 63; 765; 928; 1420; 2709; 2825; 2933; 3256; 3747; 4217; 4288   |
| HpyCH4IV    | A!CGT              | 13    | 137; 365; 494; 677; 707; 1389; 1401; 1444; 1554; 2445; 2632; 3413; 3546  |
| HpyCH4V     | TG!CA              | 22    | 54; 239; 350; 369; 675; 937; 1078; 1105; 1173; 1890; 1899; 1962; 1971; 2086; 2162; 2221; 2380; 2555; 2569; 3639; 3935; 4302  |
| HpyF3I      | C!TNAG             | 9     | 24; 715; 832; 1809; 2111; 2992; 3441; 3564; 3973   |
| HpyF10VI    | GCNNNNN!NNGC       | 27    | 185; 206; 245; 302; 575; 614; 871; 943; 1556; 1586; 1613; 1655; 1896; 1968; 2019; 2098; 2320; 2404; 2427; 2566; 2689; 2772; 3039; 3226; 3430; 3629; 4201   |
| Hsp92I      | GR!CGYC            | 6     | 107; 296; 803; 2329; 3031; 3110  |
| Hsp92II     | CATG!              | 18    | 99; 354; 699; 853; 900; 1759; 1899; 1971; 2062; 2203; 2548; 2734; 2765; 2791; 3147; 3531; 4251; 4302   |
| HspAI       | G!CGC              | 29    | 13; 107; 296; 375; 416; 578; 768; 770; 874; 1593; 1601; 1627; 1649; 1658; 1671; 1702; 2321; 2329; 2393; 2430; 2696; 2956; 3132; 3218; 3621; 3730; 3904; 4004; 4071   |
| KasI        | G!GCGCC            | 3     | 106; 295; 2328   |
| KfiI        | GG!GWCCC           | 0     |  |
| KpnI        | GGTAC!C            | 1     | 67   |
| Kpn2I       | T!CCGGA            | 1     | 811  |
| KspI        | CCGC!GG            | 2     | 70; 186  |
| Ksp22I      | T!GATCA            | 0     |  |
| KspAI       | GTT!AAC            | 1     | 1092   |
| Kzo9I       | !GATC              | 27    | 27; 78; 128; 552; 599; 633; 732; 835; 956; 969; 986; 2166; 2170; 2189; 2500; 2578; 2659; 2668; 2746; 3122; 3138; 3497; 3509; 3587; 3595; 3606; 3681  |
| LguI        | GCTCTCN!           | 0     |  |
| LguI        | !NNNNGAAGAGC       | 3     | 568; 2673; 2883  |
| LpnI        | RG!GCGY            | 8     | 14; 108; 297; 579; 1594; 1602; 2330; 4005  |
| LpnPI       | CCDGNNNNNNNNN!     | 64    | 32; 88; 124; 219; 241; 258; 276; 298; 313; 484; 550; 583; 618; 655; 766; 819; 840; 875; 922; 947; 1066; 1371; 1560; 1783; 1821; 1837; 1877; 1932; 1949; 2049; 2134; 2247; 2324; 2346; 2369; 2505; 2519; 2538; 2595; 2662; 2712; 2728; 2785; 2843; 3052; 3118; 3142; 3218; 3307; 3410; 3443; 3674; 3692; 3838; 3858; 3882; 3908; 3957; 4055; 4100; 4130; 4209; 4234; 4271 |
| LpnPI       | !NNNNNNNNNNNNNCHGG | 54    | 4; 60; 230; 249; 355; 457; 484; 531; 590; 791; 812; 847; 895; 1412; 1532; 1589; 1652; 1850; 1862; 1922; 1934; 2166; 2211; 2254; 2296; 2318; 2346; 2393; 2455; 2477; 2567; 2634; 2657; 2701; 2796; 2815; 2840; 2989; 3090; 3133; 3190; 3280; 3383; 3410; 3428; 3664; 3701; 3854; 3880; 4020; 4038; 4073; 4154; 4207   |
| Lsp1109I    | GCAGCNNNNNNNN!     | 10    | 113; 452; 620; 949; 1117; 2402; 2444; 2965; 3418; 3918   |
| Lsp1109I    | !NNNNNNNNNNNNGCTGC | 8     | 334; 878; 1611; 2276; 2460; 2553; 3619; 3825   |

| Enzyme name | Sequence                       | Count | Cutting Position (the base to the left of the cut site)   |
|-------------|--------------------------------|-------|---|
| LweI        | GCATCNNNNN!                    | 7     | 188; 1145; 1908; 1980; 2628; 2692; 2967   |
| LweI        | !NNNNNNNNNGATGC                | 6     | 192; 213; 2287; 2542; 2758; 4150  |
| MabI        | A!CCWGGT                       | 1     | 1918  |
| MaeI        | C!TAG                          | 9     | 10; 953; 984; 1178; 1596; 2152; 3167; 3501; 3754  |
| MaeII       | A!CGT                          | 13    | 137; 365; 494; 677; 707; 1389; 1401; 1444; 1554; 2445; 2632; 3413; 3546   |
| MaeIII      | !GTNAC                         | 13    | 276; 423; 726; 928; 1117; 1618; 1630; 2449; 2755; 3445; 3711; 3827; 3890  |
| MalI        | GA!TC                          | 27    | 29; 80; 130; 554; 601; 635; 734; 837; 958; 971; 988; 2168; 2172; 2191; 2502; 2580; 2661; 2670; 2748; 3124; 3140; 3499; 3511; 3589; 3597; 3608; 3683 |
| MaqI        | CRTTGACNNNNNNNNNNNNNNNNNNNNNN! | 0     |   |
| MaqI        | !NNNNNNNNNNNNNNNNNNNGTCAAYG    | 0     |   |
| MauBI       | CG!CGCGCG                      | 0     |   |
| MbiI        | CCG!CTC                        | 2     | 1753; 2942  |
| MbiI        | GAG!CGG                        | 3     | 917; 1589; 2996   |
| MboI        | !GATC                          | 27    | 27; 78; 128; 552; 599; 633; 732; 835; 956; 969; 986; 2166; 2170; 2189; 2500; 2578; 2659; 2668; 2746; 3122; 3138; 3497; 3509; 3587; 3595; 3606; 3681 |
| MboII       | GAAGANNNNNNNN!                 | 6     | 585; 1585; 1813; 2690; 2900; 3519   |
| MboII       | !NNNNNNNTCTTC                  | 4     | 2980; 3147; 3347; 3590  |
| McaTI       | GCGC!GC                        | 1     | 771   |
| MfeI        | C!AATTG                        | 1     | 1079  |
| MfiI        | R!GATCY                        | 11    | 27; 78; 128; 835; 2500; 2746; 3138; 3497; 3509; 3595; 3606  |
| MhII        | GDGCH!C                        | 16    | 38; 75; 257; 263; 271; 677; 683; 909; 1520; 2275; 2368; 2442; 2632; 2694; 3404; 3937  |
| MIsI        | TGG!CCA                        | 4     | 167; 470; 491; 2411   |
| MluI        | A!CGCGT                        | 0     |   |
| MluNI       | TGG!CCA                        | 4     | 167; 470; 491; 2411   |
| MlyI        | GAGTCNNNNN!                    | 2     | 1381; 1813  |
| MlyI        | !NNNNNGACTC                    | 5     | 16; 974; 1388; 2994; 3871   |
| MlyI13I     | GG!CGCC                        | 3     | 107; 296; 2329  |
| MmeI        | TCCRACNNNNNNNNNNNNNNNNNNNNNN!  | 1     | 1422  |
| MmeI        | !NNNNNNNNNNNNNNNNNNNGTYGGA     | 2     | 3854; 4038  |
| MnII        | CCTCNNNNNNN!                   | 7     | 1048; 2112; 2930; 3136; 3450; 3820; 4144  |
| MnII        | !NNNNNNNGAGG                   | 15    | 144; 384; 414; 1008; 1473; 1804; 2082; 2125; 2140; 2180; 2244; 2380; 2737; 3167; 4070   |
| Mph1103I    | ATGCA!T                        | 3     | 1901; 1973; 4304  |
| MreI        | CG!CCGGCG                      | 1     | 861   |
| MroI        | T!CCGGA                        | 1     | 811   |
| MroNI       | G!CCGGC                        | 4     | 861; 1546; 2829; 3112   |
| MroXI       | GAANN!NNTTC                    | 1     | 334   |
| MscI        | TGG!CCA                        | 4     | 167; 470; 491; 2411   |
| MseI        | T!TAA                          | 14    | 1030; 1091; 1212; 1233; 1244; 1256; 1267; 1284; 1382; 1653; 3472; 3486; 3491; 3543  |
| MslI        | CAYNN!NNRTG                    | 3     | 274; 643; 2766  |
| MspI        | C!CGG                          | 25    | 19; 75; 85; 245; 605; 806; 812; 827; 862; 1547; 2234; 2311; 2333; 2361; 2492; 2582; 2649; 2830; 3105; 3113; 3205; 3679; 3869; 3895; 4042            |
| Msp20I      | TGG!CCA                        | 4     | 167; 470; 491; 2411   |
| MspA1I      | CMG!CKG                        | 7     | 69; 104; 185; 1827; 2435; 3664; 3909  |
| MspCI       | C!TTAAG                        | 1     | 1211  |

| Enzyme name | Sequence                 | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|--------------------------|-------|--|
| MspJI       | CNNRNNNNNNNNNN!          | 224   | 22; 36; 49; 80; 97; 123; 138; 181; 196; 218; 239; 257; 270; 285; 298; 311; 324; 340; 354; 367; 382; 402; 420; 444; 466; 483; 505; 523; 542; 558; 572; 597; 617; 639; 653; 667; 682; 695; 708; 727; 743; 765; 781; 796; 818; 833; 846; 862; 887; 900; 914; 930; 946; 965; 989; 1002; 1017; 1036; 1053; 1066; 1107; 1121; 1134; 1152; 1190; 1205; 1223; 1264; 1288; 1308; 1326; 1347; 1370; 1402; 1417; 1439; 1457; 1470; 1491; 1508; 1521; 1534; 1550; 1563; 1593; 1608; 1633; 1651; 1664; 1679; 1703; 1717; 1741; 1766; 1782; 1820; 1836; 1875; 1889; 1903; 1931; 1947; 1961; 1975; 2004; 2017; 2038; 2047; 2069; 2099; 2115; 2133; 2163; 2186; 2210; 2234; 2253; 2281; 2307; 2323; 2336; 2359; 2373; 2393; 2406; 2420; 2439; 2453; 2466; 2482; 2497; 2510; 2526; 2541; 2557; 2582; 2601; 2617; 2630; 2643; 2661; 2675; 2689; 2705; 2727; 2743; 2762; 2784; 2800; 2823; 2842; 2856; 2872; 2886; 2906; 2922; 2935; 2957; 2990; 3004; 3024; 3037; 3051; 3105; 3118; 3141; 3154; 3173; 3190; 3217; 3253; 3286; 3305; 3330; 3351; 3378; 3391; 3408; 3422; 3437; 3453; 3513; 3538; 3554; 3576; 3589; 3603; 3634; 3648; 3673; 3691; 3706; 3719; 3738; 3751; 3766; 3795; 3814; 3832; 3857; 3872; 3891; 3907; 3926; 3944; 3960; 3974; 3993; 4020; 4043; 4065; 4084; 4099; 4112; 4129; 4149; 4176; 4189; 4208; 4233; 4256; 4270; 4283; 4300 |
| MspJI       | !NNNNNNNNNNNNNYNG        | 185   | 5; 25; 54; 71; 97; 122; 145; 162; 182; 202; 222; 242; 259; 278; 298; 328; 350; 374; 394; 416; 440; 457; 479; 496; 523; 541; 576; 598; 628; 645; 662; 682; 702; 725; 751; 772; 792; 812; 831; 848; 874; 895; 920; 939; 958; 981; 1010; 1039; 1081; 1164; 1199; 1226; 1249; 1278; 1322; 1344; 1376; 1412; 1429; 1450; 1487; 1507; 1524; 1541; 1576; 1594; 1612; 1629; 1653; 1675; 1702; 1730; 1756; 1794; 1815; 1832; 1850; 1873; 1894; 1922; 1945; 1966; 1984; 2005; 2022; 2045; 2069; 2089; 2107; 2129; 2160; 2182; 2208; 2227; 2244; 2272; 2291; 2310; 2328; 2346; 2367; 2389; 2413; 2446; 2466; 2484; 2505; 2531; 2558; 2575; 2592; 2609; 2635; 2657; 2679; 2697; 2717; 2736; 2758; 2796; 2816; 2840; 2860; 2880; 2907; 2931; 2964; 2983; 3011; 3033; 3062; 3090; 3115; 3134; 3153; 3174; 3191; 3208; 3239; 3266; 3286; 3309; 3329; 3366; 3383; 3402; 3428; 3448; 3487; 3514; 3533; 3551; 3588; 3606; 3647; 3665; 3691; 3708; 3740; 3769; 3797; 3818; 3841; 3860; 3881; 3899; 3916; 3934; 3960; 3981; 4001; 4021; 4039; 4060; 4086; 4103; 4122; 4141; 4170; 4189; 4207; 4226; 4244; 4262; 4285   |
| MspR9I      | CC!NGG                   | 19    | 75; 76; 264; 274; 472; 910; 1865; 1920; 1937; 2333; 2493; 2716; 3106; 3295; 3398; 3870; 4088; 4101; 4222   |
| MssI        | GTTT!AAAC                | 0     |  |
| MunI        | C!AATTG                  | 1     | 1079   |
| MvaI        | CC!WGG                   | 13    | 264; 274; 472; 910; 1865; 1920; 1937; 2716; 3295; 3398; 4088; 4101; 4222   |
| Mva1269I    | GAATGCN!                 | 1     | 1080   |
| Mva1269I    | G!CATTC                  | 1     | 1173   |
| MvnI        | CG!CG                    | 20    | 69; 185; 416; 455; 740; 770; 978; 1253; 1629; 1649; 1673; 1704; 2395; 2696; 3134; 3218; 3281; 3350; 3623; 4204   |
| MvrI        | CGAT!CG                  | 0     |  |
| MwoI        | GCNNNNN!NNGC             | 27    | 185; 206; 245; 302; 575; 614; 871; 943; 1556; 1586; 1613; 1655; 1896; 1968; 2019; 2098; 2320; 2404; 2427; 2566; 2689; 2772; 3039; 3226; 3430; 3629; 4201   |
| NaeI        | GCC!GGC                  | 4     | 863; 1548; 2831; 3114  |
| NarI        | GG!CGCC                  | 3     | 107; 296; 2329   |
| NciI        | CC!SGG                   | 6     | 75; 76; 2333; 2493; 3106; 3870   |
| NcoI        | C!CATGG                  | 3     | 849; 2058; 2761  |
| NdeI        | CA!TATG                  | 0     |  |
| NdeII       | !GATC                    | 27    | 27; 78; 128; 552; 599; 633; 732; 835; 956; 969; 986; 2166; 2170; 2189; 2500; 2578; 2659; 2668; 2746; 3122; 3138; 3497; 3509; 3587; 3595; 3606; 3681  |
| NgoAVIII    | GACNNNNNTGANNNNNNNNNNNN! | 1     | 302  |
| NgoAVIII    | !NNNNNNNNNNNTCANNNNNGTC  | 0     |  |
| NgoAVIII    | TCANNNNNGTCNNNNNNNNNNNN! | 0     |  |
| NgoAVIII    | !NNNNNNNNNNNGACNNNNNTGA  | 1     | 266  |
| NgoMIV      | G!CCGGC                  | 4     | 861; 1546; 2829; 3112  |
| NheI        | G!CTAGC                  | 0     |  |
| NlaIII      | CATG!                    | 18    | 99; 354; 699; 853; 900; 1759; 1899; 1971; 2062; 2203; 2548; 2734; 2765; 2791; 3147; 3531; 4251; 4302   |

| Enzyme name | Sequence                   | Count | Cutting Position (the base to the left of the cut site)   |
|-------------|----------------------------|-------|---|
| NlaIV       | GGN!NCC                    | 21    | 65; 73; 80; 108; 297; 321; 669; 810; 1484; 1505; 1517; 1708; 1869; 1941; 2330; 2365; 3213; 3292; 3337; 4180; 4219                                     |
| NlaCI       | CATCACNNNNNNNNNNNNNNNNNN!  | 4     | 405; 1161; 1476; 3068   |
| NlaCI       | !NNNNNNNNNNNNNNNNNGTGATG   | 1     | 4140  |
| Nli3877I    | CYCGR!G                    | 4     | 35; 78; 326; 686  |
| NmeAIII     | GCCGAGNNNNNNNNNNNNNNNNNN!  | 3     | 444; 2118; 2554   |
| NmeAIII     | !NNNNNNNNNNNNNNNNNNCTCGGC  | 1     | 2083  |
| NmeDI       | RCCGGYNNNNNNNN!            | 9     | 96; 256; 616; 817; 873; 1558; 2660; 2841; 3124  |
| NmeDI       | !NNNNNNNNNNNNRCCGGY        | 9     | 71; 231; 591; 792; 848; 1533; 2635; 2816; 3099  |
| NmuCI       | !GTSAC                     | 7     | 276; 423; 726; 928; 1618; 2449; 2755  |
| NotI        | GC!GGCCGC                  | 0     |   |
| NruI        | TCG!CGA                    | 0     |   |
| NsbI        | TGC!GCA                    | 1     | 2431  |
| NsiI        | ATGCA!T                    | 3     | 1901; 1973; 4304  |
| NspI        | RCATG!Y                    | 5     | 354; 1899; 1971; 2734; 4251   |
| NspV        | TT!CGAA                    | 2     | 45; 3011  |
| OliI        | CACNN!NNGTG                | 1     | 274   |
| PabI        | GTA!C                      | 6     | 66; 317; 449; 542; 689; 2636  |
| PacI        | TTAAT!TAA                  | 0     |   |
| PaeI        | GCATG!C                    | 4     | 354; 1899; 1971; 2734   |
| PaeR7I      | C!TCGAG                    | 1     | 31  |
| PagI        | T!CATGA                    | 2     | 1755; 3527  |
| PalAI       | GG!CGCGCC                  | 0     |   |
| PasI        | CC!CWGGG                   | 2     | 3294; 3397  |
| PauI        | G!CGCGC                    | 1     | 768   |
| PceI        | AGG!CCT                    | 1     | 2150  |
| PciI        | A!CATGT                    | 1     | 4247  |
| PciSI       | GCTCTCN!                   | 0     |   |
| PciSI       | !NNNNGAAGAGC               | 3     | 568; 2673; 2883   |
| PcsI        | WCGNNNN!NNNCGW             | 6     | 2623; 2700; 2943; 3017; 3410; 3928  |
| PctI        | GAATGCN!                   | 1     | 1080  |
| PctI        | G!CATTC                    | 1     | 1173  |
| PdiI        | GCC!GGC                    | 4     | 863; 1548; 2831; 3114   |
| PdmI        | GAANN!NNTTC                | 1     | 334   |
| PfeI        | G!AWTC                     | 7     | 507; 842; 2814; 2948; 3058; 3095; 4273  |
| Pfl23II     | C!GTACG                    | 0     |   |
| PfIFI       | GACN!NNGTC                 | 1     | 2447  |
| PfIMI       | CCANNNN!NTGG               | 1     | 498   |
| PfoI        | T!CCNGGA                   | 1     | 3104  |
| PhoI        | GG!CC                      | 27    | 73; 167; 320; 470; 491; 559; 569; 631; 668; 975; 1295; 1437; 2092; 2098; 2107; 2150; 2237; 2411; 2802; 2829; 3338; 3395; 3428; 3773; 4207; 4225; 4236 |
| PinAI       | A!CCGGT                    | 1     | 84  |
| PlaDI       | CATCAGNNNNNNNNNNNNNNNNNN!  | 1     | 2710  |
| PlaDI       | !NNNNNNNNNNNNNNNNNNCTGATG  | 2     | 2275; 2530  |
| PleI        | GAGTCNNNN!                 | 2     | 1380; 1812  |
| PleI        | !NNNNNGACTC                | 5     | 16; 974; 1388; 2994; 3871   |
| Ple19I      | CGAT!CG                    | 0     |   |
| PmaCI       | CAC!GTG                    | 3     | 495; 678; 708   |
| PmeI        | GTTT!AAAC                  | 0     |   |
| PmII        | CAC!GTG                    | 3     | 495; 678; 708   |
| PpiI        | GAACNNNNNNCTCNNNNNNNNNNNN! | 1     | 1411  |
| PpiI        | !NNNNNNNNNGAGNNNNNGTTC     | 1     | 3541  |
| PpiI        | GAGNNNNNGTTCNNNNNNNNNNNN!  | 1     | 3573  |
| PpiI        | !NNNNNNNGAACNNNNNNCTC      | 1     | 1379  |
| PpsI        | GAGTCNNNN!                 | 2     | 1380; 1812  |
| PpsI        | !NNNNNGACTC                | 5     | 16; 974; 1388; 2994; 3871   |
| Ppu10I      | A!TGCAT                    | 3     | 1897; 1969; 4300  |
| Ppu21I      | YAC!GTR                    | 7     | 138; 366; 495; 678; 708; 1445; 2633   |
| PpuMI       | RG!GWCCY                   | 0     |   |
| PscI        | A!CATGT                    | 1     | 4247  |
| PshAI       | GACNN!NNGTC                | 0     |   |
| PshBI       | AT!TAAT                    | 0     |   |
| PsiI        | TTA!TAA                    | 2     | 1112; 1317  |
| Psp03I      | GGWC!C                     | 3     | 927; 2848; 3293   |

| Enzyme name | Sequence                     | Count | Cutting Position (the base to the left of the cut site)   |
|-------------|------------------------------|-------|---|
| Psp5II      | RG!GWCCY                     | 0     |   |
| Psp6I       | !CCWGG                       | 13    | 262; 272; 470; 908; 1863; 1918; 1935; 2714; 3293; 3396; 4086; 4099; 4220  |
| Psp1406I    | AA!CGTT                      | 0     |   |
| Psp124BI    | GAGCT!C                      | 1     | 38  |
| PspCI       | CAC!GTG                      | 3     | 495; 678; 708   |
| PspEI       | G!GTNACC                     | 2     | 276; 423  |
| PspGI       | !CCWGG                       | 13    | 262; 272; 470; 908; 1863; 1918; 1935; 2714; 3293; 3396; 4086; 4099; 4220  |
| PspLI       | C!GTACG                      | 0     |   |
| PspN4I      | GGN!NCC                      | 21    | 65; 73; 80; 108; 297; 321; 669; 810; 1484; 1505; 1517; 1708; 1869; 1941; 2330; 2365; 3213; 3292; 3337; 4180; 4219   |
| PspOMI      | G!GGCCC                      | 1     | 71  |
| PspOMII     | CGCCARNNNNNNNNNNNNNNNNNNNNN! | 3     | 323; 2059; 3058   |
| PspOMII     | !NNNNNNNNNNNNNNNNNNNYTGGGCG  | 1     | 2461  |
| PspPI       | G!GNCC                       | 11    | 71; 72; 319; 667; 924; 1436; 2845; 3290; 3336; 3394; 3427   |
| PspPPI      | RG!GWCCY                     | 0     |   |
| PspPRI      | CCYAGNNNNNNNNNNNNNNNN!       | 3     | 1882; 1954; 3460  |
| PspPRI      | !NNNNNNNNNNNNNCTGRGG         | 3     | 532; 1795; 2990   |
| PspXI       | VC!TCGAGB                    | 0     |   |
| PsrI        | GAACNNNNNTACNNNNNNNNNNNN!    | 0     |   |
| PsrI        | !NNNNNNNGTANNNNNNGTTC        | 0     |   |
| PsrI        | GTANNNNNNGTTCNNNNNNNNNNNN!   | 0     |   |
| PsrI        | !NNNNNNNGAACNNNNNNNTAC       | 0     |   |
| PssI        | RGGNC!CY                     | 1     | 3430  |
| PstI        | CTGCA!G                      | 2     | 56; 939   |
| PstNI       | CAGNNN!CTG                   | 2     | 3430; 3838  |
| PsuI        | R!GATCY                      | 11    | 27; 78; 128; 835; 2500; 2746; 3138; 3497; 3509; 3595; 3606  |
| PsyI        | GACN!NNGTC                   | 1     | 2447  |
| PteI        | G!CGCGC                      | 1     | 768   |
| PvuI        | CGAT!CG                      | 0     |   |
| PvuII       | CAG!CTG                      | 2     | 1827; 2435  |
| RcaI        | T!CATGA                      | 2     | 1755; 3527  |
| RceI        | CATCGACNNNNNNNNNNNNNNNNNNNN! | 1     | 2844  |
| RceI        | !NNNNNNNNNNNNNNNNNNNGTCGATG  | 0     |   |
| RgaI        | GCGAT!CGC                    | 0     |   |
| RigI        | GGCCGG!CC                    | 0     |   |
| RleAI       | CCCACANNNNNNNNNNNN!          | 1     | 1058  |
| RleAI       | !NNNNNNNNNTGTGGG             | 0     |   |
| RpaBI       | CCCGCAGNNNNNNNNNNNNNNNNNNNN! | 0     |   |
| RpaBI       | !NNNNNNNNNNNNNNNNNNCTGCGGG   | 0     |   |
| RpaB5I      | CGRGGACNNNNNNNNNNNNNNNNNNNN! | 0     |   |
| RpaB5I      | !NNNNNNNNNNNNNNNNNNNGTCCYCG  | 0     |   |
| RruI        | TCG!CGA                      | 0     |   |
| RsaI        | GT!AC                        | 6     | 65; 316; 448; 541; 688; 2635  |
| RsaNI       | G!TAC                        | 6     | 64; 315; 447; 540; 687; 2634  |
| RseI        | CAYNN!NNRTG                  | 3     | 274; 643; 2766  |
| RsrII       | CG!GWCCG                     | 1     | 2845  |
| Rsr2I       | CG!GWCCG                     | 1     | 2845  |
| SacI        | GAGCT!C                      | 1     | 38  |
| SacII       | CCGC!GG                      | 2     | 70; 186   |
| SalI        | G!TCGAC                      | 1     | 57  |
| SapI        | GCTCTCN!                     | 0     |   |
| SapI        | !NNNNGAAGAGC                 | 3     | 568; 2673; 2883   |
| SaqAI       | T!TAA                        | 14    | 1030; 1091; 1212; 1233; 1244; 1256; 1267; 1284; 1382; 1653; 3472; 3486; 3491; 3543  |
| SatI        | GC!NGC                       | 41    | 102; 105; 186; 348; 441; 609; 663; 666; 783; 872; 892; 938; 976; 1106; 1625; 1647; 1661; 2099; 2238; 2290; 2301; 2391; 2396; 2433; 2474; 2561; 2564; 2567; 2803; 2899; 2940; 2954; 3068; 3407; 3422; 3633; 3839; 3842; 3907; 4050; 4205 |
| Sau96I      | G!GNCC                       | 11    | 71; 72; 319; 667; 924; 1436; 2845; 3290; 3336; 3394; 3427   |
| Sau3AI      | !GATC                        | 27    | 27; 78; 128; 552; 599; 633; 732; 835; 956; 969; 986; 2166; 2170; 2189; 2500; 2578; 2659; 2668; 2746; 3122; 3138; 3497; 3509; 3587; 3595; 3606; 3681   |
| SbfI        | CCTGCA!GG                    | 0     |   |
| ScaI        | AGT!ACT                      | 0     |   |



| Enzyme name | Sequence                    | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|-----------------------------|-------|--|
| SchI        | GAGTCNNNNN!                 | 2     | 1381; 1813   |
| SchI        | !NNNNNGACTC                 | 5     | 16; 974; 1388; 2994; 3871  |
| SciI        | CTC!GAG                     | 1     | 33   |
| ScrFI       | CC!NGG                      | 19    | 75; 76; 264; 274; 472; 910; 1865; 1920; 1937; 2333; 2493; 2716; 3106; 3295; 3398; 3870; 4088; 4101; 4222   |
| SdaI        | CCTGCA!GG                   | 0     |  |
| SdeAI       | CAGRAGNNNNNNNNNNNNNNNNNNNN! | 6     | 396; 936; 1907; 1979; 2148; 4090   |
| SdeAI       | !NNNNNNNNNNNNNNNNNNNNCTYCTG | 3     | 927; 2484; 2969  |
| SdeOSI      | GACNNNNRTGANNNNNNNNNNN!     | 0     |  |
| SdeOSI      | !NNNNNNNNNNNTCAYNNNNNGTC    | 0     |  |
| SdeOSI      | TCAYNNNNNGTCNNNNNNNNNNNN!   | 0     |  |
| SdeOSI      | !NNNNNNNNNNNGACNNNNRTGA     | 0     |  |
| SduI        | GDGCH!C                     | 16    | 38; 75; 257; 263; 271; 677; 683; 909; 1520; 2275; 2368; 2442; 2632; 2694; 3404; 3937   |
| SelI        | !CGCG                       | 20    | 67; 183; 414; 453; 738; 768; 976; 1251; 1627; 1647; 1671; 1702; 2393; 2694; 3132; 3216; 3279; 3348; 3621; 4202   |
| SetI        | ASST!                       | 60    | 38; 44; 140; 175; 221; 292; 329; 349; 368; 388; 425; 430; 464; 497; 680; 703; 710; 716; 793; 826; 1019; 1040; 1049; 1061; 1110; 1277; 1392; 1404; 1447; 1484; 1534; 1557; 1681; 1829; 1924; 2117; 2230; 2358; 2437; 2448; 2520; 2591; 2635; 2895; 3041; 3085; 3416; 3447; 3506; 3549; 3692; 3713; 3812; 3949; 3964; 3983; 3995; 4039; 4085; 4136   |
| SexAI       | A!CCWGGT                    | 1     | 1918   |
| SfaAI       | GCGAT!CGC                   | 0     |  |
| SfaNI       | GCATCNNNNN!                 | 7     | 188; 1145; 1908; 1980; 2628; 2692; 2967  |
| SfaNI       | !NNNNNNNNNGATGC             | 6     | 192; 213; 2287; 2542; 2758; 4150   |
| Sfcl        | C!TRYAG                     | 5     | 52; 935; 1664; 3791; 3982  |
| SfiI        | GGCCNNNN!NGGCC              | 1     | 2104   |
| SfoI        | GGC!GCC                     | 3     | 108; 297; 2330   |
| Sfr274I     | C!TCGAG                     | 1     | 31   |
| Sfr303I     | CCGC!GG                     | 2     | 70; 186  |
| SfuI        | TT!CGAA                     | 2     | 45; 3011   |
| SgeI        | CNNGNNNNNNNNN!              | 183   | 6; 22; 43; 80; 97; 123; 138; 196; 218; 240; 257; 270; 285; 312; 334; 354; 367; 382; 402; 420; 444; 466; 483; 505; 523; 549; 568; 582; 607; 624; 639; 654; 682; 696; 718; 732; 751; 765; 781; 798; 818; 833; 851; 870; 889; 909; 922; 946; 965; 989; 1036; 1065; 1107; 1190; 1264; 1348; 1370; 1402; 1439; 1457; 1476; 1491; 1533; 1550; 1567; 1602; 1616; 1633; 1655; 1679; 1703; 1768; 1782; 1820; 1836; 1876; 1889; 1908; 1931; 1948; 1961; 1980; 2010; 2031; 2048; 2071; 2106; 2133; 2164; 2186; 2212; 2234; 2253; 2281; 2317; 2336; 2359; 2373; 2393; 2406; 2420; 2439; 2453; 2472; 2504; 2518; 2531; 2557; 2588; 2601; 2618; 2635; 2649; 2666; 2684; 2699; 2727; 2743; 2762; 2784; 2800; 2823; 2842; 2867; 2886; 2906; 2933; 2957; 2990; 3009; 3037; 3051; 3117; 3141; 3156; 3179; 3200; 3217; 3265; 3292; 3306; 3335; 3359; 3392; 3409; 3429; 3442; 3455; 3513; 3540; 3559; 3589; 3614; 3634; 3648; 3673; 3691; 3728; 3766; 3795; 3823; 3837; 3857; 3872; 3893; 3907; 3926; 3944; 3960; 4000; 4020; 4047; 4065; 4086; 4099; 4112; 4129; 4153; 4176; 4208; 4233; 4260; 4282 |
| SgeI        | !NNNNNNNNNNNNNCNNG          | 158   | 5; 25; 54; 71; 97; 124; 170; 192; 214; 231; 249; 271; 298; 328; 352; 376; 394; 418; 440; 457; 479; 497; 523; 542; 581; 598; 628; 656; 682; 706; 725; 751; 772; 792; 813; 836; 863; 883; 904; 927; 963; 1010; 1039; 1081; 1164; 1238; 1322; 1344; 1376; 1413; 1431; 1450; 1507; 1524; 1541; 1576; 1594; 1614; 1634; 1653; 1677; 1742; 1794; 1850; 1882; 1905; 1922; 1954; 1984; 2005; 2022; 2045; 2080; 2107; 2138; 2160; 2186; 2208; 2227; 2255; 2291; 2310; 2333; 2354; 2372; 2389; 2413; 2446; 2478; 2505; 2531; 2562; 2592; 2609; 2635; 2658; 2679; 2701; 2721; 2741; 2758; 2797; 2816; 2841; 2860; 2880; 2907; 2931; 2964; 2983; 3011; 3033; 3091; 3115; 3134; 3153; 3174; 3191; 3239; 3266; 3286; 3309; 3333; 3366; 3383; 3403; 3429; 3487; 3514; 3533; 3563; 3588; 3608; 3647; 3665; 3702; 3740; 3769; 3797; 3818; 3846; 3867; 3900; 3918; 3974; 3994; 4021; 4039; 4060; 4086; 4103; 4127; 4150; 4182; 4207; 4234; 4256; 4285  |
| Sgfl        | GCGAT!CGC                   | 0     |  |
| SgrAI       | CR!CCGGYG                   | 2     | 244; 861   |
| SgrBI       | CCGC!GG                     | 2     | 70; 186  |
| SgrDI       | CG!TCGACG                   | 0     |  |
| SgsI        | GG!CGCGCC                   | 0     |  |

| Enzyme name | Sequence                     | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|------------------------------|-------|--|
| SimI        | GG!GTC                       | 3     | 1476; 3263; 4056   |
| SimI        | !GACCC                       | 4     | 410; 2757; 3325; 3573  |
| SinI        | G!GWCC                       | 3     | 924; 2845; 3290  |
| SlaI        | C!TCGAG                      | 1     | 31   |
| SmaI        | CCC!GGG                      | 1     | 76   |
| SmiI        | ATTT!AAAT                    | 0     |  |
| SmiMI       | CAYNN!NNRTG                  | 3     | 274; 643; 2766   |
| SmlI        | C!TYRAG                      | 7     | 31; 37; 1211; 2720; 3602; 3879; 4141   |
| SmoI        | C!TYRAG                      | 7     | 31; 37; 1211; 2720; 3602; 3879; 4141   |
| SmuI        | CCCGCNNNN!                   | 7     | 421; 866; 1651; 2006; 2027; 3223; 3355   |
| SmuI        | !NNNNNGCGGG                  | 7     | 62; 910; 1582; 2452; 2989; 3127; 3274  |
| SnaBI       | TAC!GTA                      | 0     |  |
| SpeI        | A!CTAGT                      | 0     |  |
| SphI        | GCATG!C                      | 4     | 354; 1899; 1971; 2734  |
| SrfI        | GCCC!GGGC                    | 0     |  |
| Sse9I       | !AATT                        | 12    | 47; 402; 519; 1079; 1143; 1221; 1247; 1258; 1905; 1977; 2069; 3487   |
| Sse8387I    | CCTGCA!GG                    | 0     |  |
| Sse8647I    | AG!GWCCT                     | 0     |  |
| SseBI       | AGG!CCT                      | 1     | 2150   |
| SsiI        | C!CGC                        | 31    | 67; 183; 414; 738; 783; 859; 976; 1644; 1647; 1661; 1751; 1999; 2011; 2020; 2032; 2042; 2053; 2099; 2238; 2301; 2803; 2848; 2914; 2940; 3065; 3068; 3216; 3348; 3655; 3799; 4295 |
| SsiI        | G!CGG                        | 28    | 69; 104; 185; 662; 665; 865; 871; 917; 1589; 1616; 1704; 1814; 2395; 2459; 2560; 2563; 2843; 2898; 2996; 3134; 3281; 3421; 3664; 3909; 4030; 4049; 4176; 4204                    |
| SspI        | AAT!ATT                      | 2     | 1237; 1790   |
| SspDI       | G!GCGCC                      | 3     | 106; 295; 2328   |
| SspD5I      | GGTGANNNNNNNN!               | 4     | 288; 435; 3400; 3516   |
| SspD5I      | !NNNNNNNNNTCACC              | 7     | 374; 515; 593; 726; 921; 1445; 2506  |
| SstI        | GAGCT!C                      | 1     | 38   |
| SstII       | CCGC!GG                      | 2     | 70; 186  |
| SstE37I     | CGAAGACNNNNNNNNNNNNNNNNNNNN! | 0     |  |
| SstE37I     | !NNNNNNNNNNNNNNNNNGTCTTCG    | 0     |  |
| Sth132I     | CCCGNNNN!                    | 20    | 81; 263; 329; 362; 420; 677; 689; 865; 1528; 1650; 2005; 2026; 2339; 2742; 2881; 2952; 3222; 3354; 3584; 4022  |
| Sth132I     | !NNNNNNNNCGGG                | 17    | 61; 909; 1529; 1581; 1682; 2409; 2451; 2484; 2988; 3097; 3126; 3273; 3377; 3408; 3861; 3905; 4113  |
| Sth302II    | CC!GG                        | 25    | 20; 76; 86; 246; 606; 807; 813; 828; 863; 1548; 2235; 2312; 2334; 2362; 2493; 2583; 2650; 2831; 3106; 3114; 3206; 3680; 3870; 3896; 4043   |
| StrI        | C!TCGAG                      | 1     | 31   |
| StsI        | GGATGNNNNNNNNNN!             | 10    | 176; 236; 500; 737; 893; 934; 2197; 2654; 2679; 3133   |
| StsI        | !NNNNNNNNNNNNNNCATCC         | 2     | 165; 2001  |
| StuI        | AGG!CCT                      | 1     | 2150   |
| StyI        | C!CWWGG                      | 5     | 849; 2058; 2151; 2761; 3166  |
| StyD4I      | !CCNGG                       | 19    | 73; 74; 262; 272; 470; 908; 1863; 1918; 1935; 2331; 2491; 2714; 3104; 3293; 3396; 3868; 4086; 4099; 4220   |
| SwaI        | ATTT!AAAT                    | 0     |  |
| TaaI        | ACN!GT                       | 11    | 63; 765; 928; 1420; 2709; 2825; 2933; 3256; 3747; 4217; 4288   |
| TaiI        | ACGT!                        | 13    | 140; 368; 497; 680; 710; 1392; 1404; 1447; 1557; 2448; 2635; 3416; 3549  |
| TaqI        | T!CGA                        | 18    | 32; 45; 58; 389; 431; 800; 840; 1478; 2169; 2442; 2598; 2622; 2658; 2820; 3011; 3056; 3311; 4149   |
| TaqII       | GACCGANNNNNNNNNN!            | 4     | 745; 1349; 2366; 3034  |
| TaqII       | !NNNNNNNNNTCGGTC             | 1     | 3278   |
| TaqII       | CACCCANNNNNNNNNN!            | 1     | 655  |
| TaqII       | !NNNNNNNNNTGGGTG             | 2     | 2233; 2825   |
| TasI        | !AATT                        | 12    | 47; 402; 519; 1079; 1143; 1221; 1247; 1258; 1905; 1977; 2069; 3487   |
| TatI        | W!GTACW                      | 2     | 446; 539   |
| TauI        | GCSG!C                       | 22    | 107; 188; 665; 668; 785; 874; 978; 1649; 1663; 2101; 2240; 2303; 2398; 2563; 2566; 2805; 2901; 2942; 3070; 3424; 4052; 4207  |
| TfiI        | G!AWTC                       | 7     | 507; 842; 2814; 2948; 3058; 3095; 4273   |
| TliI        | C!TCGAG                      | 1     | 31   |

| Enzyme name | Sequence               | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|------------------------|-------|--|
| TruII       | T!TAA                  | 14    | 1030; 1091; 1212; 1233; 1244; 1256; 1267; 1284; 1382; 1653; 3472; 3486; 3491; 3543                         |
| Tru9I       | T!TAA                  | 14    | 1030; 1091; 1212; 1233; 1244; 1256; 1267; 1284; 1382; 1653; 3472; 3486; 3491; 3543                         |
| TscAI       | CASTGNN!               | 7     | 307; 538; 1175; 2458; 3568; 3839; 3852   |
| TseI        | G!CWGC                 | 19    | 101; 347; 440; 608; 891; 937; 1105; 1624; 2289; 2390; 2432; 2473; 2566; 2953; 3406; 3632; 3838; 3841; 3906 |
| TsoI        | TARCCANNNNNNNNNN!      | 2     | 1301; 1648   |
| TsoI        | !NNNNNNNNNTGGYTA       | 3     | 350; 1106; 2858  |
| Tsp45I      | !GTSAC                 | 7     | 276; 423; 726; 928; 1618; 2449; 2755   |
| Tsp509I     | !AATT                  | 12    | 47; 402; 519; 1079; 1143; 1221; 1247; 1258; 1905; 1977; 2069; 3487   |
| TspDTI      | ATGAANNNNNNNNNN!       | 4     | 661; 1087; 2388; 3092  |
| TspDTI      | !NNNNNNNNNTTCAT        | 5     | 222; 612; 2806; 3262; 3469   |
| TspEI       | !AATT                  | 12    | 47; 402; 519; 1079; 1143; 1221; 1247; 1258; 1905; 1977; 2069; 3487   |
| TspGWI      | ACGGANNNNNNNNNN!       | 1     | 3204   |
| TspGWI      | !NNNNNNNNNTCCGT        | 0     |  |
| TspMI       | C!CCGGG                | 1     | 74   |
| TspRI       | CASTGNN!               | 7     | 307; 538; 1175; 2458; 3568; 3839; 3852   |
| TstI        | CACNNNNNTCCNNNNNNNNNN! | 0     |  |
| TstI        | !NNNNNNNGGANNNNNNGTG   | 0     |  |
| TstI        | GGANNNNNGTGNNNNNNNNNN! | 0     |  |
| TstI        | !NNNNNNNCACNNNNNTCC    | 0     |  |
| Tth111I     | GACN!NNGTC             | 1     | 2447   |
| Tth111II    | CAARCAANNNNNNNNNN!     | 1     | 3656   |
| Tth111III   | !NNNNNNNTGYTTG         | 3     | 2764; 3624; 3663   |
| UnbI        | !GGNCC                 | 11    | 70; 71; 318; 666; 923; 1435; 2844; 3289; 3335; 3393; 3426  |
| Van9II      | CCANNN!NTGG            | 1     | 498  |
| Vha464I     | C!TTAAG                | 1     | 1211   |
| VneI        | G!TGCAC                | 2     | 673; 3933  |
| VpaK11AI    | !GGWCC                 | 3     | 923; 2844; 3289  |
| VpaK11BI    | G!GWCC                 | 3     | 924; 2845; 3290  |
| VspI        | AT!TAAT                | 0     |  |
| XagI        | CCTNN!NNNAGG           | 0     |  |
| XapI        | R!AATTY                | 4     | 47; 1143; 1247; 1258   |
| XbaI        | T!CTAGA                | 1     | 983  |
| XceI        | RCATG!Y                | 5     | 354; 1899; 1971; 2734; 4251  |
| XcmI        | CCANNNN!NNNTGG         | 0     |  |
| XhoI        | C!TCGAG                | 1     | 31   |
| XhoII       | R!GATCY                | 11    | 27; 78; 128; 835; 2500; 2746; 3138; 3497; 3509; 3595; 3606   |
| XmaI        | C!CCGGG                | 1     | 74   |
| XmaJI       | C!CTAGG                | 2     | 2151; 3166   |
| XmiI        | GT!MKAC                | 1     | 58   |
| XmnI        | GAANN!NNTTC            | 1     | 334  |
| XspI        | C!TAG                  | 9     | 10; 953; 984; 1178; 1596; 2152; 3167; 3501; 3754   |
| ZraI        | GAC!GTC                | 0     |  |
| ZrmI        | AGT!ACT                | 0     |  |
| Zsp2I       | ATGCA!T                | 3     | 1901; 1973; 4304   |