

## pCasper3-BG vector

### Table of restriction sites

| Enzyme name | Sequence               | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|------------------------|-------|--|
| AanI        | TTA!TAA                | 2     | 2292; 2497   |
| AarI        | CACCTGCNNNN!           | 0     |  |
| AarI        | !NNNNNNNNGCAGGTG       | 0     |  |
| AasI        | GACNNNN!NNGTC          | 3     | 2580; 3536; 5325   |
| AatI        | AGG!CCT                | 2     | 1102; 3330   |
| AatII       | GACGT!C                | 4     | 125; 178; 261; 447   |
| AbsI        | CC!TCGAGG              | 0     |  |
| AccI        | GT!MKAC                | 2     | 640; 1253  |
| AccII       | CG!CG                  | 19    | 60; 651; 1523; 1760; 2078; 2158; 2433; 2809; 2829; 2853; 2884; 3575; 3876; 4314; 4398; 4461; 4530; 4803; 5384  |
| AccIII      | T!CCGGA                | 0     |  |
| Acc16I      | TGC!GCA                | 1     | 3611   |
| Acc36I      | ACCTGCNNNN!            | 2     | 3777; 4227   |
| Acc36I      | !NNNNNNNNGCAGGT        | 1     | 3396   |
| Acc65I      | G!GTACC                | 2     | 645; 1429  |
| AccB1I      | G!GYRCC                | 8     | 465; 645; 727; 787; 1429; 2662; 3508; 3543   |
| AccB7I      | CCANNNN!NTGG           | 2     | 1854; 2130   |
| AccBSI      | CCG!CTC                | 2     | 2933; 4122   |
| AccBSI      | GAG!CGG                | 3     | 1439; 2769; 4176   |
| AceIII      | CAGCTCNNNNNNN!         | 1     | 2465   |
| AceIII      | !NNNNNNNNNNGAGCTG      | 6     | 552; 673; 1438; 1471; 1792; 2125   |
| AciI        | C!CGC                  | 43    | 58; 86; 98; 112; 279; 507; 589; 649; 986; 1124; 1226; 1650; 1758; 1797; 1890; 1935; 2064; 2076; 2156; 2824; 2827; 2841; 2931; 3179; 3191; 3200; 3212; 3222; 3233; 3279; 3418; 3481; 3983; 4028; 4094; 4120; 4245; 4248; 4396; 4528; 4835; 4979; 5475 |
| AciI        | G!CGG                  | 33    | 370; 403; 528; 651; 821; 1133; 1175; 1439; 1523; 1760; 1952; 1955; 2152; 2769; 2796; 2884; 2994; 3575; 3639; 3740; 3743; 4023; 4078; 4176; 4314; 4461; 4601; 4844; 5089; 5210; 5229; 5356; 5384  |
| AcII        | AA!CGTT                | 0     |  |
| AcIWI       | GGATCNNNN!             | 8     | 668; 3377; 3688; 3934; 4326; 4685; 4783; 4869  |
| AcIWI       | !NNNNNGATCC            | 8     | 580; 655; 1208; 2010; 3753; 4297; 4684; 4781   |
| AcoI        | Y!GGCCR                | 8     | 1331; 1503; 1967; 2153; 3415; 3589; 3980; 4007   |
| AcsI        | R!AATY                 | 5     | 629; 1375; 2323; 2427; 2438  |
| AcuI        | CTGAAGNNNNNNNNNNNNNNN! | 4     | 1183; 1828; 3655; 4087   |
| AcuI        | !NNNNNNNNNNNNNNCTTCAG  | 2     | 2088; 4885   |
| AcvI        | CAC!GTG                | 0     |  |
| AcyI        | GR!CGYC                | 9     | 122; 175; 258; 444; 1250; 1541; 3509; 4211; 4290   |
| AdeI        | CACNNN!GTG             | 4     | 1323; 1881; 2088; 2625   |
| Afal        | GT!AC                  | 15    | 219; 244; 299; 332; 383; 540; 647; 718; 1120; 1431; 1750; 1861; 1885; 2143; 3815   |
| AfeI        | AGC!GCT                | 1     | 596  |
| AfiI        | CCNNNNN!NNGG           | 27    | 49; 784; 897; 1129; 1439; 1601; 1734; 1764; 1854; 1899; 2070; 2130; 2521; 2847; 3239; 3490; 4034; 4315; 4347; 4479; 4514; 4564; 4596; 4949; 5228; 5394; 5412   |
| AfiII       | C!TTAAG                | 1     | 2391   |
| AfiIII      | A!CRYGT                | 2     | 1880; 5427   |
| AgeI        | A!CCGGT                | 1     | 666  |
| AgsI        | TTS!AA                 | 12    | 750; 918; 1375; 1686; 1824; 1869; 1929; 2920; 2964; 2974; 3387; 4963   |
| AhdI        | GACNNN!NNGTC           | 0     |  |
| AhII        | A!CTAGT                | 0     |  |
| AjiI        | CAC!GTC                | 0     |  |
| AjiI        | GAC!GTG                | 1     | 1497   |

| Enzyme name | Sequence                   | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|----------------------------|-------|--|
| Ajnl        | !CCWGG                     | 26    | 88; 281; 848; 896; 992; 1007; 1136; 1245; 1600; 1610; 1712; 1733; 1787; 1841; 1913; 1994; 2093; 3043; 3098; 3115; 3894; 4473; 4576; 5266; 5279; 5400                   |
| AjuI        | GAANNNNNNNTTGGNNNNNNNNNN!  | 0     |  |
| AjuI        | !NNNNNNCCAANNNNNNNTTC      | 0     |  |
| AjuI        | CCAANNNNNNNTTCNNNNNNNNNN!  | 0     |  |
| AjuI        | !NNNNNNNGAANNNNNNNTTGG     | 0     |  |
| AleI        | CACNN!NNGTG                | 2     | 1612; 1789   |
| AlfI        | GCANNNNNNTGCNNNNNNNNNN!    | 2     | 3083; 3155   |
| AlfI        | !NNNNNNNNNGCANNNNNNNTGC    | 2     | 3049; 3121   |
| AloI        | GAACNNNNNNNTCCNNNNNNNNNN!  | 2     | 1074; 2591   |
| AloI        | !NNNNNNNGGANNNNNNGTTC      | 1     | 1552   |
| AloI        | GGANNNNNNGTTCNNNNNNNNNN!   | 1     | 1584   |
| AloI        | !NNNNNNNGAACNNNNNNNTCC     | 2     | 1042; 2559   |
| AluI        | AG!CT                      | 29    | 566; 618; 624; 687; 714; 860; 1167; 1371; 1410; 1452; 1485; 1557; 1590; 1806; 1854; 1865; 1965; 2139; 2288; 2455; 2712; 3007; 3295; 3615; 4073; 4870; 5127; 5173; 5263 |
| AluBI       | AG!CT                      | 29    | 566; 618; 624; 687; 714; 860; 1167; 1371; 1410; 1452; 1485; 1557; 1590; 1806; 1854; 1865; 1965; 2139; 2288; 2455; 2712; 3007; 3295; 3615; 4073; 4870; 5127; 5173; 5263 |
| AlwI        | GGATCNNNN!                 | 8     | 668; 3377; 3688; 3934; 4326; 4685; 4783; 4869  |
| AlwI        | !NNNNNGATCC                | 8     | 580; 655; 1208; 2010; 3753; 4297; 4684; 4781   |
| Alw21I      | GWGCW!C                    | 9     | 620; 757; 1502; 1664; 2041; 2093; 3622; 3812; 5117   |
| Alw26I      | GTCTCN!                    | 1     | 434  |
| Alw26I      | !NNNNNGAGAC                | 6     | 1105; 1294; 2933; 3351; 4370; 4498   |
| Alw44I      | G!TGCAC                    | 3     | 753; 1498; 5113  |
| AlwNI       | CAGNNN!CTG                 | 4     | 1340; 2111; 4610; 5018   |
| Ama87I      | C!YCGRG                    | 4     | 613; 656; 1657; 1699   |
| Aor13HI     | T!CCGGA                    | 0     |  |
| Aor51HI     | AGC!GCT                    | 1     | 596  |
| ApaI        | GGGCC!C                    | 1     | 657  |
| ApaBI       | GCANNNNN!TGC               | 4     | 1641; 3077; 3149; 4611   |
| ApaLI       | G!TGCAC                    | 3     | 753; 1498; 5113  |
| ApeKI       | G!CWGC                     | 19    | 872; 1016; 1314; 1590; 1962; 2114; 2285; 2804; 3469; 3570; 3612; 3653; 3746; 4133; 4586; 4812; 5018; 5021; 5086  |
| ApoI        | R!AATTY                    | 5     | 629; 1375; 2323; 2427; 2438  |
| ApyPI       | ATCGACNNNNNNNNNNNNNNNNNN!  | 1     | 4024   |
| ApyPI       | !NNNNNNNNNNNNNNNNNGTTCGAT  | 3     | 3818; 4471; 5309   |
| AquII       | GCCGNACNNNNNNNNNNNNNNNNNN! | 1     | 3908   |
| AquII       | !NNNNNNNNNNNNNNNNNGTNCGGC  | 0     |  |
| AquIII      | GAGGAGNNNNNNNNNNNNNNNNNN!  | 2     | 1472; 3337   |
| AquIII      | !NNNNNNNNNNNNNNNNNCTCCTC   | 0     |  |
| AquIV       | GRGGAAGNNNNNNNNNNNNNNNNNN! | 0     |  |
| AquIV       | !NNNNNNNNNNNNNNNNNCTTCCYC  | 2     | 843; 4079  |
| ArsI        | GACNNNNNNNTTYGNNNNNNNNNN!  | 0     |  |
| ArsI        | !NNNNNNCRAANNNNNNNGTC      | 0     |  |
| ArsI        | CRAANNNNNGTCCNNNNNNNNNN!   | 0     |  |
| ArsI        | !NNNNNNNGACNNNNNNNTTYG     | 0     |  |
| AscI        | GG!CGCGCC                  | 0     |  |
| AseI        | AT!TAAT                    | 1     | 7  |
| Asi256I     | G!ATC                      | 27    | 586; 610; 661; 1038; 1188; 1214; 1479; 1563; 2016; 2052; 2167; 3347; 3351; 3370; 3681; 3759; 3840; 3849; 3927; 4303; 4319; 4678; 4690; 4768; 4776; 4787; 4862          |
| AsiGI       | A!CCGGT                    | 1     | 666  |
| AsiSI       | GCGAT!CGC                  | 0     |  |
| AspI        | GACN!NNGTC                 | 1     | 3627   |
| Asp700I     | GAANN!NNTTC                | 1     | 1678   |
| Asp718I     | G!GTACC                    | 2     | 645; 1429  |
| AspA2I      | C!CTAGG                    | 2     | 3331; 4346   |
| AspEI       | GACNN!NNGTC                | 0     |  |
| AspLEI      | GCG!C                      | 25    | 597; 1523; 1693; 1721; 2110; 2775; 2783; 2809; 2831; 2840; 2853; 2884; 3503; 3511; 3575; 3612; 3878; 4138; 4314; 4400; 4803; 4912; 5086; 5186; 5253                    |
| AspS9I      | G!GNCC                     | 17    | 83; 276; 654; 823; 1066; 1158; 1604; 1845; 2005; 2061; 2073; 2616; 4025; 4470; 4516; 4574; 4607  |
| AssI        | AGT!ACT                    | 0     |  |

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|-------------|--------------------------|-------|---|
| AsuI        | TT!CGAA                  | 2     | 627; 4191   |
| AsuC2I      | CC!SGG                   | 6     | 657; 658; 3513; 3673; 4286; 5050  |
| AsuHPI      | GGTGANNNNNNNN!           | 7     | 376; 1059; 1626; 1779; 1803; 4580; 4696   |
| AsuHPI      | !NNNNNNNTCACC            | 4     | 948; 1098; 2626; 3687   |
| AsuNHI      | G!CTAGC                  | 1     | 591   |
| AvaI        | C!YCGRG                  | 4     | 613; 656; 1657; 1699  |
| AvaII       | G!GWCC                   | 3     | 2061; 4025; 4470  |
| AviII       | TGC!GCA                  | 1     | 3611  |
| AvrII       | C!CTAGG                  | 2     | 3331; 4346  |
| AxyI        | CC!TNAGG                 | 3     | 932; 2989; 4621   |
| BaeI        | ACNNNNGTAYCNNNNNNNNNN!   | 0     |   |
| BaeI        | !NNNNNNNGRTACNNNNNGT     | 0     |   |
| BaeI        | GRTACNNNNGTNNNNNNNNNNNN! | 0     |   |
| BaeI        | !NNNNNNNNNACNNNNGTAYC    | 0     |   |
| BaeGI       | GKGC!C                   | 12    | 657; 730; 757; 790; 1367; 1472; 1502; 1601; 1994; 3455; 3548; 5117                |
| BalI        | TGG!CCA                  | 2     | 1333; 3591  |
| BamHI       | G!GATCC                  | 1     | 660   |
| BanI        | G!GYRCC                  | 8     | 465; 645; 727; 787; 1429; 2662; 3508; 3543  |
| BanII       | GRGCY!C                  | 5     | 620; 657; 2700; 3874; 4584  |
| BanIII      | AT!CGAT                  | 1     | 3349  |
| BarI        | GAAGNNNNNTACNNNNNNNNNN!  | 0     |   |
| BarI        | !NNNNNNNGTANNNNNCTTC     | 0     |   |
| BarI        | GTANNNNNCTTCNNNNNNNNNN!  | 0     |   |
| BarI        | !NNNNNNNGAAGNNNNNTAC     | 0     |   |
| BasI        | CCANNN!NTGG              | 2     | 1854; 2130  |
| BauI        | C!ACGAG                  | 3     | 1318; 4227; 5254  |
| BauI        | C!TCGTG                  | 2     | 1168; 4101  |
| BbeI        | GGCG!C                   | 1     | 3512  |
| Bbr7I       | GAAGACNNNNNN!            | 1     | 979   |
| Bbr7I       | !NNNNNNNNNNNGTCTTC       | 0     |   |
| BbrPI       | CAC!GTG                  | 0     |   |
| BbsI        | GAAGACNN!                | 1     | 974   |
| BbsI        | !NNNNNNGTCTTC            | 0     |   |
| BbuI        | GCATG!C                  | 3     | 3079; 3151; 3914  |
| BbvI        | GCAGCNNNNNNNN!           | 9     | 884; 1326; 1974; 2297; 3582; 3624; 4145; 4598; 5098                               |
| BbvI        | !NNNNNNNNNNNGCTGC        | 9     | 1003; 1577; 2101; 2791; 3456; 3640; 3733; 4799; 5005                              |
| Bbv12I      | GWGCW!C                  | 9     | 620; 757; 1502; 1664; 2041; 2093; 3622; 3812; 5117                                |
| BbvCI       | CC!TCAGC                 | 0     |   |
| BbvCI       | GC!TGAGG                 | 0     |   |
| BccI        | CCATCNNNN!               | 8     | 749; 1190; 1732; 2029; 2639; 3203; 3729; 4231                                     |
| BccI        | !NNNNNGATGG              | 3     | 2607; 3387; 3815  |
| BceAI       | ACGGCNNNNNNNNNN!         | 14    | 885; 1029; 1080; 1146; 1506; 1566; 1647; 1758; 1848; 1926; 2019; 2142; 3935; 4422 |
| BceAI       | !NNNNNNNNNNNGCCGT        | 3     | 2650; 3468; 4927  |
| Bcefl       | ACGGCNNNNNNNNNN!         | 14    | 885; 1029; 1080; 1146; 1506; 1566; 1647; 1758; 1848; 1926; 2019; 2142; 3935; 4422 |
| Bcefl       | !NNNNNNNNNNNGCCGT        | 3     | 2651; 3469; 4928  |
| BcgI        | CGANNNNNNTGCNNNNNNNNNN!  | 0     |   |
| BcgI        | !NNNNNNNNNGCANNNNNTCG    | 0     |   |
| BcgI        | GCANNNNNNTCGNNNNNNNNNN!  | 0     |   |
| BcgI        | !NNNNNNNNNCGANNNNNTGC    | 0     |   |
| BciVI       | GTATCCNNNNNN!            | 3     | 2938; 3728; 5229  |
| BciVI       | !NNNNNGGATAC             | 0     |   |
| BclI        | T!GATCA                  | 0     |   |
| BcnI        | CC!SGG                   | 6     | 657; 658; 3513; 3673; 4286; 5050  |
| BcuI        | A!CTAGT                  | 0     |   |
| BdaI        | TGANNNNNNTCANNNNNNNNNNN! | 2     | 2038; 5362  |
| BdaI        | !NNNNNNNNNTGANNNNNNTCA   | 2     | 2004; 5328  |
| BfaI        | C!TAG                    | 10    | 592; 857; 1352; 2164; 2358; 2776; 3332; 4347; 4681; 4934                          |
| BfiI        | ACTGGGNNNNN!             | 2     | 1369; 3458  |
| BfiI        | !NNNCCCAGT               | 2     | 289; 3210   |
| BfmI        | C!TRYAG                  | 5     | 634; 1266; 2844; 4971; 5162   |
| BfoI        | RGCG!Y                   | 7     | 598; 1694; 2111; 2776; 2784; 3512; 5187   |
| BfrI        | C!TTAAG                  | 1     | 2391  |

| Enzyme name | Sequence                 | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|--------------------------|-------|--|
| BfuI        | GTATCCNNNNNN!            | 3     | 2938; 3728; 5229   |
| BfuI        | !NNNNNGGATAC             | 0     |  |
| BfuAI       | ACCTGCNNNN!              | 2     | 3777; 4227   |
| BfuAI       | !NNNNNNNNNGCAGGT         | 1     | 3396   |
| BfuCI       | !GATC                    | 27    | 585; 609; 660; 1037; 1187; 1213; 1478; 1562; 2015; 2051; 2166; 3346; 3350; 3369; 3680; 3758; 3839; 3848; 3926; 4302; 4318; 4677; 4689; 4767; 4775; 4786; 4861  |
| BglI        | GCCNNNN!NGGC             | 5     | 90; 212; 283; 1142; 3284   |
| BglII       | A!GATCT                  | 1     | 609  |
| BisI        | GC!NGC                   | 42    | 822; 873; 1017; 1134; 1315; 1524; 1591; 1761; 1953; 1956; 1963; 2115; 2153; 2156; 2286; 2805; 2827; 2841; 3279; 3418; 3470; 3481; 3571; 3576; 3613; 3654; 3741; 3744; 3747; 3983; 4079; 4120; 4134; 4248; 4587; 4602; 4813; 5019; 5022; 5087; 5230; 5385 |
| BlnI        | C!CTAGG                  | 2     | 3331; 4346   |
| BlpI        | GC!TNAGC                 | 0     |  |
| BlsI        | GCN!GC                   | 42    | 823; 874; 1018; 1135; 1316; 1525; 1592; 1762; 1954; 1957; 1964; 2116; 2154; 2157; 2287; 2806; 2828; 2842; 3280; 3419; 3471; 3482; 3572; 3577; 3614; 3655; 3742; 3745; 3748; 3984; 4080; 4121; 4135; 4249; 4588; 4603; 4814; 5020; 5023; 5088; 5231; 5386 |
| BmcAI       | AGT!ACT                  | 0     |  |
| Bme18I      | G!GWCC                   | 3     | 2061; 4025; 4470   |
| Bme1390I    | CC!NGG                   | 32    | 90; 283; 657; 658; 850; 898; 994; 1009; 1138; 1247; 1602; 1612; 1714; 1735; 1789; 1843; 1915; 1996; 2095; 3045; 3100; 3117; 3513; 3673; 3896; 4286; 4475; 4578; 5050; 5268; 5281; 5402   |
| BmeRI       | GACNNN!NNGTC             | 0     |  |
| BmeT110I    | C!YCGRG                  | 4     | 613; 656; 1657; 1699   |
| BmgBI       | CAC!GTC                  | 0     |  |
| BmgBI       | GAC!GTG                  | 1     | 1497   |
| BmgT120I    | GG!NCC                   | 17    | 84; 277; 655; 824; 1067; 1159; 1605; 1846; 2006; 2062; 2074; 2617; 4026; 4471; 4517; 4575; 4608  |
| BmiI        | GGN!NCC                  | 22    | 467; 647; 655; 662; 729; 789; 1180; 1431; 2007; 2664; 2685; 2697; 2888; 3049; 3121; 3510; 3545; 4393; 4472; 4517; 5360; 5399   |
| BmrI        | ACTGGGNNNNN!             | 2     | 1369; 3458   |
| BmrI        | !NNNNCCCAGT              | 2     | 289; 3210  |
| BmrFI       | CC!NGG                   | 32    | 90; 283; 657; 658; 850; 898; 994; 1009; 1138; 1247; 1602; 1612; 1714; 1735; 1789; 1843; 1915; 1996; 2095; 3045; 3100; 3117; 3513; 3673; 3896; 4286; 4475; 4578; 5050; 5268; 5281; 5402   |
| BmsI        | GCATCNNNNN!              | 10    | 911; 1472; 1643; 1808; 2325; 3088; 3160; 3808; 3872; 4147  |
| BmsI        | !NNNNNNNNNGATGC          | 7     | 357; 1064; 1232; 3467; 3722; 3938; 5330  |
| BmtI        | GCTAG!C                  | 1     | 595  |
| BmuI        | ACTGGGNNNNN!             | 2     | 1369; 3458   |
| BmuI        | !NNNNCCCAGT              | 2     | 289; 3210  |
| BoxI        | GACNN!NNGTC              | 1     | 1306   |
| BpiI        | GAAGACNN!                | 1     | 974  |
| BpiI        | !NNNNNNGTCTTC            | 0     |  |
| BpI         | GAGNNNNNCTCNNNNNNNNNNNN! | 2     | 3298; 4197   |
| BpI         | !NNNNNNNNNGAGNNNNNCTC    | 2     | 3266; 4165   |
| BpmI        | CTGGAGNNNNNNNNNNNNNNNN!  | 5     | 1579; 1876; 1936; 2116; 4349   |
| BpmI        | !NNNNNNNNNNNNNNNCTCCAG   | 2     | 991; 4292  |
| Bpu10I      | CC!TNAGC                 | 1     | 2035   |
| Bpu10I      | GC!TNAGG                 | 0     |  |
| Bpu14I      | TT!CGAA                  | 2     | 627; 4191  |
| Bpu1102I    | GC!TNAGC                 | 0     |  |
| BpuAI       | GAAGACNN!                | 1     | 974  |
| BpuAI       | !NNNNNNGTCTTC            | 0     |  |
| BpuEI       | CTTGAGNNNNNNNNNNNNNNNN!  | 2     | 4803; 5342   |
| BpuEI       | !NNNNNNNNNNNNNNNCTCAAG   | 4     | 604; 1222; 3885; 5044  |
| BpuMI       | CC!SGG                   | 6     | 657; 658; 3513; 3673; 4286; 5050   |
| BpvUI       | CGAT!CG                  | 0     |  |
| BsaI        | GGTCTCN!                 | 0     |  |
| BsaI        | !NNNNNGAGACC             | 2     | 1294; 4498   |
| Bsa29I      | AT!CGAT                  | 1     | 3349   |

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|-------------|---------------------------|-------|---|
| BsaAI       | YAC!GTR                   | 3     | 340; 2625; 3813   |
| BsaBI       | GATNN!NNATC               | 3     | 2020; 2171; 3368  |
| BsaHI       | GR!CGYC                   | 9     | 122; 175; 258; 444; 1250; 1541; 3509; 4211; 4290  |
| BsaJI       | C!CNNGG                   | 31    | 360; 649; 656; 731; 761; 896; 897; 992; 1600; 1610; 1700; 1758; 1787; 1842; 1994; 1995; 2124; 3043; 3115; 3238; 3273; 3282; 3331; 3672; 3941; 4346; 4473; 4474; 4576; 4577; 5267                                  |
| BsaMI       | GAATGCN!                  | 1     | 2260  |
| BsaMI       | G!CATTC                   | 1     | 2353  |
| BsaWI       | W!CCGGW                   | 6     | 600; 666; 3540; 4384; 5074; 5221  |
| BsaXI       | ACNNNNNCTCCNNNNNNNNNN!    | 1     | 2589  |
| BsaXI       | !NNNNNNNGGAGNNNNNGT       | 1     | 1552  |
| BsaXI       | GGAGNNNNNGTNNNNNNNNNN!    | 1     | 1582  |
| BsaXI       | !NNNNNNNNNACNNNNNCTCC     | 1     | 2559  |
| BsbI        | CAACACNNNNNNNNNNNNNNNNNN! | 0     |   |
| BsbI        | !NNNNNNNNNNNNNNNNNGTGTTG  | 2     | 2510; 4417  |
| Bsc4I       | CCNNNNN!NNGG              | 27    | 49; 784; 897; 1129; 1439; 1601; 1734; 1764; 1854; 1899; 2070; 2130; 2521; 2847; 3239; 3490; 4034; 4315; 4347; 4479; 4514; 4564; 4596; 4949; 5228; 5394; 5412  |
| BscAI       | GCATCNNNN!                | 10    | 910; 1471; 1642; 1807; 2324; 3087; 3159; 3807; 3871; 4146   |
| BscAI       | !NNNNNGATGC               | 7     | 360; 1067; 1235; 3470; 3725; 3941; 5333   |
| BseI        | ACTGGN!                   | 5     | 1277; 1364; 3453; 3654; 4900  |
| BseI        | C!CAGT                    | 7     | 295; 1638; 1728; 2538; 3216; 5012; 5025   |
| Bse8I       | GATNN!NNATC               | 3     | 2020; 2171; 3368  |
| Bse21I      | CC!TNAGG                  | 3     | 932; 2989; 4621   |
| Bse118I     | R!CCGGY                   | 7     | 666; 1459; 1582; 2726; 3828; 4009; 4292   |
| BseAI       | T!CCGGA                   | 0     |   |
| BseBI       | CC!WGG                    | 26    | 90; 283; 850; 898; 994; 1009; 1138; 1247; 1602; 1612; 1714; 1735; 1789; 1843; 1915; 1996; 2095; 3045; 3100; 3117; 3896; 4475; 4578; 5268; 5281; 5402  |
| BseCI       | AT!CGAT                   | 1     | 3349  |
| BseDI       | C!CNNGG                   | 31    | 360; 649; 656; 731; 761; 896; 897; 992; 1600; 1610; 1700; 1758; 1787; 1842; 1994; 1995; 2124; 3043; 3115; 3238; 3273; 3282; 3331; 3672; 3941; 4346; 4473; 4474; 4576; 4577; 5267                                  |
| Bse3DI      | GCAATGNN!                 | 1     | 3742  |
| Bse3DI      | !CATTGC                   | 0     |   |
| BseGI       | GGATGNN!                  | 4     | 3369; 3826; 3851; 4305  |
| BseGI       | !CATCC                    | 10    | 757; 845; 902; 1057; 1634; 1709; 1724; 1838; 1886; 3195   |
| BseJI       | GATNN!NNATC               | 3     | 2020; 2171; 3368  |
| BseLI       | CCNNNNN!NNGG              | 27    | 49; 784; 897; 1129; 1439; 1601; 1734; 1764; 1854; 1899; 2070; 2130; 2521; 2847; 3239; 3490; 4034; 4315; 4347; 4479; 4514; 4564; 4596; 4949; 5228; 5394; 5412  |
| BseMI       | GCAATGNN!                 | 1     | 3742  |
| BseMI       | !CATTGC                   | 0     |   |
| BseMII      | CTCAGNNNNNNNNNN!          | 3     | 620; 2056; 4635   |
| BseMII      | !NNNNNNNNCTGAG            | 7     | 923; 2026; 2980; 3282; 4163; 4735; 5144   |
| BseNI       | ACTGGN!                   | 5     | 1277; 1364; 3453; 3654; 4900  |
| BseNI       | C!CAGT                    | 7     | 295; 1638; 1728; 2538; 3216; 5012; 5025   |
| BsePI       | G!CGCGC                   | 0     |   |
| BseRI       | GAGGAGNNNNNNNNNN!         | 2     | 1462; 3327  |
| BseRI       | !NNNNNNNNCTCCTC           | 0     |   |
| BseSI       | GKGCMI!C                  | 12    | 657; 730; 757; 790; 1367; 1472; 1502; 1601; 1994; 3455; 3548; 5117  |
| BseXI       | GCAGCNNNNNNNN!            | 9     | 884; 1326; 1974; 2297; 3582; 3624; 4145; 4598; 5098   |
| BseXI       | !NNNNNNNNNNNGCTGC         | 9     | 1003; 1577; 2101; 2791; 3456; 3640; 3733; 4799; 5005  |
| BseX3I      | C!GGCCG                   | 2     | 2153; 3415  |
| BseYI       | C!CCAGC                   | 3     | 3052; 3124; 5123  |
| BseYI       | G!CTGGG                   | 2     | 1094; 4013  |
| BsgI        | GTGCAGNNNNNNNNNNNNNNNN!   | 1     | 1981  |
| BsgI        | !NNNNNNNNNNNNNNCTGCAC     | 1     | 1560  |
| Bsh1236I    | CG!CG                     | 19    | 60; 651; 1523; 1760; 2078; 2158; 2433; 2809; 2829; 2853; 2884; 3575; 3876; 4314; 4398; 4461; 4530; 4803; 5384   |
| Bsh1285I    | CGRY!CG                   | 4     | 671; 2156; 3418; 5093   |
| BshFI       | GG!CC                     | 36    | 84; 277; 655; 824; 1067; 1102; 1136; 1159; 1291; 1333; 1505; 1605; 1847; 1903; 1913; 1969; 2006; 2074; 2155; 2475; 2617; 3272; 3278; 3287; 3330; 3417; 3591; 3982; 4009; 4518; 4575; 4608; 4953; 5387; 5405; 5416 |
| BshNI       | G!GYRCC                   | 8     | 465; 645; 727; 787; 1429; 2662; 3508; 3543  |

| Enzyme name | Sequence                 | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|--------------------------|-------|--|
| BshTI       | A!CCGGT                  | 1     | 666  |
| BshVI       | AT!CGAT                  | 1     | 3349   |
| BsiEI       | CGRY!CG                  | 4     | 671; 2156; 3418; 5093  |
| BsiHKAI     | GWGCW!C                  | 9     | 620; 757; 1502; 1664; 2041; 2093; 3622; 3812; 5117   |
| BsiHKCI     | C!YCGRG                  | 4     | 613; 656; 1657; 1699   |
| BsiSI       | C!CGG                    | 21    | 601; 657; 667; 1460; 1583; 2727; 3414; 3491; 3513; 3541; 3672; 3762; 3829; 4010; 4285; 4293; 4385; 4859; 5049; 5075; 5222  |
| BsiWI       | C!GTACG                  | 0     |  |
| BsII        | CCNNNNN!NNGG             | 27    | 49; 784; 897; 1129; 1439; 1601; 1734; 1764; 1854; 1899; 2070; 2130; 2521; 2847; 3239; 3490; 4034; 4315; 4347; 4479; 4514; 4564; 4596; 4949; 5228; 5394; 5412   |
| BsIFI       | GGGACNNNNNNNNNN!         | 6     | 175; 326; 494; 3660; 4192; 4301  |
| BsIFI       | !NNNNNNNNNNNNNGTCCC      | 4     | 3025; 3097; 3161; 4456   |
| BsmI        | GAATGCN!                 | 1     | 2260   |
| BsmI        | G!CATTC                  | 1     | 2353   |
| BsmAI       | GTCTCN!                  | 1     | 434  |
| BsmAI       | !NNNNNGAGAC              | 6     | 1105; 1294; 2933; 3351; 4370; 4498   |
| BsmBI       | CGTCTCN!                 | 0     |  |
| BsmBI       | !NNNNNGAGACG             | 1     | 1105   |
| BsmFI       | GGGACNNNNNNNNNN!         | 6     | 175; 326; 494; 3660; 4192; 4301  |
| BsmFI       | !NNNNNNNNNNNNNGTCCC      | 4     | 3025; 3097; 3161; 4456   |
| BsnI        | GG!CC                    | 36    | 84; 277; 655; 824; 1067; 1102; 1136; 1159; 1291; 1333; 1505; 1605; 1847; 1903; 1913; 1969; 2006; 2074; 2155; 2475; 2617; 3272; 3278; 3287; 3330; 3417; 3591; 3982; 4009; 4518; 4575; 4608; 4953; 5387; 5405; 5416                                    |
| Bso3II      | GGTCTCN!                 | 0     |  |
| Bso3II      | !NNNNNGAGACC             | 2     | 1294; 4498   |
| BsoBI       | C!YCGRG                  | 4     | 613; 656; 1657; 1699   |
| Bsp13I      | T!CCGGA                  | 0     |  |
| Bsp19I      | C!CATGG                  | 3     | 360; 3238; 3941  |
| Bsp24I      | GACNNNNNTGGNNNNNNNNNNNN! | 2     | 289; 4312  |
| Bsp24I      | !NNNNNNCCANNNNNGTC       | 2     | 4733; 4911   |
| Bsp24I      | CCANNNNNGTCNNNNNNNNNNNN! | 2     | 4765; 4943   |
| Bsp24I      | !NNNNNNNGACNNNNNTGG      | 2     | 257; 4280  |
| Bsp68I      | TCG!CGA                  | 0     |  |
| Bsp119I     | TT!CGAA                  | 2     | 627; 4191  |
| Bsp120I     | G!GGCCC                  | 1     | 653  |
| Bsp143I     | !GATC                    | 27    | 585; 609; 660; 1037; 1187; 1213; 1478; 1562; 2015; 2051; 2166; 3346; 3350; 3369; 3680; 3758; 3839; 3848; 3926; 4302; 4318; 4677; 4689; 4767; 4775; 4786; 4861  |
| Bsp1286I    | GDGCH!C                  | 21    | 620; 657; 730; 757; 790; 1367; 1472; 1502; 1601; 1664; 1994; 2041; 2093; 2700; 3455; 3548; 3622; 3812; 3874; 4584; 5117  |
| Bsp1407I    | T!GTACA                  | 3     | 716; 1883; 2141  |
| Bsp1720I    | GC!TNAGC                 | 0     |  |
| BspACI      | C!CGC                    | 43    | 58; 86; 98; 112; 279; 507; 589; 649; 986; 1124; 1226; 1650; 1758; 1797; 1890; 1935; 2064; 2076; 2156; 2824; 2827; 2841; 2931; 3179; 3191; 3200; 3212; 3222; 3233; 3279; 3418; 3481; 3983; 4028; 4094; 4120; 4245; 4248; 4396; 4528; 4835; 4979; 5475 |
| BspACI      | G!CGG                    | 33    | 370; 403; 528; 651; 821; 1133; 1175; 1439; 1523; 1760; 1952; 1955; 2152; 2769; 2796; 2884; 2994; 3575; 3639; 3740; 3743; 4023; 4078; 4176; 4314; 4461; 4601; 4844; 5089; 5210; 5229; 5356; 5384  |
| BspCNI      | CTCAGNNNNNNNNNN!         | 3     | 619; 2055; 4634  |
| BspCNI      | !NNNNNNNCTGAG            | 7     | 924; 2027; 2981; 3283; 4164; 4736; 5145  |
| BspDI       | AT!CGAT                  | 1     | 3349   |
| BspD6I      | GACTCNNNN!               | 6     | 418; 612; 2168; 2582; 4188; 5065   |
| BspD6I      | !NNNNNNGAGTC             | 4     | 946; 2091; 2545; 2977  |
| BspEI       | T!CCGGA                  | 0     |  |
| BspFNI      | CG!CG                    | 19    | 60; 651; 1523; 1760; 2078; 2158; 2433; 2809; 2829; 2853; 2884; 3575; 3876; 4314; 4398; 4461; 4530; 4803; 5384  |
| BspHI       | T!CATGA                  | 2     | 2935; 4707   |
| BspLI       | GGN!NCC                  | 22    | 467; 647; 655; 662; 729; 789; 1180; 1431; 2007; 2664; 2685; 2697; 2888; 3049; 3121; 3510; 3545; 4393; 4472; 4517; 5360; 5399   |
| BspMI       | ACCTGCNNNN!              | 2     | 3777; 4227   |

| Enzyme name | Sequence        | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|-----------------|-------|--|
| BspMI       | !NNNNNNNNGCAGGT | 1     | 3396   |
| BspOI       | GCTAG!C         | 1     | 595  |
| BspPI       | GGATCNNNN!      | 8     | 668; 3377; 3688; 3934; 4326; 4685; 4783; 4869  |
| BspPI       | !NNNNNGATCC     | 8     | 580; 655; 1208; 2010; 3753; 4297; 4684; 4781   |
| BspQI       | GCTCTTCN!       | 0     |  |
| BspQI       | !NNNNGAAGAGC    | 2     | 3853; 4063   |
| BspTI       | C!TTAAG         | 1     | 2391   |
| BspT104I    | TT!CGAA         | 2     | 627; 4191  |
| BspT107I    | G!GYRCC         | 8     | 465; 645; 727; 787; 1429; 2662; 3508; 3543   |
| BspTNI      | GGTCTCN!        | 0     |  |
| BspTNI      | !NNNNNGAGACC    | 2     | 1294; 4498   |
| BsrI        | ACTGGN!         | 5     | 1277; 1364; 3453; 3654; 4900   |
| BsrI        | C!CAGT          | 7     | 295; 1638; 1728; 2538; 3216; 5012; 5025  |
| BsrBI       | CCG!CTC         | 2     | 2933; 4122   |
| BsrBI       | GAG!CGG         | 3     | 1439; 2769; 4176   |
| BsrDI       | GCAATGNN!       | 1     | 3742   |
| BsrDI       | !CATTGC         | 0     |  |
| BsrFI       | R!CCGGY         | 7     | 666; 1459; 1582; 2726; 3828; 4009; 4292  |
| BsrGI       | T!GTACA         | 3     | 716; 1883; 2141  |
| BsrSI       | ACTGGN!         | 5     | 1277; 1364; 3453; 3654; 4900   |
| BsrSI       | C!CAGT          | 7     | 295; 1638; 1728; 2538; 3216; 5012; 5025  |
| BssAI       | R!CCGGY         | 7     | 666; 1459; 1582; 2726; 3828; 4009; 4292  |
| BssECI      | C!CNNGG         | 31    | 360; 649; 656; 731; 761; 896; 897; 992; 1600; 1610; 1700; 1758; 1787; 1842; 1994; 1995; 2124; 3043; 3115; 3238; 3273; 3282; 3331; 3672; 3941; 4346; 4473; 4474; 4576; 4577; 5267   |
| BssHII      | G!CGCGC         | 0     |  |
| BssKI       | !CCNNGG         | 32    | 88; 281; 655; 656; 848; 896; 992; 1007; 1136; 1245; 1600; 1610; 1712; 1733; 1787; 1841; 1913; 1994; 2093; 3043; 3098; 3115; 3511; 3671; 3894; 4284; 4473; 4576; 5048; 5266; 5279; 5400   |
| BssMI       | !GATC           | 27    | 585; 609; 660; 1037; 1187; 1213; 1478; 1562; 2015; 2051; 2166; 3346; 3350; 3369; 3680; 3758; 3839; 3848; 3926; 4302; 4318; 4677; 4689; 4767; 4775; 4786; 4861  |
| BssNI       | GR!CGYC         | 9     | 122; 175; 258; 444; 1250; 1541; 3509; 4211; 4290   |
| BssNAI      | GTA!TAC         | 0     |  |
| BssSI       | C!ACGAG         | 3     | 1318; 4227; 5254   |
| BssSI       | C!TCGTG         | 2     | 1168; 4101   |
| BssT1I      | C!CWWGG         | 5     | 360; 3238; 3331; 3941; 4346  |
| Bst6I       | CTCTTCN!        | 0     |  |
| Bst6I       | !NNNNGAAGAG     | 3     | 2976; 3853; 4063   |
| Bst98I      | C!TTAAG         | 1     | 2391   |
| Bst1107I    | GTA!TAC         | 0     |  |
| BstACI      | GR!CGYC         | 9     | 122; 175; 258; 444; 1250; 1541; 3509; 4211; 4290   |
| BstAFI      | C!TTAAG         | 1     | 2391   |
| BstAPI      | GCANNNN!NTGC    | 4     | 1640; 3076; 3148; 4610   |
| BstAUI      | T!GTACA         | 3     | 716; 1883; 2141  |
| BstBI       | TT!CGAA         | 2     | 627; 4191  |
| Bst2BI      | C!ACGAG         | 3     | 1318; 4227; 5254   |
| Bst2BI      | C!TCGTG         | 2     | 1168; 4101   |
| BstBAI      | YAC!GTR         | 3     | 340; 2625; 3813  |
| Bst4CI      | ACN!GT          | 15    | 76; 198; 269; 543; 581; 645; 732; 2600; 3889; 4005; 4113; 4436; 4927; 5397; 5468   |
| BstC8I      | GCN!NGC         | 41    | 86; 279; 593; 653; 685; 775; 979; 1461; 1519; 1555; 1588; 1650; 1960; 1967; 2076; 2112; 2728; 2771; 2785; 3058; 3077; 3130; 3149; 3403; 3589; 3808; 3874; 3880; 3908; 3912; 3953; 3957; 4011; 4294; 4481; 4528; 4584; 4606; 4817; 5377; 5414 |
| BstDEI      | C!TNAG          | 11    | 606; 932; 1229; 2035; 2042; 2989; 3291; 4172; 4621; 4744; 5153   |
| BstDSI      | C!CRYGG         | 7     | 360; 649; 731; 1758; 2124; 3238; 3941  |
| BstEII      | G!GTNACC        | 1     | 1614   |
| BstENI      | CCTNN!NNNAGG    | 0     |  |
| BstF5I      | GGATGNN!        | 4     | 3369; 3826; 3851; 4305   |
| BstF5I      | !CATCC          | 10    | 757; 845; 902; 1057; 1634; 1709; 1724; 1838; 1886; 3195  |
| BstFNI      | CG!CG           | 19    | 60; 651; 1523; 1760; 2078; 2158; 2433; 2809; 2829; 2853; 2884; 3575; 3876; 4314; 4398; 4461; 4530; 4803; 5384  |
| BstH2I      | RGCGC!Y         | 7     | 598; 1694; 2111; 2776; 2784; 3512; 5187  |

| Enzyme name | Sequence             | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|----------------------|-------|--|
| BstHHI      | GCG!C                | 25    | 597; 1523; 1693; 1721; 2110; 2775; 2783; 2809; 2831; 2840; 2853; 2884; 3503; 3511; 3575; 3612; 3878; 4138; 4314; 4400; 4803; 4912; 5086; 5186; 5253  |
| BstKTI      | GAT!C                | 27    | 588; 612; 663; 1040; 1190; 1216; 1481; 1565; 2018; 2054; 2169; 3349; 3353; 3372; 3683; 3761; 3842; 3851; 3929; 4305; 4321; 4680; 4692; 4770; 4778; 4789; 4864  |
| BstMAI      | GTCTCN!              | 1     | 434  |
| BstMAI      | !NNNNNGAGAC          | 6     | 1105; 1294; 2933; 3351; 4370; 4498   |
| BstMBI      | !GATC                | 27    | 585; 609; 660; 1037; 1187; 1213; 1478; 1562; 2015; 2051; 2166; 3346; 3350; 3369; 3680; 3758; 3839; 3848; 3926; 4302; 4318; 4677; 4689; 4767; 4775; 4786; 4861  |
| BstMCI      | CGRY!CG              | 4     | 671; 2156; 3418; 5093  |
| BstMWI      | GCNNNNN!NNGC         | 35    | 90; 212; 244; 283; 376; 400; 711; 1142; 1482; 1523; 1583; 1596; 1640; 1871; 2063; 2114; 2736; 2766; 2793; 2835; 3076; 3148; 3199; 3278; 3500; 3584; 3607; 3746; 3869; 3952; 4219; 4406; 4610; 4809; 5381   |
| BstNI       | CC!WGG               | 26    | 90; 283; 850; 898; 994; 1009; 1138; 1247; 1602; 1612; 1714; 1735; 1789; 1843; 1915; 1996; 2095; 3045; 3100; 3117; 3896; 4475; 4578; 5268; 5281; 5402   |
| BstNSI      | RCATG!Y              | 5     | 705; 3079; 3151; 3914; 5431  |
| BstOI       | CC!WGG               | 26    | 90; 283; 850; 898; 994; 1009; 1138; 1247; 1602; 1612; 1714; 1735; 1789; 1843; 1915; 1996; 2095; 3045; 3100; 3117; 3896; 4475; 4578; 5268; 5281; 5402   |
| BstPI       | G!GTNACC             | 1     | 1614   |
| BstPAI      | GACNN!NNGTC          | 1     | 1306   |
| BstSCI      | !CCNGG               | 32    | 88; 281; 655; 656; 848; 896; 992; 1007; 1136; 1245; 1600; 1610; 1712; 1733; 1787; 1841; 1913; 1994; 2093; 3043; 3098; 3115; 3511; 3671; 3894; 4284; 4473; 4576; 5048; 5266; 5279; 5400   |
| BstSFI      | C!TRYAG              | 5     | 634; 1266; 2844; 4971; 5162  |
| BstSLI      | GKGCMI!C             | 12    | 657; 730; 757; 790; 1367; 1472; 1502; 1601; 1994; 3455; 3548; 5117   |
| BstSNI      | TAC!GTA              | 1     | 340  |
| BstUI       | CG!CG                | 19    | 60; 651; 1523; 1760; 2078; 2158; 2433; 2809; 2829; 2853; 2884; 3575; 3876; 4314; 4398; 4461; 4530; 4803; 5384  |
| Bst2UI      | CC!WGG               | 26    | 90; 283; 850; 898; 994; 1009; 1138; 1247; 1602; 1612; 1714; 1735; 1789; 1843; 1915; 1996; 2095; 3045; 3100; 3117; 3896; 4475; 4578; 5268; 5281; 5402   |
| BstV1I      | GCAGCNNNNNNNN!       | 9     | 884; 1326; 1974; 2297; 3582; 3624; 4145; 4598; 5098  |
| BstV1I      | !NNNNNNNNNNNGCTGC    | 9     | 1003; 1577; 2101; 2791; 3456; 3640; 3733; 4799; 5005   |
| BstV2I      | GAAGACNN!            | 1     | 974  |
| BstV2I      | !NNNNNNGTCTTC        | 0     |  |
| BstXI       | CCANNNNN!NTGG        | 0     |  |
| BstX2I      | R!GATCY              | 11    | 585; 609; 660; 1213; 3680; 3926; 4318; 4677; 4689; 4775; 4786  |
| BstYI       | R!GATCY              | 11    | 585; 609; 660; 1213; 3680; 3926; 4318; 4677; 4689; 4775; 4786  |
| BstZI       | C!GGCCG              | 2     | 2153; 3415   |
| BstZ17I     | GTA!TAC              | 0     |  |
| BsuI        | GTATCCNNNNNN!        | 3     | 2938; 3728; 5229   |
| BsuI        | !NNNNNGGATAC         | 0     |  |
| Bsu15I      | AT!CGAT              | 1     | 3349   |
| Bsu36I      | CC!TNAGG             | 3     | 932; 2989; 4621  |
| BsuRI       | GG!CC                | 36    | 84; 277; 655; 824; 1067; 1102; 1136; 1159; 1291; 1333; 1505; 1605; 1847; 1903; 1913; 1969; 2006; 2074; 2155; 2475; 2617; 3272; 3278; 3287; 3330; 3417; 3591; 3982; 4009; 4518; 4575; 4608; 4953; 5387; 5405; 5416  |
| BsuTUI      | AT!CGAT              | 1     | 3349   |
| BtgI        | C!CRYGG              | 7     | 360; 649; 731; 1758; 2124; 3238; 3941  |
| BtgZI       | GCGATGNNNNNNNNN!     | 2     | 2626; 3961   |
| BtgZI       | !NNNNNNNNNNNNNCATCGC | 3     | 334; 3780; 4124  |
| BthCI       | GCNG!C               | 42    | 824; 875; 1019; 1136; 1317; 1526; 1593; 1763; 1955; 1958; 1965; 2117; 2155; 2158; 2288; 2807; 2829; 2843; 3281; 3420; 3472; 3483; 3573; 3578; 3615; 3656; 3743; 3746; 3749; 3985; 4081; 4122; 4136; 4250; 4589; 4604; 4815; 5021; 5024; 5089; 5232; 5387 |
| BtrI        | CAC!GTC              | 0     |  |
| BtrI        | GAC!GTG              | 1     | 1497   |
| BtsI        | GCAGTGNN!            | 1     | 1334   |
| BtsI        | !CACTGC              | 1     | 2348   |

| Enzyme name | Sequence                    | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|-----------------------------|-------|--|
| BtsCI       | GGATGNN!                    | 4     | 3369; 3826; 3851; 4305   |
| BtsCI       | !CATCC                      | 10    | 757; 845; 902; 1057; 1634; 1709; 1724; 1838; 1886; 3195  |
| BtuMI       | TCG!CGA                     | 0     |  |
| BveI        | ACCTGCNNNN!                 | 2     | 3777; 4227   |
| BveI        | !NNNNNNNNGCAGGT             | 1     | 3396   |
| Cac8I       | GCN!NGC                     | 41    | 86; 279; 593; 653; 685; 775; 979; 1461; 1519; 1555; 1588; 1650; 1960; 1967; 2076; 2112; 2728; 2771; 2785; 3058; 3077; 3130; 3149; 3403; 3589; 3808; 3874; 3880; 3908; 3912; 3953; 3957; 4011; 4294; 4481; 4528; 4584; 4606; 4817; 5377; 5414 |
| CaiI        | CAGNNN!CTG                  | 4     | 1340; 2111; 4610; 5018   |
| CciI        | T!CATGA                     | 2     | 2935; 4707   |
| CciNI       | GC!GGCCGC                   | 1     | 2153   |
| CdiI        | CATC!G                      | 8     | 352; 1467; 1803; 1947; 3798; 3803; 4001; 4142  |
| CdiI        | C!GATG                      | 2     | 2612; 3947   |
| CdpI        | GCGGAGNNNNNNNNNNNNNNNNNNNN! | 1     | 5381   |
| CdpI        | !NNNNNNNNNNNNNNNNNNNNCTCCGC | 3     | 486; 3170; 3212  |
| CelII       | GC!TNAGC                    | 0     |  |
| CfoI        | GCG!C                       | 25    | 597; 1523; 1693; 1721; 2110; 2775; 2783; 2809; 2831; 2840; 2853; 2884; 3503; 3511; 3575; 3612; 3878; 4138; 4314; 4400; 4803; 4912; 5086; 5186; 5253  |
| CfrI        | Y!GGCCR                     | 8     | 1331; 1503; 1967; 2153; 3415; 3589; 3980; 4007   |
| Cfr9I       | C!CCGGG                     | 1     | 656  |
| Cfr10I      | R!CCGGY                     | 7     | 666; 1459; 1582; 2726; 3828; 4009; 4292  |
| Cfr13I      | G!GNCC                      | 17    | 83; 276; 654; 823; 1066; 1158; 1604; 1845; 2005; 2061; 2073; 2616; 4025; 4470; 4516; 4574; 4607  |
| Cfr42I      | CCGC!GG                     | 2     | 652; 1761  |
| ChaI        | GATC!                       | 27    | 589; 613; 664; 1041; 1191; 1217; 1482; 1566; 2019; 2055; 2170; 3350; 3354; 3373; 3684; 3762; 3843; 3852; 3930; 4306; 4322; 4681; 4693; 4771; 4779; 4790; 4865  |
| CjeI        | CCANNNNNGTNNNNNNNNNNNNNNNN! | 5     | 1900; 3326; 4766; 4866; 4944   |
| CjeI        | !NNNNNNNNNACNNNNNTGG        | 7     | 64; 257; 2993; 3088; 4280; 4830; 5385  |
| CjeI        | ACNNNNNTGGNNNNNNNNNNNNNNNN! | 7     | 98; 291; 3027; 3122; 4314; 4864; 5419  |
| CjeI        | !NNNNNNNNCCANNNNNGT         | 5     | 1866; 3292; 4732; 4832; 4910   |
| CjePI       | CCANNNNNNNTCNNNNNNNNNNNNNN! | 2     | 4766; 4944   |
| CjePI       | !NNNNNNNGANNNNNNNTGG        | 5     | 257; 794; 1139; 4280; 4879   |
| CjePI       | GANNNNNNNTGGNNNNNNNNNNNNNN! | 5     | 290; 827; 1172; 4313; 4912   |
| CjePI       | !NNNNNNNNCCANNNNNNNTC       | 2     | 4733; 4911   |
| Clal        | AT!CGAT                     | 1     | 3349   |
| CpoI        | CG!GWCCG                    | 1     | 4025   |
| CseI        | GACGCNNNNN!                 | 5     | 526; 1122; 1549; 4219; 4298  |
| CseI        | !NNNNNNNNNGCGTC             | 4     | 1239; 2842; 4737; 5315   |
| CsiI        | A!CCWGGT                    | 1     | 3098   |
| CspI        | CG!GWCCG                    | 1     | 4025   |
| Csp6I       | G!TAC                       | 15    | 218; 243; 298; 331; 382; 539; 646; 717; 1119; 1430; 1749; 1860; 1884; 2142; 3814   |
| Csp45I      | TT!CGAA                     | 2     | 627; 4191  |
| CspAI       | A!CCGGT                     | 1     | 666  |
| CspCI       | CAANNNNNGTGNNNNNNNNNNNNNN!  | 1     | 411  |
| CspCI       | !NNNNNNNNNNCCACNNNNNTTG     | 0     |  |
| CspCI       | CCACNNNNNTTGNNNNNNNNNNNNNN! | 0     |  |
| CspCI       | !NNNNNNNNNNCAANNNNNGTG      | 1     | 376  |
| CstMI       | AAGGAGNNNNNNNNNNNNNNNNNNNN! | 5     | 719; 1310; 1850; 2789; 4398  |
| CstMI       | !NNNNNNNNNNNNNNNNNNNNCTCCTT | 0     |  |
| CviAII      | C!ATG                       | 27    | 301; 361; 678; 702; 708; 720; 798; 944; 1155; 1434; 1665; 1695; 2085; 2130; 2936; 3076; 3148; 3239; 3380; 3725; 3911; 3942; 3968; 4324; 4708; 5428; 5479   |

| Enzyme name | Sequence                       | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|--------------------------------|-------|--|
| CviJI       | RG!CY                          | 115   | 42; 84; 93; 277; 566; 618; 624; 655; 687; 714; 777; 824; 853; 860; 938; 1004; 1016; 1067; 1094; 1102; 1136; 1159; 1167; 1181; 1291; 1333; 1371; 1410; 1452; 1485; 1505; 1557; 1590; 1605; 1706; 1806; 1847; 1854; 1865; 1874; 1903; 1913; 1921; 1965; 1969; 2006; 2074; 2139; 2155; 2178; 2288; 2455; 2475; 2617; 2698; 2712; 2726; 3007; 3048; 3120; 3243; 3272; 3278; 3287; 3295; 3318; 3330; 3336; 3417; 3434; 3442; 3469; 3494; 3578; 3587; 3591; 3615; 3653; 3729; 3746; 3765; 3828; 3872; 3882; 3899; 3982; 4009; 4013; 4050; 4073; 4089; 4269; 4296; 4357; 4479; 4518; 4575; 4582; 4589; 4608; 4619; 4870; 4899; 4942; 4953; 5018; 5097; 5122; 5127; 5173; 5263; 5361; 5387; 5405; 5416 |
| CviKI-1     | RG!CY                          | 115   | 42; 84; 93; 277; 566; 618; 624; 655; 687; 714; 777; 824; 853; 860; 938; 1004; 1016; 1067; 1094; 1102; 1136; 1159; 1167; 1181; 1291; 1333; 1371; 1410; 1452; 1485; 1505; 1557; 1590; 1605; 1706; 1806; 1847; 1854; 1865; 1874; 1903; 1913; 1921; 1965; 1969; 2006; 2074; 2139; 2155; 2178; 2288; 2455; 2475; 2617; 2698; 2712; 2726; 3007; 3048; 3120; 3243; 3272; 3278; 3287; 3295; 3318; 3330; 3336; 3417; 3434; 3442; 3469; 3494; 3578; 3587; 3591; 3615; 3653; 3729; 3746; 3765; 3828; 3872; 3882; 3899; 3982; 4009; 4013; 4050; 4073; 4089; 4269; 4296; 4357; 4479; 4518; 4575; 4582; 4589; 4608; 4619; 4870; 4899; 4942; 4953; 5018; 5097; 5122; 5127; 5173; 5263; 5361; 5387; 5405; 5416 |
| CviQI       | G!TAC                          | 15    | 218; 243; 298; 331; 382; 539; 646; 717; 1119; 1430; 1749; 1860; 1884; 2142; 3814   |
| DdeI        | C!TNAG                         | 11    | 606; 932; 1229; 2035; 2042; 2989; 3291; 4172; 4621; 4744; 5153   |
| DinI        | GGC!GCC                        | 1     | 3510   |
| DpnI        | GA!TC                          | 27    | 587; 611; 662; 1039; 1189; 1215; 1480; 1564; 2017; 2053; 2168; 3348; 3352; 3371; 3682; 3760; 3841; 3850; 3928; 4304; 4320; 4679; 4691; 4769; 4777; 4788; 4863  |
| DpnII       | !GATC                          | 27    | 585; 609; 660; 1037; 1187; 1213; 1478; 1562; 2015; 2051; 2166; 3346; 3350; 3369; 3680; 3758; 3839; 3848; 3926; 4302; 4318; 4677; 4689; 4767; 4775; 4786; 4861  |
| DraI        | TTT!AAA                        | 3     | 2211; 4653; 4672   |
| DraII       | RG!GNCCY                       | 1     | 4607   |
| DraIII      | CACNNN!GTG                     | 4     | 1323; 1881; 2088; 2625   |
| DraRI       | CAAGNACNNNNNNNNNNNNNNNNNNNNNN! | 5     | 266; 1772; 1841; 2084; 4989  |
| DraRI       | !NNNNNNNNNNNNNNNNNNNGTNCTTG    | 0     |  |
| DrdI        | GACNNN!NNGTC                   | 3     | 2580; 3536; 5325   |
| DrdIV       | TACGACNNNNNNNNNNNNNNNNNNNNNN!  | 0     |  |
| DrdIV       | !NNNNNNNNNNNNNNNNNNNGTCGTA     | 1     | 476  |
| DriI        | GACNNN!NNGTC                   | 0     |  |
| DseDI       | GACNNN!NNGTC                   | 3     | 2580; 3536; 5325   |
| EaeI        | Y!GGCCR                        | 8     | 1331; 1503; 1967; 2153; 3415; 3589; 3980; 4007   |
| EagI        | C!GGCCG                        | 2     | 2153; 3415   |
| Eam1104I    | CTCTTCN!                       | 0     |  |
| Eam1104I    | !NNNNGAAGAG                    | 3     | 2976; 3853; 4063   |
| Eam1105I    | GACNNN!NNGTC                   | 0     |  |
| EarI        | CTCTTCN!                       | 0     |  |
| EarI        | !NNNNGAAGAG                    | 3     | 2976; 3853; 4063   |
| EciI        | GGCGANNNNNNNNNNNNNN!           | 4     | 3009; 4038; 5225; 5371   |
| EciI        | !NNNNNNNNNTCCGCC               | 5     | 496; 1879; 3180; 3201; 3222  |
| Ecl136II    | GAG!CTC                        | 1     | 618  |
| EclXI       | C!GGCCG                        | 2     | 2153; 3415   |
| Eco24I      | GRGY!C                         | 5     | 620; 657; 2700; 3874; 4584   |
| Eco31I      | GGTCTCN!                       | 0     |  |
| Eco31I      | !NNNNNGAGACC                   | 2     | 1294; 4498   |
| Eco32I      | GAT!ATC                        | 0     |  |
| Eco47I      | G!GWCC                         | 3     | 2061; 4025; 4470   |
| Eco47III    | AGC!GCT                        | 1     | 596  |
| Eco52I      | C!GGCCG                        | 2     | 2153; 3415   |
| Eco57I      | CTGAAGNNNNNNNNNNNNNNNN!        | 4     | 1183; 1828; 3655; 4087   |
| Eco57I      | !NNNNNNNNNNNNNNNNCTTCAG        | 2     | 2088; 4885   |
| Eco72I      | CAC!GTG                        | 0     |  |
| Eco81I      | CC!TNAGG                       | 3     | 932; 2989; 4621  |
| Eco88I      | C!YCGRG                        | 4     | 613; 656; 1657; 1699   |

| Enzyme name | Sequence                | Count | Cutting Position (the base to the left of the cut site)   |
|-------------|-------------------------|-------|---|
| Eco91I      | G!GTNACC                | 1     | 1614  |
| Eco105I     | TAC!GTA                 | 1     | 340   |
| Eco130I     | C!CWWGG                 | 5     | 360; 3238; 3331; 3941; 4346   |
| Eco147I     | AGG!CCT                 | 2     | 1102; 3330  |
| EcoHI       | !CCSGG                  | 6     | 655; 656; 3511; 3671; 4284; 5048  |
| EcoICRI     | GAG!CTC                 | 1     | 618   |
| Eco57MI     | CTGRAGNNNNNNNNNNNNNNNN! | 9     | 1183; 1579; 1828; 1876; 1936; 2116; 3655; 4087; 4349  |
| Eco57MI     | !NNNNNNNNNNNNNNCTYCAG   | 4     | 991; 2088; 4292; 4885   |
| EcoNI       | CCTNN!NNNAGG            | 0     |   |
| Eco065I     | G!GTNACC                | 1     | 1614  |
| EcoO109I    | RG!GNCCY                | 1     | 4607  |
| EcoRI       | G!AATTC                 | 2     | 629; 1375   |
| EcoRII      | !CCWGG                  | 26    | 88; 281; 848; 896; 992; 1007; 1136; 1245; 1600; 1610; 1712; 1733; 1787; 1841; 1913; 1994; 2093; 3043; 3098; 3115; 3894; 4473; 4576; 5266; 5279; 5400  |
| EcoRV       | GAT!ATC                 | 0     |   |
| EcoT14I     | C!CWWGG                 | 5     | 360; 3238; 3331; 3941; 4346   |
| EcoT22I     | ATGCA!T                 | 3     | 3081; 3153; 5484  |
| EcoT38I     | GRGCY!C                 | 5     | 620; 657; 2700; 3874; 4584  |
| Eco53kI     | GAG!CTC                 | 1     | 618   |
| EgeI        | GGC!GCC                 | 1     | 3510  |
| EheI        | GGC!GCC                 | 1     | 3510  |
| ErhI        | C!CWWGG                 | 5     | 360; 3238; 3331; 3941; 4346   |
| EsaBC3I     | TC!GA                   | 21    | 615; 628; 641; 816; 843; 1311; 1482; 1776; 1803; 1947; 2659; 3350; 3623; 3779; 3803; 3839; 4001; 4192; 4237; 4492; 5330   |
| Esp3I       | CGTCTCN!                | 0     |   |
| Esp3I       | !NNNNNGAGACG            | 1     | 1105  |
| FaeI        | CATG!                   | 27    | 304; 364; 681; 705; 711; 723; 801; 947; 1158; 1437; 1668; 1698; 2088; 2133; 2939; 3079; 3151; 3242; 3383; 3728; 3914; 3945; 3971; 4327; 4711; 5431; 5482  |
| Fail        | YA!TR                   | 67    | 39; 46; 50; 67; 138; 146; 234; 236; 291; 302; 310; 362; 555; 557; 679; 703; 709; 721; 799; 945; 963; 1156; 1209; 1211; 1259; 1435; 1666; 1696; 2086; 2131; 2171; 2181; 2247; 2292; 2497; 2925; 2937; 3068; 3077; 3140; 3149; 3173; 3240; 3264; 3381; 3445; 3726; 3751; 3912; 3943; 3969; 4041; 4257; 4325; 4403; 4455; 4616; 4633; 4635; 4637; 4709; 4987; 5176; 5292; 5365; 5429; 5480 |
| FaII        | AAGNNNNNCTNNNNNNNNNNNN! | 2     | 942; 3849   |
| FaII        | !NNNNNNNNAAGNNNNNCTT    | 2     | 910; 3817   |
| FaqI        | GGGACNNNNNNNNNN!        | 6     | 175; 326; 494; 3660; 4192; 4301   |
| FaqI        | !NNNNNNNNNNNNNGTCCC     | 4     | 3025; 3097; 3161; 4456  |
| FatI        | !CATG                   | 27    | 300; 360; 677; 701; 707; 719; 797; 943; 1154; 1433; 1664; 1694; 2084; 2129; 2935; 3075; 3147; 3238; 3379; 3724; 3910; 3941; 3967; 4323; 4707; 5427; 5478  |
| FauI        | CCCGCNNNN!              | 14    | 93; 119; 286; 1131; 1233; 1657; 1765; 1942; 2083; 2831; 3186; 3207; 4403; 4535  |
| FauI        | !NNNNNNNGCGGG           | 8     | 644; 1168; 1432; 2762; 3632; 4169; 4307; 4454   |
| FauNDI      | CA!TATG                 | 1     | 234   |
| FbaI        | T!GATCA                 | 0     |   |
| FblI        | GT!MKAC                 | 2     | 640; 1253   |
| FmuI        | GGNC!C                  | 17    | 86; 279; 657; 826; 1069; 1161; 1607; 1848; 2008; 2064; 2076; 2619; 4028; 4473; 4519; 4577; 4610   |
| Fnu4HI      | GC!NGC                  | 42    | 822; 873; 1017; 1134; 1315; 1524; 1591; 1761; 1953; 1956; 1963; 2115; 2153; 2156; 2286; 2805; 2827; 2841; 3279; 3418; 3470; 3481; 3571; 3576; 3613; 3654; 3741; 3744; 3747; 3983; 4079; 4120; 4134; 4248; 4587; 4602; 4813; 5019; 5022; 5087; 5230; 5385  |
| FokI        | GGATGNNNNNNNNNN!        | 4     | 3376; 3833; 3858; 4312  |
| FokI        | !NNNNNNNNNNNNNCATCC     | 9     | 744; 832; 889; 1044; 1621; 1696; 1825; 1873; 3182   |
| FriOI       | GRGCY!C                 | 5     | 620; 657; 2700; 3874; 4584  |
| FseI        | GGCCGG!CC               | 0     |   |
| FspI        | TGC!GCA                 | 1     | 3611  |
| FspAI       | RTGC!GCAY               | 0     |   |
| FspBI       | C!TAG                   | 10    | 592; 857; 1352; 2164; 2358; 2776; 3332; 4347; 4681; 4934  |

| Enzyme name | Sequence                 | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|--------------------------|-------|--|
| FspEI       | CCNNNNNNNNNNNN!          | 184   | 56; 71; 98; 114; 128; 129; 156; 182; 220; 252; 291; 307; 333; 373; 450; 451; 482; 501; 520; 593; 614; 662; 677; 744; 774; 791; 805; 838; 852; 877; 894; 909; 939; 972; 999; 1014; 1033; 1074; 1116; 1135; 1150; 1173; 1195; 1218; 1238; 1259; 1305; 1347; 1363; 1446; 1473; 1519; 1557; 1593; 1607; 1623; 1651; 1669; 1707; 1726; 1741; 1770; 1800; 1855; 1888; 1903; 1917; 1947; 1983; 1998; 2020; 2034; 2061; 2077; 2095; 2115; 2131; 2169; 2192; 2231; 2245; 2386; 2481; 2505; 2528; 2551; 2569; 2591; 2612; 2631; 2650; 2679; 2700; 2714; 2740; 2828; 2854; 2903; 2944; 2962; 3001; 3017; 3055; 3112; 3127; 3184; 3204; 3218; 3235; 3249; 3286; 3301; 3344; 3427; 3494; 3525; 3545; 3560; 3605; 3619; 3685; 3699; 3718; 3734; 3775; 3795; 3842; 3892; 3908; 3928; 3953; 3973; 3996; 4023; 4041; 4067; 4107; 4133; 4157; 4213; 4227; 4255; 4298; 4319; 4354; 4398; 4486; 4510; 4532; 4552; 4568; 4589; 4603; 4622; 4706; 4726; 4754; 4769; 4803; 4845; 4872; 4887; 4932; 4956; 4970; 4992; 5018; 5038; 5062; 5088; 5136; 5155; 5174; 5200; 5235; 5280; 5310; 5324; 5375; 5389; 5414; 5430; 5451; 5480 |
| FspEI       | !NNNNNNNNNNNNNGG         | 154   | 8; 35; 59; 144; 166; 197; 252; 295; 347; 375; 400; 435; 463; 509; 529; 552; 586; 628; 652; 679; 706; 748; 769; 793; 835; 854; 883; 919; 956; 979; 998; 1028; 1049; 1076; 1115; 1141; 1160; 1232; 1258; 1306; 1345; 1364; 1397; 1424; 1445; 1471; 1508; 1535; 1568; 1587; 1616; 1687; 1720; 1745; 1763; 1795; 1813; 1840; 1885; 1903; 1933; 1951; 1981; 2044; 2071; 2111; 2130; 2180; 2280; 2349; 2379; 2457; 2506; 2528; 2556; 2592; 2637; 2666; 2702; 2721; 2749; 2769; 2832; 2855; 2963; 2995; 3013; 3042; 3085; 3114; 3225; 3254; 3297; 3318; 3346; 3379; 3399; 3424; 3451; 3476; 3498; 3526; 3552; 3573; 3624; 3645; 3663; 3711; 3747; 3803; 3828; 3852; 3881; 3904; 3928; 3954; 3976; 3995; 4020; 4060; 4096; 4161; 4246; 4270; 4299; 4333; 4354; 4374; 4419; 4446; 4465; 4498; 4550; 4581; 4608; 4660; 4758; 4829; 4874; 4935; 5000; 5034; 5060; 5078; 5114; 5184; 5207; 5229; 5253; 5286; 5334; 5369; 5387; 5444  |
| Fsp4HI      | GC!NGC                   | 42    | 822; 873; 1017; 1134; 1315; 1524; 1591; 1761; 1953; 1956; 1963; 2115; 2153; 2156; 2286; 2805; 2827; 2841; 3279; 3418; 3470; 3481; 3571; 3576; 3613; 3654; 3741; 3744; 3747; 3983; 4079; 4120; 4134; 4248; 4587; 4602; 4813; 5019; 5022; 5087; 5230; 5385   |
| GdiII       | C!GGCCR                  | 3     | 1503; 2153; 3415   |
| GdiII       | Y!GGCCG                  | 5     | 1967; 2153; 3415; 3980; 4007   |
| GlaI        | GC!GC                    | 25    | 596; 1522; 1692; 1720; 2109; 2774; 2782; 2808; 2830; 2839; 2852; 2883; 3502; 3510; 3574; 3611; 3877; 4137; 4313; 4399; 4802; 4911; 5085; 5185; 5252  |
| GluI        | GC!NGC                   | 42    | 822; 873; 1017; 1134; 1315; 1524; 1591; 1761; 1953; 1956; 1963; 2115; 2153; 2156; 2286; 2805; 2827; 2841; 3279; 3418; 3470; 3481; 3571; 3576; 3613; 3654; 3741; 3744; 3747; 3983; 4079; 4120; 4134; 4248; 4587; 4602; 4813; 5019; 5022; 5087; 5230; 5385   |
| GsaI        | CCCAG!C                  | 3     | 3056; 3128; 5127   |
| GsaI        | GCTGG!G                  | 2     | 1098; 4017   |
| GsuI        | CTGGAGNNNNNNNNNNNNNN!    | 5     | 1579; 1876; 1936; 2116; 4349   |
| GsuI        | !NNNNNNNNNNNNNCTCCAG     | 2     | 991; 4292  |
| HaeI        | WGG!CCW                  | 9     | 1102; 1291; 1333; 1903; 3330; 3591; 4953; 5405; 5416   |
| HaeII       | RGCG!Y                   | 7     | 598; 1694; 2111; 2776; 2784; 3512; 5187  |
| HaeIII      | GG!CC                    | 36    | 84; 277; 655; 824; 1067; 1102; 1136; 1159; 1291; 1333; 1505; 1605; 1847; 1903; 1913; 1969; 2006; 2074; 2155; 2475; 2617; 3272; 3278; 3287; 3330; 3417; 3591; 3982; 4009; 4518; 4575; 4608; 4953; 5387; 5405; 5416  |
| HaeIV       | GAYNNNNNRTCNNNNNNNNNNNN! | 2     | 2184; 3705   |
| HaeIV       | !NNNNNNNNNGAYNNNNNRTC    | 2     | 2150; 3671   |
| HaeIV       | GAYNNNNNRTCNNNNNNNNNNNN! | 2     | 2183; 3704   |
| HaeIV       | !NNNNNNNGAYNNNNNRTC      | 2     | 2152; 3673   |
| HapII       | C!CGG                    | 21    | 601; 657; 667; 1460; 1583; 2727; 3414; 3491; 3513; 3541; 3672; 3762; 3829; 4010; 4285; 4293; 4385; 4859; 5049; 5075; 5222  |
| HgaI        | GACGCNNNNN!              | 5     | 526; 1122; 1549; 4219; 4298  |
| HgaI        | !NNNNNNNNNNNGCGTC        | 4     | 1239; 2842; 4737; 5315   |
| HhaI        | GCG!C                    | 25    | 597; 1523; 1693; 1721; 2110; 2775; 2783; 2809; 2831; 2840; 2853; 2884; 3503; 3511; 3575; 3612; 3878; 4138; 4314; 4400; 4803; 4912; 5086; 5186; 5253  |
| HinII       | GR!CGYC                  | 9     | 122; 175; 258; 444; 1250; 1541; 3509; 4211; 4290   |

| Enzyme name | Sequence                  | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|---------------------------|-------|--|
| Hin1II      | CATG!                     | 27    | 304; 364; 681; 705; 711; 723; 801; 947; 1158; 1437; 1668; 1698; 2088; 2133; 2939; 3079; 3151; 3242; 3383; 3728; 3914; 3945; 3971; 4327; 4711; 5431; 5482   |
| Hin4I       | GAYNNNNNVTCNNNNNNNNNNNN!  | 2     | 2183; 3704   |
| Hin4I       | !NNNNNNNGABNNNNNNRTC      | 3     | 2151; 3672; 3702   |
| Hin4I       | GABNNNNNNRTCNNNNNNNNNNNN! | 3     | 2183; 3704; 3734   |
| Hin4I       | !NNNNNNNGAYNNNNNVTC       | 2     | 2151; 3672   |
| Hin6I       | G!CGC                     | 25    | 595; 1521; 1691; 1719; 2108; 2773; 2781; 2807; 2829; 2838; 2851; 2882; 3501; 3509; 3573; 3610; 3876; 4136; 4312; 4398; 4801; 4910; 5084; 5184; 5251  |
| HinP1I      | G!CGC                     | 25    | 595; 1521; 1691; 1719; 2108; 2773; 2781; 2807; 2829; 2838; 2851; 2882; 3501; 3509; 3573; 3610; 3876; 4136; 4312; 4398; 4801; 4910; 5084; 5184; 5251  |
| HincII      | GTY!RAC                   | 2     | 641; 2272  |
| HindII      | GTY!RAC                   | 2     | 641; 2272  |
| HindIII     | A!AGCTT                   | 3     | 622; 1369; 1408  |
| HinfI       | G!ANTC                    | 17    | 410; 604; 803; 953; 1280; 2098; 2160; 2552; 2574; 2984; 3994; 4128; 4180; 4238; 4275; 5057; 5453   |
| HpaI        | GTT!AAC                   | 1     | 2272   |
| HpaII       | C!CGG                     | 21    | 601; 657; 667; 1460; 1583; 2727; 3414; 3491; 3513; 3541; 3672; 3762; 3829; 4010; 4285; 4293; 4385; 4859; 5049; 5075; 5222  |
| HphI        | GGTGANNNNNNNN!            | 7     | 376; 1059; 1626; 1779; 1803; 4580; 4696  |
| HphI        | !NNNNNNNTCACC             | 4     | 948; 1098; 2626; 3687  |
| Hpy8I       | GTN!NAC                   | 17    | 201; 539; 577; 641; 735; 755; 1050; 1254; 1263; 1500; 1794; 1884; 2272; 2556; 2573; 2628; 5115   |
| Hpy99I      | CGWCG!                    | 13    | 645; 1312; 1498; 1543; 1744; 2005; 3599; 3627; 3921; 3936; 4213; 4598; 5331  |
| Hpy166II    | GTN!NAC                   | 17    | 201; 539; 577; 641; 735; 755; 1050; 1254; 1263; 1500; 1794; 1884; 2272; 2556; 2573; 2628; 5115   |
| Hpy188I     | TCN!GA                    | 17    | 585; 609; 762; 1042; 1187; 1395; 2045; 2683; 3292; 3476; 3820; 4173; 4274; 4753; 4887; 5240; 5318  |
| Hpy188III   | TC!NNGA                   | 26    | 613; 1035; 1200; 1239; 1686; 1929; 2164; 2936; 2988; 3301; 3354; 3527; 3843; 3852; 3935; 3991; 4035; 4125; 4158; 4227; 4708; 4782; 4865; 4963; 5061; 5195  |
| HpyAV       | CCTTCNNNNNN!              | 8     | 843; 891; 936; 1113; 2112; 4154; 4261; 4940  |
| HpyAV       | !NNNNNGAAGG               | 10    | 763; 1135; 1803; 2742; 3637; 4366; 4382; 4565; 4882; 5192  |
| HpyCH4III   | ACN!GT                    | 15    | 76; 198; 269; 543; 581; 645; 732; 2600; 3889; 4005; 4113; 4436; 4927; 5397; 5468   |
| HpyCH4IV    | A!CGT                     | 19    | 122; 134; 175; 258; 339; 444; 1031; 1307; 1496; 1880; 1988; 2569; 2581; 2624; 2734; 3625; 3812; 4593; 4726   |
| HpyCH4V     | TG!CA                     | 23    | 636; 705; 755; 1077; 1500; 1577; 1962; 2258; 2285; 2353; 3070; 3079; 3142; 3151; 3266; 3342; 3401; 3560; 3735; 3749; 4819; 5115; 5482  |
| HpyF3I      | C!TNAG                    | 11    | 606; 932; 1229; 2035; 2042; 2989; 3291; 4172; 4621; 4744; 5153   |
| HpyF10VI    | GCNNNNN!NNGC              | 35    | 90; 212; 244; 283; 376; 400; 711; 1142; 1482; 1523; 1583; 1596; 1640; 1871; 2063; 2114; 2736; 2766; 2793; 2835; 3076; 3148; 3199; 3278; 3500; 3584; 3607; 3746; 3869; 3952; 4219; 4406; 4610; 4809; 5381 |
| Hsp92I      | GR!CGYC                   | 9     | 122; 175; 258; 444; 1250; 1541; 3509; 4211; 4290   |
| Hsp92II     | CATG!                     | 27    | 304; 364; 681; 705; 711; 723; 801; 947; 1158; 1437; 1668; 1698; 2088; 2133; 2939; 3079; 3151; 3242; 3383; 3728; 3914; 3945; 3971; 4327; 4711; 5431; 5482   |
| HspAI       | G!CGC                     | 25    | 595; 1521; 1691; 1719; 2108; 2773; 2781; 2807; 2829; 2838; 2851; 2882; 3501; 3509; 3573; 3610; 3876; 4136; 4312; 4398; 4801; 4910; 5084; 5184; 5251  |
| KasI        | G!GCGCC                   | 1     | 3508   |
| KfII        | GG!GWCCC                  | 0     |  |
| KpnI        | GGTAC!C                   | 2     | 649; 1433  |
| Kpn2I       | T!CCGGA                   | 0     |  |
| KspI        | CCGC!GG                   | 2     | 652; 1761  |
| Ksp22I      | T!GATCA                   | 0     |  |
| KspAI       | GTT!AAC                   | 1     | 2272   |
| Kzo9I       | !GATC                     | 27    | 585; 609; 660; 1037; 1187; 1213; 1478; 1562; 2015; 2051; 2166; 3346; 3350; 3369; 3680; 3758; 3839; 3848; 3926; 4302; 4318; 4677; 4689; 4767; 4775; 4786; 4861  |
| LguI        | GCTCTCN!                  | 0     |  |

| Enzyme name | Sequence                     | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|------------------------------|-------|--|
| LguI        | !NNNNGAAGAGC                 | 2     | 3853; 4063   |
| LpnI        | RGC!GCGY                     | 7     | 596; 1692; 2109; 2774; 2782; 3510; 5185  |
| LpnPI       | CCDGNNNNNNNNN!               | 73    | 102; 295; 614; 670; 805; 862; 910; 944; 1006; 1021; 1082; 1150; 1174; 1259; 1347; 1473; 1596; 1614; 1651; 1726; 1741; 1801; 1855; 1927; 1993; 2008; 2047; 2107; 2124; 2246; 2551; 2740; 2963; 3001; 3017; 3057; 3112; 3129; 3229; 3314; 3427; 3504; 3526; 3549; 3685; 3699; 3718; 3775; 3842; 3892; 3908; 3965; 4023; 4232; 4298; 4322; 4398; 4487; 4590; 4623; 4854; 4872; 5018; 5038; 5062; 5088; 5137; 5235; 5280; 5310; 5389; 5414; 5451 |
| LpnPI       | !NNNNNNNNNNNNNNCHGG          | 70    | 75; 268; 552; 586; 642; 835; 883; 979; 1080; 1123; 1232; 1258; 1345; 1373; 1406; 1445; 1471; 1543; 1568; 1587; 1699; 1720; 1774; 1828; 1900; 1951; 1981; 2080; 2130; 2592; 2712; 2769; 2832; 3030; 3042; 3102; 3114; 3346; 3391; 3434; 3476; 3498; 3526; 3573; 3635; 3657; 3747; 3814; 3837; 3881; 3976; 3995; 4020; 4169; 4270; 4313; 4370; 4460; 4563; 4590; 4608; 4844; 4881; 5034; 5060; 5200; 5218; 5253; 5334; 5387                    |
| Lsp1109I    | GCAGCNNNNNNNN!               | 9     | 884; 1326; 1974; 2297; 3582; 3624; 4145; 4598; 5098  |
| Lsp1109I    | !NNNNNNNNNNNGCTGC            | 9     | 1003; 1577; 2101; 2791; 3456; 3640; 3733; 4799; 5005   |
| LweI        | GCATCNNNNN!                  | 10    | 911; 1472; 1643; 1808; 2325; 3088; 3160; 3808; 3872; 4147  |
| LweI        | !NNNNNNNNNGATGC              | 7     | 357; 1064; 1232; 3467; 3722; 3938; 5330  |
| MabI        | A!CCWGGT                     | 1     | 3098   |
| MaeI        | C!TAG                        | 10    | 592; 857; 1352; 2164; 2358; 2776; 3332; 4347; 4681; 4934   |
| MaeII       | A!CGT                        | 19    | 122; 134; 175; 258; 339; 444; 1031; 1307; 1496; 1880; 1988; 2569; 2581; 2624; 2734; 3625; 3812; 4593; 4726   |
| MaeIII      | !GTNAC                       | 14    | 61; 148; 497; 954; 1614; 2297; 2798; 2810; 3629; 3935; 4625; 4891; 5007; 5070  |
| MalI        | GA!TC                        | 27    | 587; 611; 662; 1039; 1189; 1215; 1480; 1564; 2017; 2053; 2168; 3348; 3352; 3371; 3682; 3760; 3841; 3850; 3928; 4304; 4320; 4679; 4691; 4769; 4777; 4788; 4863  |
| MaqI        | CRTTGACNNNNNNNNNNNNNNNNNNNN! | 4     | 144; 197; 466; 540   |
| MaqI        | !NNNNNNNNNNNNNNNNNNNGTCAAYG  | 3     | 157; 240; 426  |
| MauBI       | CG!CGCGCG                    | 0     |  |
| MbiI        | CCG!CTC                      | 2     | 2933; 4122   |
| MbiI        | GAG!CGG                      | 3     | 1439; 2769; 4176   |
| MboI        | !GATC                        | 27    | 585; 609; 660; 1037; 1187; 1213; 1478; 1562; 2015; 2051; 2166; 3346; 3350; 3369; 3680; 3758; 3839; 3848; 3926; 4302; 4318; 4677; 4689; 4767; 4775; 4786; 4861  |
| MboII       | GAAGANNNNNNNN!               | 8     | 979; 1092; 1680; 2765; 2993; 3870; 4080; 4699  |
| MboII       | !NNNNNNNTCTTC                | 6     | 906; 1674; 4160; 4327; 4527; 4770  |
| McaTI       | GCGC!GC                      | 0     |  |
| MfeI        | C!AATTG                      | 1     | 2259   |
| MfII        | R!GATCY                      | 11    | 585; 609; 660; 1213; 3680; 3926; 4318; 4677; 4689; 4775; 4786  |
| MhII        | GDGCH!C                      | 21    | 620; 657; 730; 757; 790; 1367; 1472; 1502; 1601; 1664; 1994; 2041; 2093; 2700; 3455; 3548; 3622; 3812; 3874; 4584; 5117  |
| MisI        | TGG!CCA                      | 2     | 1333; 3591   |
| MluI        | A!CGCGT                      | 0     |  |
| MluNI       | TGG!CCA                      | 2     | 1333; 3591   |
| MlyI        | GAGTCNNNNN!                  | 4     | 962; 2107; 2561; 2993  |
| MlyI        | !NNNNNGACTC                  | 6     | 404; 598; 2154; 2568; 4174; 5051   |
| Mly113I     | GG!CGCC                      | 1     | 3509   |
| MmeI        | TCCRACNNNNNNNNNNNNNNNNNNNN!  | 2     | 1085; 2602   |
| MmeI        | !NNNNNNNNNNNNNNNNNNNGTYGGA   | 2     | 5034; 5218   |
| MnII        | CCTCNNNNNNN!                 | 14    | 836; 874; 1015; 1030; 1246; 1357; 1633; 2228; 3292; 4110; 4316; 4630; 5000; 5324   |
| MnII        | !NNNNNGAGG                   | 31    | 541; 717; 756; 777; 810; 927; 1036; 1092; 1281; 1314; 1440; 1521; 1695; 1758; 1770; 1821; 1911; 1941; 2064; 2188; 2653; 2984; 3262; 3305; 3320; 3360; 3424; 3560; 3917; 4347; 5250   |
| Mph1103I    | ATGCA!T                      | 3     | 3081; 3153; 5484   |
| MreI        | CG!CCGGCG                    | 0     |  |
| MroI        | T!CCGGA                      | 0     |  |
| MroNI       | G!CCGGC                      | 4     | 1459; 2726; 4009; 4292   |
| MroXI       | GAANN!NNTTC                  | 1     | 1678   |
| MscI        | TGG!CCA                      | 2     | 1333; 3591   |
| MseI        | T!TAA                        | 16    | 7; 692; 2210; 2271; 2392; 2413; 2424; 2436; 2447; 2464; 2562; 2833; 4652; 4666; 4671; 4723   |
| MsiI        | CAYNN!NNRTG                  | 6     | 365; 706; 1612; 1669; 1789; 3946   |

| Enzyme name | Sequence         | Count | Cutting Position (the base to the left of the cut site)   |
|-------------|------------------|-------|---|
| MspI        | C!CGG            | 21    | 601; 657; 667; 1460; 1583; 2727; 3414; 3491; 3513; 3541; 3672; 3762; 3829; 4010; 4285; 4293; 4385; 4859; 5049; 5075; 5222   |
| Msp20I      | TGG!CCA          | 2     | 1333; 3591  |
| MspA1I      | CMG!CKG          | 8     | 651; 1126; 1760; 1965; 3007; 3615; 4844; 5089   |
| MspCI       | C!TTAAG          | 1     | 2391  |
| MspJI       | CNNRRNNNNNNNN!   | 283   | 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; 613; 627; 640; 662; 679; 696; 714; 732; 762; 780; 795; 810; 828; 861; 888; 904; 919; 943; 956; 972; 990; 1004; 1020; 1033; 1047; 1068; 1081; 1102; 1121; 1135; 1149; 1167; 1180; 1198; 1212; 1229; 1249; 1281; 1296; 1311; 1330; 1346; 1363; 1378; 1400; 1423; 1439; 1452; 1472; 1485; 1498; 1513; 1530; 1545; 1566; 1595; 1608; 1623; 1650; 1669; 1698; 1711; 1725; 1740; 1758; 1771; 1788; 1801; 1815; 1836; 1854; 1867; 1888; 1906; 1926; 1941; 1959; 1978; 1992; 2007; 2020; 2046; 2060; 2077; 2095; 2123; 2137; 2157; 2176; 2192; 2216; 2233; 2246; 2287; 2301; 2314; 2332; 2370; 2385; 2403; 2444; 2468; 2488; 2506; 2527; 2550; 2582; 2597; 2619; 2637; 2650; 2671; 2688; 2701; 2714; 2730; 2743; 2773; 2788; 2813; 2831; 2844; 2859; 2883; 2897; 2921; 2946; 2962; 3000; 3016; 3055; 3069; 3083; 3111; 3127; 3141; 3155; 3184; 3197; 3218; 3227; 3249; 3279; 3295; 3313; 3343; 3366; 3390; 3414; 3433; 3461; 3487; 3503; 3516; 3539; 3553; 3573; 3586; 3600; 3619; 3633; 3646; 3662; 3677; 3690; 3706; 3721; 3737; 3762; 3781; 3797; 3810; 3823; 3841; 3855; 3869; 3885; 3907; 3923; 3942; 3964; 3980; 4003; 4022; 4036; 4052; 4066; 4086; 4102; 4115; 4137; 4170; 4184; 4204; 4217; 4231; 4285; 4298; 4321; 4334; 4353; 4370; 4397; 4433; 4466; 4485; 4510; 4531; 4558; 4571; 4588; 4602; 4617; 4633; 4693; 4718; 4734; 4756; 4769; 4783; 4814; 4828; 4853; 4871; 4886; 4899; 4918; 4931; 4946; 4975; 4994; 5012; 5037; 5052; 5071; 5087; 5106; 5124; 5140; 5154; 5173; 5200; 5223; 5245; 5264; 5279; 5292; 5309; 5329; 5356; 5369; 5388; 5413; 5436; 5450; 5463; 5480 |
| MspJI       | !NNNNNNNNNNNNYNG | 235   | 8; 35; 59; 76; 97; 123; 145; 168; 197; 221; 269; 281; 310; 330; 347; 377; 400; 437; 465; 492; 511; 531; 553; 574; 593; 612; 636; 653; 670; 688; 706; 736; 760; 778; 802; 821; 843; 862; 883; 904; 930; 950; 979; 1021; 1055; 1076; 1096; 1123; 1141; 1158; 1186; 1204; 1225; 1244; 1270; 1292; 1320; 1338; 1358; 1379; 1397; 1420; 1442; 1459; 1483; 1500; 1519; 1540; 1560; 1580; 1597; 1616; 1635; 1672; 1699; 1720; 1738; 1760; 1789; 1810; 1828; 1867; 1885; 1915; 1933; 1952; 1975; 1994; 2020; 2040; 2061; 2080; 2097; 2116; 2135; 2161; 2190; 2219; 2261; 2344; 2379; 2406; 2429; 2458; 2502; 2524; 2556; 2592; 2609; 2630; 2667; 2687; 2704; 2721; 2756; 2774; 2792; 2809; 2833; 2855; 2882; 2910; 2936; 2974; 2995; 3012; 3030; 3053; 3074; 3102; 3125; 3146; 3164; 3185; 3202; 3225; 3249; 3269; 3287; 3309; 3340; 3362; 3388; 3407; 3424; 3452; 3471; 3490; 3508; 3526; 3547; 3569; 3593; 3626; 3646; 3664; 3685; 3711; 3738; 3755; 3772; 3789; 3815; 3837; 3859; 3877; 3897; 3916; 3938; 3976; 3996; 4020; 4040; 4060; 4087; 4111; 4144; 4163; 4191; 4213; 4242; 4270; 4295; 4314; 4333; 4354; 4371; 4388; 4419; 4446; 4466; 4489; 4509; 4546; 4563; 4582; 4608; 4628; 4667; 4694; 4713; 4731; 4768; 4786; 4827; 4845; 4871; 4888; 4920; 4949; 4977; 4998; 5021; 5040; 5061; 5079; 5096; 5114; 5140; 5161; 5181; 5201; 5219; 5240; 5266; 5283; 5302; 5321; 5350; 5369; 5387; 5406; 5424; 5442; 5465   |
| MspR9I      | CC!NGG           | 32    | 90; 283; 657; 658; 850; 898; 994; 1009; 1138; 1247; 1602; 1612; 1714; 1735; 1789; 1843; 1915; 1996; 2095; 3045; 3100; 3117; 3513; 3673; 3896; 4286; 4475; 4578; 5050; 5268; 5281; 5402  |
| MssI        | GTTT!AAC         | 0     |   |
| MunI        | C!AATTG          | 1     | 2259  |
| MvaI        | CC!WGG           | 26    | 90; 283; 850; 898; 994; 1009; 1138; 1247; 1602; 1612; 1714; 1735; 1789; 1843; 1915; 1996; 2095; 3045; 3100; 3117; 3896; 4475; 4578; 5268; 5281; 5402  |
| Mva1269I    | GAATGCN!         | 1     | 2260  |
| Mva1269I    | G!CATTC          | 1     | 2353  |
| MvnI        | CG!CG            | 19    | 60; 651; 1523; 1760; 2078; 2158; 2433; 2809; 2829; 2853; 2884; 3575; 3876; 4314; 4398; 4461; 4530; 4803; 5384   |
| MvrI        | CGAT!CG          | 0     |   |

| Enzyme name | Sequence                  | Count | Cutting Position (the base to the left of the cut site)   |
|-------------|---------------------------|-------|---|
| MwoI        | GCNNNNN!NNGC              | 35    | 90; 212; 244; 283; 376; 400; 711; 1142; 1482; 1523; 1583; 1596; 1640; 1871; 2063; 2114; 2736; 2766; 2793; 2835; 3076; 3148; 3199; 3278; 3500; 3584; 3607; 3746; 3869; 3952; 4219; 4406; 4610; 4809; 5381          |
| NaeI        | GCC!GGC                   | 4     | 1461; 2728; 4011; 4294  |
| NarI        | GG!CGCC                   | 1     | 3509  |
| NciI        | CC!SGG                    | 6     | 657; 658; 3513; 3673; 4286; 5050  |
| NcoI        | C!CATGG                   | 3     | 360; 3238; 3941   |
| NdeI        | CA!TATG                   | 1     | 234   |
| NdeII       | !GATC                     | 27    | 585; 609; 660; 1037; 1187; 1213; 1478; 1562; 2015; 2051; 2166; 3346; 3350; 3369; 3680; 3758; 3839; 3848; 3926; 4302; 4318; 4677; 4689; 4767; 4775; 4786; 4861   |
| NgoAVIII    | GACNNNNNTGANNNNNNNNNNNN!  | 1     | 280   |
| NgoAVIII    | !NNNNNNNNNNNTCANNNNNGTC   | 1     | 794   |
| NgoAVIII    | TCANNNNNGTCNNNNNNNNNNNN!  | 1     | 830   |
| NgoAVIII    | !NNNNNNNNNNNGACNNNNNTGA   | 1     | 244   |
| NgoMIV      | G!CCGGC                   | 4     | 1459; 2726; 4009; 4292  |
| NheI        | G!CTAGC                   | 1     | 591   |
| NlaII       | CATG!                     | 27    | 304; 364; 681; 705; 711; 723; 801; 947; 1158; 1437; 1668; 1698; 2088; 2133; 2939; 3079; 3151; 3242; 3383; 3728; 3914; 3945; 3971; 4327; 4711; 5431; 5482  |
| NlaIV       | GGN!NCC                   | 22    | 467; 647; 655; 662; 729; 789; 1180; 1431; 2007; 2664; 2685; 2697; 2888; 3049; 3121; 3510; 3545; 4393; 4472; 4517; 5360; 5399  |
| NlaCI       | CATCACNNNNNNNNNNNNNNNN!   | 4     | 766; 2341; 2656; 4248   |
| NlaCI       | !NNNNNNNNNNNNNNNNNGTGATG  | 3     | 347; 1054; 5320   |
| Nli3877I    | CYCGR!G                   | 4     | 617; 660; 1661; 1703  |
| NmeAIII     | GCCGAGNNNNNNNNNNNNNNNN!   | 2     | 3298; 3734  |
| NmeAIII     | !NNNNNNNNNNNNNNNNNNCTCGGC | 2     | 1070; 3263  |
| NmeDI       | RCCGGYNNNNNN!             | 7     | 678; 1471; 1594; 2738; 3840; 4021; 4304   |
| NmeDI       | !NNNNNNNNNNNNRCCGGY       | 7     | 653; 1446; 1569; 2713; 3815; 3996; 4279   |
| NmuCI       | !GTSAC                    | 5     | 954; 1614; 2798; 3629; 3935   |
| NotI        | GC!GGCCGC                 | 1     | 2153  |
| NruI        | TCG!CGA                   | 0     |   |
| NsbI        | TGC!GCA                   | 1     | 3611  |
| NsiI        | ATGCA!T                   | 3     | 3081; 3153; 5484  |
| NspI        | RCATG!Y                   | 5     | 705; 3079; 3151; 3914; 5431   |
| NspV        | TT!CGAA                   | 2     | 627; 4191   |
| OliI        | CACNN!NNGTG               | 2     | 1612; 1789  |
| PabI        | GTA!C                     | 15    | 220; 245; 300; 333; 384; 541; 648; 719; 1121; 1432; 1751; 1862; 1886; 2144; 3816  |
| PacI        | TTAAT!TAA                 | 0     |   |
| PaeI        | GCATG!C                   | 3     | 3079; 3151; 3914  |
| PaeR7I      | C!TCGAG                   | 1     | 613   |
| PagI        | T!CATGA                   | 2     | 2935; 4707  |
| PaIAI       | GG!CGCGCC                 | 0     |   |
| PasI        | CC!CWGGG                  | 4     | 897; 1995; 4474; 4577   |
| PauI        | G!CGCGC                   | 0     |   |
| PceI        | AGG!CCT                   | 2     | 1102; 3330  |
| PciI        | A!CATGT                   | 1     | 5427  |
| PciSI       | GCTCTTCN!                 | 0     |   |
| PciSI       | !NNNNGAAGAGC              | 2     | 3853; 4063  |
| PcsI        | WCGNNNN!NNNCGW            | 10    | 1304; 1463; 2075; 2132; 3803; 3880; 4123; 4197; 4590; 5108  |
| PctI        | GAATGCN!                  | 1     | 2260  |
| PctI        | G!CATTC                   | 1     | 2353  |
| PdiI        | GCC!GGC                   | 4     | 1461; 2728; 4011; 4294  |
| PdmI        | GAANN!NNTTC               | 1     | 1678  |
| PfeI        | G!AWTC                    | 7     | 803; 1280; 3994; 4128; 4238; 4275; 5453   |
| Pfl23II     | C!GTACG                   | 0     |   |
| PfIFI       | GACN!NNGTC                | 1     | 3627  |
| PfIMI       | CCANNNN!NTGG              | 2     | 1854; 2130  |
| PfoI        | T!CCNGGA                  | 5     | 1007; 1712; 1733; 2093; 4284  |
| PhoI        | GG!CC                     | 36    | 84; 277; 655; 824; 1067; 1102; 1136; 1159; 1291; 1333; 1505; 1605; 1847; 1903; 1913; 1969; 2006; 2074; 2155; 2475; 2617; 3272; 3278; 3287; 3330; 3417; 3591; 3982; 4009; 4518; 4575; 4608; 4953; 5387; 5405; 5416 |

| Enzyme name | Sequence                      | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|-------------------------------|-------|--|
| PinAI       | A!CCGGT                       | 1     | 666  |
| PlaDI       | CATCAGNNNNNNNNNNNNNNNNNNNNNN! | 1     | 3890   |
| PlaDI       | !NNNNNNNNNNNNNNNNNNNNCTGATG   | 3     | 1374; 3455; 3710   |
| PleI        | GAGTCNNNN!                    | 4     | 961; 2106; 2560; 2992  |
| PleI        | !NNNNNGACTC                   | 6     | 404; 598; 2154; 2568; 4174; 5051   |
| Ple19I      | CGAT!CG                       | 0     |  |
| PmaCI       | CAC!GTG                       | 0     |  |
| PmeI        | GTTT!AAAC                     | 0     |  |
| PmlI        | CAC!GTG                       | 0     |  |
| PpiI        | GAACNNNNNCTCNNNNNNNNNNNNNN!   | 1     | 2591   |
| PpiI        | !NNNNNNNNNGAGNNNNNGTTC        | 4     | 1438; 1552; 1756; 4721   |
| PpiI        | GAGNNNNNGTTCNNNNNNNNNNNNNN!   | 4     | 1470; 1584; 1788; 4753   |
| PpiI        | !NNNNNNNGAACNNNNNNCTC         | 1     | 2559   |
| PpsI        | GAGTCNNNN!                    | 4     | 961; 2106; 2560; 2992  |
| PpsI        | !NNNNNGACTC                   | 6     | 404; 598; 2154; 2568; 4174; 5051   |
| Ppu10I      | A!TGCAT                       | 3     | 3077; 3149; 5480   |
| Ppu21I      | YAC!GTR                       | 3     | 340; 2625; 3813  |
| PpuMI       | RG!GWCCY                      | 0     |  |
| PscI        | A!CATGT                       | 1     | 5427   |
| PshAI       | GACNN!NNGTC                   | 1     | 1306   |
| PshBI       | AT!TAAT                       | 1     | 7  |
| PsiI        | TTA!TAA                       | 2     | 2292; 2497   |
| Psp03I      | GGWC!C                        | 3     | 2064; 4028; 4473   |
| Psp5II      | RG!GWCCY                      | 0     |  |
| Psp6I       | !CCWGG                        | 26    | 88; 281; 848; 896; 992; 1007; 1136; 1245; 1600; 1610; 1712; 1733; 1787; 1841; 1913; 1994; 2093; 3043; 3098; 3115; 3894; 4473; 4576; 5266; 5279; 5400 |
| Psp1406I    | AA!CGTT                       | 0     |  |
| Psp124BI    | GAGCT!C                       | 1     | 620  |
| PspCI       | CAC!GTG                       | 0     |  |
| PspEI       | G!GTNACC                      | 1     | 1614   |
| PspGI       | !CCWGG                        | 26    | 88; 281; 848; 896; 992; 1007; 1136; 1245; 1600; 1610; 1712; 1733; 1787; 1841; 1913; 1994; 2093; 3043; 3098; 3115; 3894; 4473; 4576; 5266; 5279; 5400 |
| PspLI       | C!GTACG                       | 0     |  |
| PspN4I      | GGN!NCC                       | 22    | 467; 647; 655; 662; 729; 789; 1180; 1431; 2007; 2664; 2685; 2697; 2888; 3049; 3121; 3510; 3545; 4393; 4472; 4517; 5360; 5399                         |
| PspOMI      | G!GGCCC                       | 1     | 653  |
| PspOMII     | CGCCARNNNNNNNNNNNNNNNNNNNNN!  | 3     | 125; 3239; 4238  |
| PspOMII     | !NNNNNNNNNNNNNNNNNNNYTGGGCG   | 2     | 1977; 3641   |
| PspPI       | G!GNCC                        | 17    | 83; 276; 654; 823; 1066; 1158; 1604; 1845; 2005; 2061; 2073; 2616; 4025; 4470; 4516; 4574; 4607  |
| PspPPI      | RG!GWCCY                      | 0     |  |
| PspPRI      | CCYAGNNNNNNNNNNNNNNNNNN!      | 3     | 3062; 3134; 4640   |
| PspPRI      | !NNNNNNNNNNNNNNNNCTGRGG       | 4     | 918; 1346; 2975; 4170  |
| PspXI       | VC!TCGAGB                     | 0     |  |
| PsrI        | GAACNNNNNTACNNNNNNNNNNNNNN!   | 0     |  |
| PsrI        | !NNNNNNNGTANNNNNNGTTC         | 0     |  |
| PsrI        | GTANNNNNNGTTCNNNNNNNNNNNNNN!  | 0     |  |
| PsrI        | !NNNNNNNGAACNNNNNNNTAC        | 0     |  |
| PssI        | RGGNC!CY                      | 1     | 4610   |
| PstI        | CTGCA!G                       | 1     | 638  |
| PstNI       | CAGNNN!CTG                    | 4     | 1340; 2111; 4610; 5018   |
| PsuI        | R!GATCY                       | 11    | 585; 609; 660; 1213; 3680; 3926; 4318; 4677; 4689; 4775; 4786  |
| PsyI        | GACN!NNGTC                    | 1     | 3627   |
| PteI        | G!CGCGC                       | 0     |  |
| PvuI        | CGAT!CG                       | 0     |  |
| PvuII       | CAG!CTG                       | 3     | 1965; 3007; 3615   |
| RcaI        | T!CATGA                       | 2     | 2935; 4707   |
| RceI        | CATCGACNNNNNNNNNNNNNNNNNNNN!  | 1     | 4024   |
| RceI        | !NNNNNNNNNNNNNNNNNGTCGATG     | 0     |  |
| RgaI        | GCGAT!CGC                     | 0     |  |
| RigI        | GGCCGG!CC                     | 0     |  |
| RleAI       | CCCACANNNNNNNNNNNNNN!         | 1     | 2238   |
| RleAI       | !NNNNNNNNNTGTGGG              | 0     |  |

| Enzyme name | Sequence                       | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|--------------------------------|-------|--|
| RpaBI       | CCCGCAGNNNNNNNNNNNNNNNNNNNNNN! | 0     |  |
| RpaBI       | !NNNNNNNNNNNNNNNNNNNNCTGCGGG   | 0     |  |
| RpaB5I      | CGRGGACNNNNNNNNNNNNNNNNNNNNNN! | 0     |  |
| RpaB5I      | !NNNNNNNNNNNNNNNNNNNNGTCCYCG   | 0     |  |
| RruI        | TCG!CGA                        | 0     |  |
| RsaI        | GT!AC                          | 15    | 219; 244; 299; 332; 383; 540; 647; 718; 1120; 1431; 1750; 1861; 1885; 2143; 3815   |
| RsaNI       | G!TAC                          | 15    | 218; 243; 298; 331; 382; 539; 646; 717; 1119; 1430; 1749; 1860; 1884; 2142; 3814   |
| RseI        | CAYNN!NNRTG                    | 6     | 365; 706; 1612; 1669; 1789; 3946   |
| RsrII       | CG!GWCCG                       | 1     | 4025   |
| Rsr2I       | CG!GWCCG                       | 1     | 4025   |
| SacI        | GAGCT!C                        | 1     | 620  |
| SacII       | CCGC!GG                        | 2     | 652; 1761  |
| SaI         | G!TCGAC                        | 1     | 639  |
| SapI        | GCTCTCN!                       | 0     |  |
| SapI        | !NNNNGAAGAGC                   | 2     | 3853; 4063   |
| SaqAI       | T!TAA                          | 16    | 7; 692; 2210; 2271; 2392; 2413; 2424; 2436; 2447; 2464; 2562; 2833; 4652; 4666; 4671; 4723   |
| SatI        | GC!NGC                         | 42    | 822; 873; 1017; 1134; 1315; 1524; 1591; 1761; 1953; 1956; 1963; 2115; 2153; 2156; 2286; 2805; 2827; 2841; 3279; 3418; 3470; 3481; 3571; 3576; 3613; 3654; 3741; 3744; 3747; 3983; 4079; 4120; 4134; 4248; 4587; 4602; 4813; 5019; 5022; 5087; 5230; 5385   |
| Sau96I      | G!GNCC                         | 17    | 83; 276; 654; 823; 1066; 1158; 1604; 1845; 2005; 2061; 2073; 2616; 4025; 4470; 4516; 4574; 4607  |
| Sau3AI      | !GATC                          | 27    | 585; 609; 660; 1037; 1187; 1213; 1478; 1562; 2015; 2051; 2166; 3346; 3350; 3369; 3680; 3758; 3839; 3848; 3926; 4302; 4318; 4677; 4689; 4767; 4775; 4786; 4861  |
| SbfI        | CCTGCA!GG                      | 0     |  |
| ScaI        | AGT!ACT                        | 0     |  |
| SchI        | GAGTCNNNNN!                    | 4     | 962; 2107; 2561; 2993  |
| SchI        | !NNNNNGACTC                    | 6     | 404; 598; 2154; 2568; 4174; 5051   |
| SciI        | CTC!GAG                        | 1     | 615  |
| ScrFI       | CC!NGG                         | 32    | 90; 283; 657; 658; 850; 898; 994; 1009; 1138; 1247; 1602; 1612; 1714; 1735; 1789; 1843; 1915; 1996; 2095; 3045; 3100; 3117; 3513; 3673; 3896; 4286; 4475; 4578; 5050; 5268; 5281; 5402   |
| SdaI        | CCTGCA!GG                      | 0     |  |
| SdeAI       | CAGRAGNNNNNNNNNNNNNNNNNNNNNN!  | 6     | 1104; 1740; 3087; 3159; 3328; 5270   |
| SdeAI       | !NNNNNNNNNNNNNNNNNNNNCTYCTG    | 3     | 2072; 3664; 4149   |
| SdeOSI      | GACNNNNRTGANNNNNNNNNNNNN!      | 1     | 279  |
| SdeOSI      | !NNNNNNNNNNNTCAYNNNNNGTC       | 0     |  |
| SdeOSI      | TCAYNNNNNGTCNNNNNNNNNNNN!      | 0     |  |
| SdeOSI      | !NNNNNNNNNNNGACNNNNRTGA        | 1     | 245  |
| SduI        | GDGCH!C                        | 21    | 620; 657; 730; 757; 790; 1367; 1472; 1502; 1601; 1664; 1994; 2041; 2093; 2700; 3455; 3548; 3622; 3812; 3874; 4584; 5117  |
| SelI        | !CGCG                          | 19    | 58; 649; 1521; 1758; 2076; 2156; 2431; 2807; 2827; 2851; 2882; 3573; 3874; 4312; 4396; 4459; 4528; 4801; 5382  |
| SetI        | ASST!                          | 80    | 125; 137; 178; 261; 308; 342; 447; 552; 568; 620; 626; 689; 716; 812; 862; 883; 1034; 1169; 1238; 1306; 1310; 1325; 1349; 1373; 1412; 1454; 1487; 1499; 1559; 1592; 1769; 1808; 1856; 1867; 1883; 1967; 1991; 2036; 2141; 2149; 2199; 2220; 2229; 2241; 2290; 2457; 2572; 2584; 2627; 2664; 2714; 2737; 2861; 3009; 3104; 3297; 3410; 3538; 3617; 3628; 3700; 3771; 3815; 4075; 4221; 4265; 4596; 4627; 4686; 4729; 4872; 4893; 4992; 5129; 5144; 5163; 5175; 5219; 5265; 5316 |
| SexAI       | A!CCWGGT                       | 1     | 3098   |
| SfaAI       | GCGAT!CGC                      | 0     |  |
| SfaNI       | GCATCNNNNN!                    | 10    | 911; 1472; 1643; 1808; 2325; 3088; 3160; 3808; 3872; 4147  |
| SfaNI       | !NNNNNNNNNGATGC                | 7     | 357; 1064; 1232; 3467; 3722; 3938; 5330  |
| Sfcl        | C!TRYAG                        | 5     | 634; 1266; 2844; 4971; 5162  |
| SfiI        | GGCCNNNN!NGGCC                 | 1     | 3284   |
| SfoI        | GGC!GCC                        | 1     | 3510   |
| Sfr274I     | C!TCGAG                        | 1     | 613  |
| Sfr303I     | CCGC!GG                        | 2     | 652; 1761  |
| SfuI        | TT!CGAA                        | 2     | 627; 4191  |

| Enzyme name | Sequence         | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|------------------|-------|--|
| SgeI        | CNNGNNNNNNNNNN!  | 232   | 6; 36; 71; 97; 123; 223; 236; 252; 290; 307; 336; 373; 407; 426; 491; 548; 579; 604; 625; 662; 679; 696; 714; 732; 762; 786; 804; 819; 861; 888; 909; 930; 943; 956; 984; 1005; 1020; 1047; 1081; 1102; 1122; 1135; 1149; 1167; 1180; 1212; 1230; 1251; 1285; 1311; 1330; 1346; 1364; 1380; 1400; 1423; 1439; 1452; 1472; 1485; 1498; 1513; 1530; 1545; 1566; 1595; 1608; 1623; 1650; 1669; 1698; 1711; 1725; 1740; 1758; 1771; 1788; 1801; 1815; 1836; 1854; 1867; 1893; 1906; 1926; 1941; 1959; 1978; 1992; 2007; 2020; 2046; 2061; 2082; 2097; 2123; 2137; 2157; 2176; 2216; 2245; 2287; 2370; 2444; 2528; 2550; 2582; 2619; 2637; 2656; 2671; 2713; 2730; 2747; 2782; 2796; 2813; 2835; 2859; 2883; 2948; 2962; 3000; 3016; 3056; 3069; 3088; 3111; 3128; 3141; 3160; 3190; 3211; 3228; 3251; 3286; 3313; 3344; 3366; 3392; 3414; 3433; 3461; 3497; 3516; 3539; 3553; 3573; 3586; 3600; 3619; 3633; 3652; 3684; 3698; 3711; 3737; 3768; 3781; 3798; 3815; 3829; 3846; 3864; 3879; 3907; 3923; 3942; 3964; 3980; 4003; 4022; 4047; 4066; 4086; 4113; 4137; 4170; 4189; 4217; 4231; 4297; 4321; 4336; 4359; 4380; 4397; 4445; 4472; 4486; 4515; 4539; 4572; 4589; 4609; 4622; 4635; 4693; 4720; 4739; 4769; 4794; 4814; 4828; 4853; 4871; 4908; 4946; 4975; 5003; 5017; 5037; 5052; 5073; 5087; 5106; 5124; 5140; 5180; 5200; 5227; 5245; 5266; 5279; 5292; 5309; 5333; 5356; 5388; 5413; 5440; 5462 |
| SgeI        | !NNNNNNNNNNNNCNG | 198   | 10; 45; 71; 97; 197; 226; 264; 281; 310; 347; 381; 400; 465; 522; 553; 578; 599; 636; 653; 670; 688; 706; 736; 760; 778; 802; 835; 862; 883; 904; 930; 958; 979; 1021; 1055; 1076; 1096; 1123; 1141; 1162; 1186; 1204; 1225; 1259; 1285; 1304; 1338; 1358; 1397; 1420; 1446; 1468; 1487; 1504; 1540; 1569; 1587; 1624; 1643; 1672; 1699; 1720; 1738; 1762; 1789; 1810; 1828; 1867; 1885; 1915; 1933; 1952; 1975; 1994; 2020; 2044; 2061; 2080; 2097; 2116; 2143; 2190; 2219; 2261; 2344; 2418; 2502; 2524; 2556; 2593; 2611; 2630; 2687; 2704; 2721; 2756; 2774; 2794; 2814; 2833; 2857; 2922; 2974; 3030; 3062; 3085; 3102; 3134; 3164; 3185; 3202; 3225; 3260; 3287; 3318; 3340; 3366; 3388; 3407; 3435; 3471; 3490; 3513; 3534; 3552; 3569; 3593; 3626; 3658; 3685; 3711; 3742; 3772; 3789; 3815; 3838; 3859; 3881; 3901; 3921; 3938; 3977; 3996; 4021; 4040; 4060; 4087; 4111; 4144; 4163; 4191; 4213; 4271; 4295; 4314; 4333; 4354; 4371; 4419; 4446; 4466; 4489; 4513; 4546; 4563; 4583; 4609; 4667; 4694; 4713; 4743; 4768; 4788; 4827; 4845; 4882; 4920; 4949; 4977; 4998; 5026; 5047; 5080; 5098; 5154; 5174; 5201; 5219; 5240; 5266; 5283; 5307; 5330; 5362; 5387; 5414; 5436; 5465  |
| SgfI        | GCGAT!CGC        | 0     |  |
| SgrAI       | CR!CCGGYG        | 0     |  |
| SgrBI       | CCGC!GG          | 2     | 652; 1761  |
| SgrDI       | CG!TCGACG        | 0     |  |
| SgsI        | GG!CGCGCC        | 0     |  |
| SimI        | GG!GTC           | 4     | 27; 2656; 4443; 5236   |
| SimI        | !GACCC           | 6     | 106; 1754; 1931; 3937; 4505; 4753  |
| SinI        | G!GWCC           | 3     | 2061; 4025; 4470   |
| SlaI        | C!TCGAG          | 1     | 613  |
| SmaI        | CCC!GGG          | 1     | 658  |
| SmiI        | ATTT!AAAT        | 0     |  |
| SmiMI       | CAYNN!NNRTG      | 6     | 365; 706; 1612; 1669; 1789; 3946   |
| SmlI        | C!TYRAG          | 8     | 613; 619; 1237; 2391; 3900; 4782; 5059; 5321   |
| SmoI        | C!TYRAG          | 8     | 613; 619; 1237; 2391; 3900; 4782; 5059; 5321   |
| SmuI        | CCCGCNNNN!       | 14    | 93; 119; 286; 1131; 1233; 1657; 1765; 1942; 2083; 2831; 3186; 3207; 4403; 4535   |
| SmuI        | !NNNNNNGCGGG     | 8     | 644; 1168; 1432; 2762; 3632; 4169; 4307; 4454  |
| SnaBI       | TAC!GTA          | 1     | 340  |
| SpeI        | A!CTAGT          | 0     |  |
| SphI        | GCATG!C          | 3     | 3079; 3151; 3914   |
| SrfI        | GCCC!GGGC        | 0     |  |
| Sse9I       | !AATT            | 12    | 18; 629; 1375; 2259; 2323; 2401; 2427; 2438; 3085; 3157; 3249; 4667  |
| Sse8387I    | CCTGCA!GG        | 0     |  |
| Sse8647I    | AG!GWCCT         | 0     |  |
| SseBI       | AGG!CCT          | 2     | 1102; 3330   |

| Enzyme name | Sequence                     | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|------------------------------|-------|--|
| SsiI        | C!CGC                        | 43    | 58; 86; 98; 112; 279; 507; 589; 649; 986; 1124; 1226; 1650; 1758; 1797; 1890; 1935; 2064; 2076; 2156; 2824; 2827; 2841; 2931; 3179; 3191; 3200; 3212; 3222; 3233; 3279; 3418; 3481; 3983; 4028; 4094; 4120; 4245; 4248; 4396; 4528; 4835; 4979; 5475 |
| SsiI        | G!CGG                        | 33    | 370; 403; 528; 651; 821; 1133; 1175; 1439; 1523; 1760; 1952; 1955; 2152; 2769; 2796; 2884; 2994; 3575; 3639; 3740; 3743; 4023; 4078; 4176; 4314; 4461; 4601; 4844; 5089; 5210; 5229; 5356; 5384  |
| SspI        | AAT!ATT                      | 2     | 2417; 2970   |
| SspDI       | G!GCGCC                      | 1     | 3508   |
| SspD5I      | GGTGANNNNNNNN!               | 7     | 376; 1059; 1626; 1779; 1803; 4580; 4696  |
| SspD5I      | !NNNNNNNNNTCACC              | 4     | 947; 1097; 2625; 3686  |
| SstI        | GAGCT!C                      | 1     | 620  |
| SstII       | CCG!GG                       | 2     | 652; 1761  |
| SstE37I     | CGAAGACNNNNNNNNNNNNNNNNNNNN! | 1     | 992  |
| SstE37I     | !NNNNNNNNNNNNNNNNNNNGTCTCG   | 0     |  |
| Sth132I     | CCCGNNNN!                    | 29    | 92; 118; 285; 663; 914; 1130; 1232; 1478; 1601; 1656; 1664; 1706; 1764; 1901; 1941; 2015; 2082; 2708; 2830; 3185; 3206; 3519; 3922; 4061; 4132; 4402; 4534; 4764; 5202   |
| Sth132I     | !NNNNNNNNCCGGG               | 22    | 15; 407; 470; 643; 963; 1167; 1431; 2709; 2761; 2862; 3589; 3631; 3664; 4168; 4277; 4306; 4453; 4557; 4588; 5041; 5085; 5293   |
| Sth302II    | CC!GG                        | 21    | 602; 658; 668; 1461; 1584; 2728; 3415; 3492; 3514; 3542; 3673; 3763; 3830; 4011; 4286; 4294; 4386; 4860; 5050; 5076; 5223  |
| StrI        | C!TCGAG                      | 1     | 613  |
| StsI        | GGATGNNNNNNNNNN!             | 4     | 3377; 3834; 3859; 4313   |
| StsI        | !NNNNNNNNNNNNNNNNCATCC       | 9     | 743; 831; 888; 1043; 1620; 1695; 1824; 1872; 3181  |
| StuI        | AGG!CCT                      | 2     | 1102; 3330   |
| StyI        | C!CWGGG                      | 5     | 360; 3238; 3331; 3941; 4346  |
| StyD4I      | !CCNGG                       | 32    | 88; 281; 655; 656; 848; 896; 992; 1007; 1136; 1245; 1600; 1610; 1712; 1733; 1787; 1841; 1913; 1994; 2093; 3043; 3098; 3115; 3511; 3671; 3894; 4284; 4473; 4576; 5048; 5266; 5279; 5400   |
| SwaI        | ATTT!AAAT                    | 0     |  |
| TaaI        | ACN!GT                       | 15    | 76; 198; 269; 543; 581; 645; 732; 2600; 3889; 4005; 4113; 4436; 4927; 5397; 5468   |
| TaiI        | ACGT!                        | 19    | 125; 137; 178; 261; 342; 447; 1034; 1310; 1499; 1883; 1991; 2572; 2584; 2627; 2737; 3628; 3815; 4596; 4729   |
| TaqI        | T!CGA                        | 21    | 614; 627; 640; 815; 842; 1310; 1481; 1775; 1802; 1946; 2658; 3349; 3622; 3778; 3802; 3838; 4000; 4191; 4236; 4491; 5329  |
| TaqII       | GACCGANNNNNNNNNN!            | 3     | 2529; 3546; 4214   |
| TaqII       | !NNNNNNNNNTCGGTC             | 1     | 4458   |
| TaqII       | CACCCANNNNNNNNNN!            | 3     | 805; 910; 2137   |
| TaqII       | !NNNNNNNNNTGGGTG             | 3     | 172; 3413; 4005  |
| TasI        | !AATT                        | 12    | 18; 629; 1375; 2259; 2323; 2401; 2427; 2438; 3085; 3157; 3249; 4667  |
| TatI        | W!GTACW                      | 9     | 217; 297; 330; 381; 716; 1748; 1859; 1883; 2141  |
| TauI        | GCSG!C                       | 23    | 824; 1136; 1526; 1763; 1955; 1958; 2155; 2158; 2829; 2843; 3281; 3420; 3483; 3578; 3743; 3746; 3985; 4081; 4122; 4250; 4604; 5232; 5387  |
| TfiI        | G!AWTC                       | 7     | 803; 1280; 3994; 4128; 4238; 4275; 5453  |
| ThiI        | C!TCGAG                      | 1     | 613  |
| TruII       | T!TAA                        | 16    | 7; 692; 2210; 2271; 2392; 2413; 2424; 2436; 2447; 2464; 2562; 2833; 4652; 4666; 4671; 4723   |
| Tru9I       | T!TAA                        | 16    | 7; 692; 2210; 2271; 2392; 2413; 2424; 2436; 2447; 2464; 2562; 2833; 4652; 4666; 4671; 4723   |
| TscAI       | CASTGNN!                     | 7     | 1334; 1645; 2355; 3638; 4748; 5019; 5032   |
| TseI        | G!CWGC                       | 19    | 872; 1016; 1314; 1590; 1962; 2114; 2285; 2804; 3469; 3570; 3612; 3653; 3746; 4133; 4586; 4812; 5018; 5021; 5086  |
| TsoI        | TARCCANNNNNNNNNN!            | 2     | 2481; 2828   |
| TsoI        | !NNNNNNNNNTGGYTA             | 3     | 841; 2286; 4038  |
| Tsp45I      | !GTSAC                       | 5     | 954; 1614; 2798; 3629; 3935  |
| Tsp509I     | !AATT                        | 12    | 18; 629; 1375; 2259; 2323; 2401; 2427; 2438; 3085; 3157; 3249; 4667  |
| TspDTI      | ATGAANNNNNNNNNN!             | 6     | 724; 1412; 1681; 2267; 3568; 4272  |
| TspDTI      | !NNNNNNNNNTTCAT              | 6     | 26; 873; 1560; 3986; 4442; 4649  |

| Enzyme name | Sequence               | Count | Cutting Position (the base to the left of the cut site)   |
|-------------|------------------------|-------|---|
| TspEI       | !AATT                  | 12    | 18; 629; 1375; 2259; 2323; 2401; 2427; 2438; 3085; 3157; 3249; 4667                             |
| TspGWI      | ACGGANNNNNNNNNNN!      | 1     | 4384  |
| TspGWI      | !NNNNNNNNNTCCGT        | 0     |   |
| TspMI       | C!CCGGG                | 1     | 656   |
| TspRI       | CASTGNN!               | 7     | 1334; 1645; 2355; 3638; 4748; 5019; 5032  |
| TstI        | CACNNNNNTCCNNNNNNNNNN! | 0     |   |
| TstI        | !NNNNNNNGGANNNNNGTG    | 0     |   |
| TstI        | GGANNNNNGTGNNNNNNNNNN! | 0     |   |
| TstI        | !NNNNNNNCCACNNNNNTCC   | 0     |   |
| Tth111I     | GACN!NNGTC             | 1     | 3627  |
| Tth111II    | CAARCANNNNNNNNNN!      | 3     | 934; 1209; 4836   |
| Tth111II    | !NNNNNNNNNTGYTTG       | 3     | 3944; 4804; 4843  |
| UnbI        | !GGNCC                 | 17    | 82; 275; 653; 822; 1065; 1157; 1603; 1844; 2004; 2060; 2072; 2615; 4024; 4469; 4515; 4573; 4606 |
| Van91I      | CCANNNN!NTGG           | 2     | 1854; 2130  |
| Vha464I     | C!TTAAG                | 1     | 2391  |
| VneI        | G!TGCAC                | 3     | 753; 1498; 5113   |
| VpaK11AI    | !GGWCC                 | 3     | 2060; 4024; 4469  |
| VpaK11BI    | G!GWCC                 | 3     | 2061; 4025; 4470  |
| VspI        | AT!TAAT                | 1     | 7   |
| XagI        | CCTNN!NNNAGG           | 0     |   |
| XapI        | R!AATTY                | 5     | 629; 1375; 2323; 2427; 2438   |
| XbaI        | T!CTAGA                | 1     | 2163  |
| XceI        | RCATG!Y                | 5     | 705; 3079; 3151; 3914; 5431   |
| XcmI        | CCANNNN!NNNTGG         | 2     | 1911; 1992  |
| XhoI        | C!TCGAG                | 1     | 613   |
| XhoII       | R!GATCY                | 11    | 585; 609; 660; 1213; 3680; 3926; 4318; 4677; 4689; 4775; 4786                                   |
| XmaI        | C!CCGGG                | 1     | 656   |
| XmaJI       | C!CTAGG                | 2     | 3331; 4346  |
| XmiI        | GT!MKAC                | 2     | 640; 1253   |
| XmnI        | GAANN!NNTTC            | 1     | 1678  |
| XspI        | C!TAG                  | 10    | 592; 857; 1352; 2164; 2358; 2776; 3332; 4347; 4681; 4934  |
| ZraI        | GAC!GTC                | 4     | 123; 176; 259; 445  |
| ZrmI        | AGT!ACT                | 0     |   |
| Zsp2I       | ATGCA!T                | 3     | 3081; 3153; 5484  |