

# Effect of site-specific modification on restriction endonucleases and DNA modification methyltransferases

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## ABSTRACT

**Restriction endonucleases have site-specific interactions with DNA that can often be inhibited by site-specific DNA methylation and other site-specific DNA modifications. However, such inhibition cannot generally be predicted. The empirically acquired data on these effects are tabulated for over 320 restriction endonucleases. In addition, a table of known site-specific DNA modification methyltransferases and their specificities is presented along with EMBL database accession numbers for cloned genes.**

We present in Table I an updated list of the sensitivities of over 320 restriction endonucleases to site-specific modification at 4-methylcytosine ( $m^4C$ ), 5-methylcytosine ( $m^5C$ ), 5-hydroxymethylcytosine ( $hm^5C$ ), and 6-methyladenine ( $m^6A$ ), four modifications that are common in the DNA of prokaryotes, eukaryotes, and their viruses (Mc2, Mc5, Mc8, Mc11, Ne3, Ne4, Mc14, Ne14).

Knowledge of the sensitivity of restriction endonucleases to site-specific modification can be used to study cellular DNA methylation. Several restriction-modification enzymes share the same recognition sequence specificity, but differ in their sensitivities to site-specific methylation. Table II lists 33 known isoschizomer pairs and one isomethylator pair, along with the modified recognition sites at which they differ. Table III lists over 240 characterized DNA methyltransferases. A detailed list of cloned restriction-modification genes can be found in Wilson (Wi4).

The data presented here and two other tables are available in printed form or as a text file on a 3.5' Macintosh diskette. The extra tables include Table IV which lists the sensitivities of 24 Type II DNA methyltransferases to  $m^4C$ ,  $m^5C$ ,  $hm^5C$ , and  $m^6A$  modification. Most methyltransferases are sensitive to non-canonical modifications within their recognition sequences (Bu9, Mc10, Ne3, Po4), and this sensitivity often differs from that of their restriction endonuclease partners. Table V gives a list of restriction systems in this review alphabetized by recognition sequence.

## MOLECULAR BASIS FOR SENSITIVITY RESTRICTION ENZYMES TO METHYLATION

$m^4C$ ,  $m^5C$ ,  $hm^5C$ ,  $hm^5U$  and  $m^6A$  are bulky alkyl substitutions in the major groove of DNA. Site-specific DNA methylation can interfere with many sequence-specific DNA binding proteins (e.g. St2, Wa8), including restriction endonucleases and DNA methyltransferases. At the molecular level, the inability of restriction enzymes to cut modified DNA can be explained using *EcoRI* and *EcoRV* endonucleases as instructive models. DNA modification can interfere with substrate binding and/or conformational changes of the enzyme: substrate complex.

Based on the *EcoRI*: DNA co-crystal structure (Mc15, Ro8), methylation of either adenine ( $G^{m6}AATT$  or  $GA^{m6}ATT$ ) perturbs essential hydrogen bond contacts to Glu-144 and Arg-145. Therefore aminomethylation of either adenine inhibits DNA cleavage at the level of *EcoRI*: substrate binding (Br2). In contrast, cytosine ring methylation at  $GAATT^{m5}C$  would not be expected to interfere with critical DNA: protein contacts inferred from the X-ray crystal structure (Mc15). Therefore, the reduced rate of *EcoRI* cleavage at  $GAATT^{m5}C$  can be attributed to steric distortions of the enzyme:substrate complex during catalysis (He3).

In contrast, the X-ray structure of *EcoRV* endonuclease (Wi5) predicts that hydrogen bonding of Asn-185 to the first adenine of GATATC is perturbed by 'canonical' methylation at the  $G^{m6}ATATC$ . However, it is thought that *EcoRV* cannot cleave canonically modified  $G^{m6}ATATC$  sites because non-productive enzyme: substrate complexes are formed (Ta4, Ne12). Therefore the mechanism by which canonical DNA methylation inhibits cleavage is very different for *EcoRI* and *EcoRV* endonucleases.

Although DNA modification often results in complete inhibition of restriction enzyme cleavage, a range of rate effects are observed when *non-canonically* modified DNA is used as a substrate, as listed in the footnotes to Table I. Rate effects at  $m^5C$ -hemimethylated restriction endonuclease target sites are listed in Ne10.

## RATE OF CLEAVAGE AT METHYLATED RESTRICTION SITES

A range of rate effects are observed when modified substrates are used in endonuclease cleavage reactions. However, in general, results can be summarized as follows.

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(1) Canonical site-specific methylation *always* inhibits DNA cleavage by a restriction endonuclease. For example, M·*Bam*HI methylase modifies GGAT<sup>m4</sup>CC; and *Bam*HI endonuclease cannot cut this methylated sequence.

(2) In about one half of the cases tested, methylation at non-canonical sites inhibits the rate of duplex DNA cleavage at least ten-fold (Table I). However, in other cases non-canonical methylation has no effect on restriction cleavage. For example, *Bam*HI cuts DNA which has been modified at GGATC<sup>m4</sup>C or GGATC<sup>m5</sup>C, but cannot cut DNA methylated at GGAT<sup>m5</sup>CC.

(3) There are a few examples in which non-canonical methylation slows the rate of cleavage or permits nicking of one strand of a hemimethylated duplex. Examples of such effects are presented in footnotes to Table I. Such nicking has proved useful in site-directed mutagenesis (US Biochemicals Inc.).

(4) Sometimes base modifications which lie *outside* a recognition sequence can influence the rate of DNA cleavage by a restriction enzyme. For example, *Nar*I does not cut at overlapping M·*Mva*I–*Nar*I GGCGCC<sup>m4</sup>CCWGG sites (Ne14), *Hae*III cannot cut certain GGCC<sup>mT</sup> sites, where <sup>mT</sup> are modified thymine residues (Wi1), and *Msp*I, *Hpa*II, *Sma*I, and *Hha*I are unable to cut DNAs in which bases adjacent to their recognition sequences are modified with hydroxymethyluracil (Ho1). Such methylation-induced ‘action at a distance’ may be more common than has been previously appreciated. We have tested only a few enzymes for sensitivity to base modifications *outside* their canonical recognition sequences.

#### DNA MODIFICATIONS OTHER THAN <sup>m4</sup>C, <sup>m5</sup>C, <sup>hm5</sup>C, <sup>hm5</sup>U, AND <sup>m6</sup>A

The effects of several other site-specific DNA modifications on the rate of restriction endonuclease cleavage, such as 5-bromo-deoxycytidine, 5-bromodeoxyuridine, 5-iododeoxycytidine, deoxyinosine, 2-aminopurine, 2,6-diaminopurine, 2-chloroadenosine, 7-deazaguanosine, and deoxynucleotide phosphorothioates, are listed elsewhere (Bo1,Be6,Mo4,Br2,Ta5,He4,Gr3,Se4,Vo2).

#### EFFECT OF <sup>m5</sup>CG AND <sup>m5</sup>CNG ON RESTRICTION ENDONUCLEASES

Enzymes that are *not* sensitive to site-specific methylation are particularly useful for achieving complete digestion of methylated DNA. For instance, endonucleases that are unaffected by <sup>m5</sup>CG and <sup>m5</sup>CNG are useful for the digestion of plant DNA, which is frequently methylated at these positions. Endonucleases that are unaffected by these two cytosine modifications include: *Acc*III, *Afl*II, *Aha*III, *Ase*I, *Asp*700I *Asu*II, *Bbv*I, *Bcl*I, *Bsp*HI, *Bsp*NI, *Bst*EII, *Bst*NI, *Cvi*QI, *Dpn*I, *Dra*I, *Eco*RV, *Hin*CII, *Hpa*I, *Kpn*I, *Mbo*II, *Mse*I, *Nde*I, *Nde*II, *Pac*I, *Rsa*I, *Rsp*XI, *Sfi*I, *Spe*I, *Sph*I, *Ssp*I, *Sw*I, *Taq*I, *Tsp*509I, *Tth*HBI and *Xmn*I. However, adenine methylation may also occur in plants.

CpG sequences occur infrequently and are often methylated in mammalian genomes (Mc9). Almost all the enzymes that could generate large fragments of mammalian DNA are blocked by this <sup>m5</sup>CpG modification at overlapping sites, including *Aat*II, *Apel*, *Asc*I, *Avi*II, *Bbe*I, *Bma*DI, *Bsr*BI, *Bss*HII, *Bsp*MII, *Bst*BI, *Cla*I, *Csp*I, *Csp*45I, *Eag*I, *Ecl*XI, *Eco*47III, *Fse*I, *Fsp*I, *Kpn*2I, *Mlu*I, *Mlu*9273I, *Mlu*9273II, *Mro*I, *Nae*I, *Nar*I, *Not*I, *Nru*I, *Pfu*I, *Pml*I, *Ppu*AI, *Pvu*I, *Rsr*II, *Sal*I, *Sal*DI, *Sbo*13I, *Sfi*I, *Sma*I, *Sna*BI, *SpI*, *Spe*I, *Srf*I, *Xba*I and *Xor*II (see Table I). Only ten enzymes suitable for pulsed field mapping of eukaryotic chromosomes are

known to cut <sup>m5</sup>CG-modified DNA: *Acc*III, *Asu*II, *Bsp*EI, *Cfr*9I, *Pac*I, *Pme*I, *Sfi*I, *Sse*8387I, *Sw*I, and *Xma*I.

#### <sup>m4</sup>C, <sup>m5</sup>C, AND <sup>hm5</sup>C CYTOSINE MODIFICATIONS

In some cases, a restriction enzyme may differ in sensitivity to <sup>m4</sup>C, <sup>m5</sup>C or <sup>hm5</sup>C at a particular sequence. For example, *Bst*NI and *Mva*I cut <sup>m5</sup>C, but not <sup>m4</sup>C modified CCWGG sequences. *Rsa*I cuts GTAm<sup>m5</sup>C but not GTAm<sup>m4</sup>C. *Kpn*I cuts GGTAC<sup>m5</sup>C but not GGTAC<sup>m4</sup>C. *Bst*YI cuts RGAT<sup>m5</sup>CY but not RGAT<sup>m4</sup>CY. Similarly, *Cvi*SIII cuts T<sup>m5</sup>CGA but not T<sup>hm5</sup>CGA. These endonucleases may be used to distinguish among these modifications.

#### EFFECT OF SITE-SPECIFIC METHYLATION ON DNA METHYLTRANSFERASES

Twenty-three Type II methyltransferases have been tested for sensitivity to *non-canonical* DNA modifications, of which nine were blocked (Mc10 and Table IV). As with restriction endonucleases, rate effects are sometimes seen with DNA methyltransferases at non-canonically modified sequences. For example, *E.coli Dam* methyltransferase is unaffected by GAT<sup>m4</sup>C, but methylates GAT<sup>m5</sup>C relatively slowly. Such data is summarized in Table IV and footnotes to Table I.

#### METHYLATION-DEPENDENT RESTRICTION SYSTEMS IN BACTERIA

*E.coli K-12* contains at least three different methylation-dependent restriction systems which selectively restrict methylated target sequences: *mrr* (<sup>m6</sup>A), *mcrA* (<sup>m5</sup>CG), *mcrB* (R<sup>m5</sup>C) (Br5,Di1, He2,Ra1,Ra2). *In vivo* or *in vitro* modified DNA is inefficiently cloned into *E.coli*. For example, human DNA which is extensively methylated at <sup>m5</sup>CpG is restricted by *mcrA* (Wo3) and other systems (Bu2). Appropriate non-restricting strains of *E.coli* (Go2,Kr2,Ra1,Ra2) should be chosen for efficient transformation and cloning of methylated DNA. Other species also have such methyl-dependent restriction systems (e.g. Ma2).

#### ENGINEERED DNA METHYLTRANSFERASE SPECIFICITIES

Many DNA methyltransferase genes have now been sequenced. Extensive homologies between closely related enzymes (Wi3) or common motifs (Po5,Sm3) allow new specificities to be engineered (e.g. Ba4,Tr4).

#### DATA IN ELECTRONIC FORM

This paper is available as a text file on a 3.5' Macintosh diskette. The data can be supplied as a Microsoft Word, Macwrite or MS-DOS file. Please contact Michael McClelland at CIBR, phone (619) 535 5486, FAX (619) 535 5472.

#### ACKNOWLEDGEMENTS

This work is supported by grants AI34829, NS33377 and HG00456 to MM from the U.S. National Institutes of Health. We thank Andrew Bradbury for helpful comments and corrections.

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**Table I.** Methylation sensitivity of restriction endonucleases<sup>a</sup>

Restriction enzyme	Recognition sequence	Sites cut	Sites not cut	References
<i>AaiI</i>	AGGCCT	?	AGG <sup>m5</sup> CCT AGG <sup>m5</sup> CT AGG <sup>m4</sup> CT	Ne14 So3 Ne14
<i>AatII</i>	GACGTC	?	GA <sup>m5</sup> CGTC GACGT <sup>m5</sup> C	Fo1 Ne14
<i>AccI</i>	GTMKAC	?	GTMK <sup>m6</sup> AC <sup>#</sup> GTMKA <sup>m5</sup> C <sup>b</sup>	Lu2,Mc3
<i>AccII</i>	CGCG	?	<sup>m5</sup> CGCG	Ga2
<i>AccIII</i>	TCCGGA	T <sup>m5</sup> CCGGA <sup>b</sup> TC <sup>m5</sup> CGGA <sup>b</sup>	TCCGG <sup>m6</sup> A	Ke3,La2,Sc2
<i>Acc65I</i>	GGTACC	?	GGTAC <sup>m5</sup> C	Ne5
<i>AciI</i>	CCGC	?	C <sup>m5</sup> CGC	Fo1
<i>AflI</i>	GGWCC	GGWC <sup>m5</sup> C GGWC <sup>m4</sup> C <sup>b</sup>	?	Mc11,Wh2
<i>AfII</i>	CTTAAG	?	<sup>m5</sup> CTTAAG CTTA <sup>m6</sup> AG	Ne14
<i>AfIII</i>	ACRYGT	?	A <sup>m5</sup> CRYGT	Ne14
<i>AgeI</i>	ACCGGT	?	A <sup>m5</sup> CCGGT AC <sup>m5</sup> CGGT	Ne14
<i>AhaII</i>	GRCGYC <sup>b</sup>	?	GR <sup>m5</sup> CGYC GRCGY <sup>m5</sup> C	Ka2,Hu1
<i>AluI</i>	AGCT	?	<sup>m6</sup> AGCT AG <sup>m4</sup> CT AG <sup>m5</sup> CT <sup>#</sup> AG <sup>m5</sup> CT	Gr5,Mc11,Ne2 Hu1,Wo1,Zh1 Bu9
<i>AlwI</i>	GGATC	?	GG <sup>m6</sup> ATC GGAT <sup>m4</sup> C	Ne4
<i>Alw26I</i>	GTCTC	?	GT <sup>m5</sup> CTC <sup>#</sup> GAG <sup>m6</sup> AC <sup>#</sup>	Bi7
<i>Alw44I</i>	GTGCAC	GTGC <sup>m6</sup> AC	GTG <sup>m5</sup> CAC	Ne14
<i>AlwNI</i>	CAGN <sub>3</sub> CTG	?	CAGN <sub>2</sub> C <sup>m5</sup> CTG	Bo5,Ne5
<i>Amal</i>	TCGCGA	TCGCG <sup>m6</sup> A	?	Mc13
<i>AosII</i>	GRCGYC	?	GR <sup>m5</sup> CGYC	Eh2,Gr5,Va3
<i>Apal</i>	GGGCC	?	GGG <sup>m5</sup> CCC <sup>#</sup> GGGCC <sup>m5</sup> C	La9,Gu9
<i>ApalI</i>	GTGCAC	GTGC <sup>m6</sup> AC GTG <sup>m5</sup> CAC	GTGCA <sup>m5</sup> C	Fo1,Ho2,Ho3 Ne14
<i>Apel</i>	ACCGGT	?	A <sup>m5</sup> CGCGT	Ne14,Qi2
<i>ApyI</i>	CCWGG	C <sup>m5</sup> CWGG	<sup>m5</sup> CCWGG	Kl1,Mc11,Ra3
<i>AquI</i>	CYCGRG	?	<sup>m5</sup> CYCGRG <sup>#</sup> GG <sup>m5</sup> CGCGCC	Ka7,Ka8
<i>Ascl</i>	GGCGCGCC	?	GGCG <sup>m5</sup> CGCC GGCGCG <sup>m5</sup> CC GGCGCGC <sup>m5</sup> C	Si2
<i>AspI</i>	GWCGWC	?	GW <sup>m5</sup> CGWC	Ne14
<i>AspMDI</i>	GATC	G <sup>m6</sup> ATC	?	Ch4
<i>Asp700I</i>	GAAN <sub>4</sub> TTC	GA <sup>m6</sup> AN <sub>4</sub> TTC GAAN <sub>4</sub> TT <sup>m5</sup> C	G <sup>m6</sup> AAN <sub>4</sub> TTC	Ne14
<i>Asp718I</i>	GGTACC	GGT <sup>m6</sup> A <sup>m5</sup> CC <sup>b</sup> GGTA <sup>m5</sup> CC <sup>b</sup>	GGTAC <sup>m5</sup> C GGTA <sup>m5</sup> C <sup>m5</sup> C <sup>b</sup>	Mu2,Ne4
<i>AsuI</i>	GGNCC	GGNC <sup>m5</sup> C	?	Pr4
<i>AsuII</i>	TTCGAA	TT <sup>m5</sup> CGAA	?	Ne14
<i>AtuCI</i>	TGATCA	?	TG <sup>m6</sup> ATCA	Ro3,Sc12
<i>Aval</i>	CYCGRG	C <sup>m5</sup> CCGGG	<sup>m5</sup> CYCGRG CY <sup>m5</sup> CGRG CTCG <sup>m6</sup> AG <sup>b</sup>	Eh2,Ne14 Ka4,Ka7,Mc11 Ne2
<i>AvaII</i>	GGWCC	GGWC <sup>m4</sup> C <sup>b</sup>	GGW <sup>m5</sup> CC GGWC <sup>m5</sup> C GGW <sup>hm5</sup> C <sup>hm5</sup> C	Ba3,Ko3 Mc10,Mc11 Hu1
<i>AviII</i>	TGCGCA	?	TG <sup>m5</sup> CGCA	Ne14
<i>BaeI</i>	ACN <sub>4</sub> GTAYC	?	ACN <sub>4</sub> GTYA <sup>m5</sup> C	Fo1
<i>Ball</i>	TGGCCA	?	TGG <sup>m5</sup> CCA <sup>#</sup> TGGC <sup>m5</sup> CA <sup>b</sup>	Gi1,Gu9
<i>BamHI</i>	GGATCC	GGATC <sup>m5</sup> C GG <sup>m6</sup> ATCC GG <sup>m6</sup> ATC <sup>m5</sup> C GGATC <sup>m4</sup> C	GGAT <sup>m4</sup> CC <sup>#</sup> GGAT <sup>m5</sup> CC GGAT <sup>hm5</sup> C <sup>hm5</sup> C GGA <sup>hm5</sup> UCC	Br8,Dr1,Ha3,Hu1 La7
<i>BamFI</i>	GGATCC	GG <sup>m6</sup> ATCC	GGAT <sup>m4</sup> CC	An1,Sh1
<i>BamKI</i>	GGATCC	GG <sup>m6</sup> ATCC	GGAT <sup>m4</sup> CC	An1,Sh1
<i>BanI</i>	GGYRCC <sup>b</sup>	GG <sup>m5</sup> CGCC	?	Co3,Ka2,Ma12,Su1

Table I. (cont.)

Restriction enzyme	Recognition sequence	Sites cut	Sites not cut	References
<i>Ban</i> II	GRGCYC	GGYRC <sup>m4</sup> C	GRG <sup>m5</sup> CYC	Fo1,Ne2,Ne6
<i>Ban</i> III	ATCGAT	GRGCY <sup>m5</sup> C	ATCG <sup>m6</sup> AT <sup>#</sup>	Su1
<i>Bbe</i> I	GGCGCC	GGCG <sup>m5</sup> CC	GG <sup>m5</sup> CGCC	Co3,Ne2,Sh2
<i>Bbv</i> II	GRCGYC	?	GR <sup>m5</sup> CGYC	Co3
<i>Bbr</i> PI	CACGTG	?	CA <sup>m5</sup> CGTG	Wo1,Ka11
<i>Bbs</i> I	GAAGAC	GAAGA <sup>m5</sup> C	?	Fo1
<i>Bbu</i> I	GCATGC	GCATG <sup>m5</sup> C	GC <sup>m6</sup> ATGC	Ne14
<i>Bbv</i> I	GCAGC	?	GM <sup>m5</sup> CAGC <sup>#</sup>	Do1,Ha3,Va5
<i>Bca</i> 77I	WCCGGW	WC <sup>m5</sup> CGGW	W <sup>m5</sup> CCGGW	Sc10
<i>Bcg</i> I	CGCN <sub>5</sub> TGC	?	CG <sup>m6</sup> AN <sub>5</sub> TGC <sup>#</sup>	Ra4
<i>Bcl</i> I	TGATCA	TGAT <sup>m5</sup> CA	TG <sup>m6</sup> ATCA	Bi4,Br8,Eh3,Ro3
<i>Bcn</i> I	CCSGG	m <sup>5</sup> CCSGG	TGAT <sup>hm5</sup> CA	Hu1
<i>Bep</i> I	CGCG	?	C <sup>m4</sup> CSGG <sup>#</sup>	Ja3,Ja6,Kl1
<i>Bfr</i> I	CTTAAG	?	C <sup>m5</sup> CSGG	Kr3
<i>Bgl</i> I	GCCN <sub>5</sub> GGC	GC <sup>m5</sup> CN <sub>5</sub> GGC <sup>b</sup>	m <sup>5</sup> CGCG	Ku3
<i>Bgl</i> II	AGATCT	AG <sup>m6</sup> ATCT	m <sup>5</sup> CTTAAG	Wo1
<i>Bgl</i> III	AGATCT	AGA <sup>hm5</sup> UC <sup>hm5</sup> U	GC <sup>m5</sup> CCN <sub>5</sub> GGC	Kl1,Ko3,Mc11,Ne2
<i>Bin</i> I	GGATC	?	GCCN <sub>5</sub> GG <sup>m5</sup> C <sup>b</sup>	
<i>Bma</i> DI	CGATCG	CG <sup>m6</sup> ATCG	GC <sup>m4</sup> CN <sub>4</sub> GGC <sup>#</sup> <sup>b</sup>	
<i>Bme</i> 216I	GGWCC	?	AGAT <sup>hm5</sup> CT	Bi4,Br8,Dr1,
<i>Bna</i> I	GGATCC	GG <sup>m6</sup> ATCC	Dy1,Eh3	
<i>Bsa</i> I	GGTCTC	GAGA <sup>m5</sup> C <sup>m5</sup> C	AGAT <sup>hm5</sup> CT	Hu1,Pi6,Ho1
<i>Bsa</i> AI	YACGTR	?	GG <sup>m6</sup> ATC	Bo2
<i>Bsa</i> BI	GATN <sub>4</sub> ATC	?	CGAT <sup>m6</sup> CG	Qi2
<i>Bsa</i> HI	GRGCYC	?	GGWC <sup>m5</sup> C	Ma9
<i>Bsa</i> JI	CCNNGG	C <sup>m5</sup> CNNGG	GGAT <sup>m4</sup> CC	Ne14
<i>Bsa</i> WI	WCCGGW	WC <sup>m5</sup> CGGW	GGAT <sup>m5</sup> CC <sup>#</sup>	Ki1
<i>Bse</i> CI	ATCGAT	?	GGTCT <sup>m5</sup> C	Fo1,Ne5
<i>Bsg</i> I	CTGCAC	?	Y <sup>m5</sup> CGTR	Fo1
<i>Bsh</i> 1365I	GATN <sub>4</sub> ATC	?	GATN <sub>4</sub> AT <sup>m5</sup> C	Fo1
<i>Bsi</i> BI	GATN <sub>4</sub> ATC	?	G <sup>m6</sup> ATN <sub>4</sub> m <sup>6</sup> ATC	
<i>Bsi</i> EI	CGRYCG	?	GR <sup>m5</sup> CGYC	Fo1
<i>Bsi</i> LI	CCWGG	?	?	Fo1
<i>Bsi</i> MI	TCCGGA	?	?	Fo1
<i>Bsi</i> WI	CGTAGC	?	ATCG <sup>m6</sup> AT <sup>#</sup>	Ri3,Ri4
<i>Bsi</i> XI	ATCGAT	?	CTGCA <sup>m5</sup> C	Fo1
<i>Bsi</i> XII	ATCGAT	?	G <sup>m6</sup> ATN <sub>4</sub> ATC	Ja3
<i>Bsi</i> XI	ATCGAT	?	GATN <sub>4</sub> m <sup>6</sup> ATC	
<i>Bsi</i> XI	CCN <sub>7</sub> GG	?	G <sup>m6</sup> AT <sub>4</sub> ATC	
<i>Bsm</i> I	GAATGC	GAATG <sup>m5</sup> C	G <sup>m5</sup> CN <sub>7</sub> GG	Im1
<i>Bsm</i> AI	GTCTC	G <sup>m6</sup> AGAC	G <sup>m6</sup> ATAGC	Fo1,Ne14
<i>Bsp</i> DI	ATCGAT	?	GTCT <sup>m5</sup> C	Fo1,Ne14
<i>Bsp</i> EI	TCCGGA	TC <sup>m5</sup> CGGA <sup>b</sup>	AT <sup>m5</sup> CGAT	Fo1
<i>Bsp</i> HI	TCATGA	?	m <sup>6</sup> ATCG <sup>m6</sup> AT	Pa2,Se3
<i>Bsp</i> MI	ACCTGC	ACCTG <sup>m5</sup> C	TCCGG <sup>m6</sup> A	Mc1
<i>Bsp</i> MII	TCCGGA	TCCGG <sup>m6</sup> A	TC <sup>m5</sup> CGGA	La2,Sc2
<i>Bsp</i> XI	ATCGAT	?	TC <sup>m5</sup> CGGA	
<i>Bsp</i> XII	TGATCA	?	ATCG <sup>m6</sup> AT	Zi1
<i>Bsp</i> 106I	ATCGAT	?	TG <sup>m6</sup> ATCA	Zi1
<i>Bsp</i> 143I	GATC	?	ATCG <sup>m5</sup> AT <sup>#</sup>	Ne5
<i>Bsp</i> 1286I	GDGCHC	GDGCH <sup>m5</sup> C	G <sup>m6</sup> ATC	Ne14
<i>Bsr</i> BI	GAGCGG	GAG <sup>m5</sup> CGG <sup>b</sup>	GDG <sup>m5</sup> CHC	Fo1,Ne2,Ne6
<i>Bsr</i> FI	RCCGGY	?	?	Fo1
<i>Bss</i> HII	GCGCGC <sup>b</sup>	?	RC <sup>m5</sup> CGGY	Fo1
<i>Bst</i> I	GGATCC	GG <sup>m6</sup> ATCC	G <sup>m5</sup> CGCGC	Ne4,Qi3
<i>Bst</i> VI	GGATCC	GGATC <sup>m5</sup> C	GGAT <sup>m4</sup> CC	Ne4
<i>Bst</i> BI	CTCGAG	?	GGAT <sup>m5</sup> CC	
<i>Bst</i> BI	TTCGAA	?	GGATC <sup>m4</sup> C	Ne14
<i>Bst</i> VI	CTCGAG	?	CTCG <sup>m6</sup> AG <sup>#</sup>	Ba7
<i>Bst</i> BI	TTCGAA	?	TT <sup>m5</sup> CGAA	Ne4
<i>Bst</i> VI	TTCGAA	?	TT <sup>m5</sup> CGAA	Wo1

Table I. (cont.)

Restriction enzyme	Recognition sequence	Sites cut	Sites not cut	References
<i>Bst</i> EII	GGTNACC	GGTNA <sup>m5</sup> C <sup>m5</sup> C	GGTNA <sup>hm5</sup> C <sup>hm5</sup> C GGTNAC <sup>m4</sup> C	Hu1,Mc11 Ne14
<i>Bst</i> EIII	GATC <sup>b</sup>	?	G <sup>m6</sup> ATC	My1,Ro3
<i>Bst</i> GI	TGATCA	?	TG <sup>m6</sup> ATCA	Ro3
<i>Bst</i> NI	CCWGG <sup>b</sup>	<sup>m5</sup> CCWGG <sup>b</sup> C <sup>m5</sup> CWGG <sup>m5</sup> C <sup>m5</sup> CWGG <sup>b</sup>	hm <sup>5</sup> C hm <sup>5</sup> CWGG C <sup>m4</sup> CWGG #	Gr5,Hu1,Mc11 Ba12,Br8,Ne14,Ro3
<i>Bst</i> OI	CCWGG	C <sup>m5</sup> CWGG	?	Sc10
<i>Bst</i> UI	CGCG	?	m <sup>5</sup> CGCG CG <sup>m5</sup> CG	Ne5 Ne14
<i>Bst</i> XI	CCAN <sub>6</sub> TGG	C <sup>m5</sup> CAN <sub>6</sub> TGG	m <sup>5</sup> CCAN <sub>6</sub> TGG	Ne14,Ne2
<i>Bst</i> YI	RGATCY	RG <sup>m6</sup> ATCY	RGAT <sup>m4</sup> CY RGAT <sup>m5</sup> CY	Ne4 Ne14
<i>Bsu</i> 1107I	GTATAC	?	GTATA <sup>m5</sup> C	Fo1
<i>Bsu</i> BI	CTGCAG	?	CTGC <sup>m6</sup> AG #	Gal,Je1,Sh1,St5
<i>Bsu</i> EII	CGCG	?	m <sup>5</sup> CGCG #	Gal,Je1,Sh1,St5
<i>Bsu</i> FI	CCGG	?	m <sup>5</sup> CCGG #	Je1
<i>Bsu</i> MI	CTCGAG	?	CT <sup>m5</sup> CGAG #	Je1
<i>Bsu</i> RI	GGCC	?	GG <sup>m5</sup> CC # <sup>b</sup>	Gu8,Ki2,Ki3
<i>Bsu</i> 15I	ATCGAT	?	ATCG <sup>m6</sup> AT #	Re6
<i>Bsu</i> 36I	CCTNAGG	CCTN <sup>m6</sup> AGG	m <sup>5</sup> CCTNAGG	Ne14,Ne5
<i>Cbi</i> I	TTCGAA	TTCG <sup>m6</sup> AA	?	Mu1
<i>Ccr</i> I	CTCGAG	?	CTCG <sup>m6</sup> AG	Ne14
<i>Cfo</i> I	GCGC	?	G <sup>m5</sup> CGC GCG <sup>m5</sup> C G <sup>hm5</sup> CG <sup>hm5</sup> C	Eh1 Ne14 Hu1
<i>Cfr</i> I	YGGCCR	?	YGG <sup>m5</sup> CCR #	Kl1
<i>Cfr</i> AI	GCAN <sub>8</sub> GTGG	?	GC <sup>m6</sup> AN <sub>8</sub> GTGG	Ne14
<i>Cfr</i> BI	CCWWGG	?	m <sup>4</sup> CCWWGG #	Za2
<i>Cfr</i> 6I	CAGCTG	?	CAG <sup>m4</sup> CTG # CAG <sup>m5</sup> CTG	Bu9
<i>Cfr</i> 9I	CCCGGG <sup>b</sup>	C <sup>m5</sup> CCGGG CC <sup>m5</sup> CCGG	m <sup>4</sup> CCCGGG m <sup>5</sup> CCCGGG C <sup>m4</sup> CCGGG # CC <sup>m4</sup> CCGG	Bu10 Kl2
<i>Cfr</i> 10I	RCCGGY	?	R <sup>m5</sup> CCGGY #	Bi5,Kl1
<i>Cfr</i> 13I	GGNCC	?	RC <sup>m5</sup> CGGY	Ne14
<i>Clal</i>	ATCGAT	?	GGN <sup>m5</sup> CC # m <sup>6</sup> ATCGAT	Bi5,Kl1 Ca4,Mc11, Mc12,Ne4 Wo1 Mc3
<i>Cpe</i> I	TGATCA	?	AT <sup>m5</sup> CGAT <sup>b</sup>	Fi1,Ro3
<i>Csp</i> I	CGGWCCG	CGGW <sup>m5</sup> CG	ATCG <sup>m6</sup> AT # TG <sup>m6</sup> ATCA CGGW <sup>m5</sup> CCG m <sup>5</sup> CGGWCCG	Mc11
<i>Csp</i> 45I	TTCGAA	?	TTCC <sup>m6</sup> AA	Ne4,Sc11
<i>Cty</i> I	GATC	?	G <sup>m6</sup> ATC #	Ri2
<i>Cvi</i> AI	GATC	GAT <sup>m5</sup> C	G <sup>m6</sup> ATC #	Ne14,Xi1,Xi6
<i>Cvi</i> All	CATG	<sup>m5</sup> CATG	C <sup>m6</sup> ATG #	Ne14,Zh2
<i>Cvi</i> BI	GANTC	?	G <sup>m6</sup> ANTC #	Xi3
<i>Cvi</i> JI	RGCY	?	RG <sup>m5</sup> CY #	Sh3,Xi2
<i>Cvi</i> PI	CC	C <sup>m5</sup> C	m <sup>5</sup> CC #	Xi4
<i>Cvi</i> QI	GTAC	GT <sup>m5</sup> C	GT <sup>m6</sup> AC #	Xi5
<i>Cvi</i> QII	GANTC	?	G <sup>m6</sup> ANTC #	Ne14
<i>Cvi</i> QIII	CATG	?	C <sup>m6</sup> ATG	Ne14
<i>Cvi</i> RI	TGCA	?	TGC <sup>m6</sup> A #	Ne14
<i>Cvi</i> RII	GTAC	?	TG <sup>m5</sup> CA	
<i>Cvi</i> SIII	TCGA	T <sup>m5</sup> CGA	GT <sup>m6</sup> AC # TCG <sup>m6</sup> A # T <sup>hm5</sup> CGA	Ne14 Ne14
<i>Cvi</i> SIV	GATC	?	G <sup>m6</sup> ATC #	Ri2
<i>Dde</i> I	CTNAG	?	m <sup>5</sup> CTNAG # hm <sup>5</sup> CTNAG CTN <sup>m6</sup> AG	Ho4,Ne2 Hu1 Ne14
<i>Dpn</i> I	G <sup>m6</sup> ATC <sup>b</sup>	G <sup>m6</sup> ATC G <sup>m6</sup> AT <sup>m5</sup> C <sup>b</sup> G <sup>m6</sup> AT <sup>m4</sup> C	GATC GAT <sup>m4</sup> C GAT <sup>m5</sup> C G <sup>m6</sup> ATC #	La3,Mc11,Wo1 Ne4 Ne5
<i>Dpn</i> II	GATC	?	G <sup>m6</sup> ATC #	De1,La3,La4, La5,Ma6,Wo1
<i>Dra</i> I	TTTAAA	TTTA <sup>m6</sup> AA	?	Ne14
<i>Dra</i> II	RGGNCCY	?	RGGNC <sup>m5</sup> CY	Sc8
<i>Drd</i> I	GACN <sub>6</sub> GTC	?	GA <sup>m5</sup> CN <sub>6</sub> GT <sup>m5</sup> C	Fo1
<i>Dsa</i> V	CCNGG	?	C <sup>m5</sup> CNGG	N1
<i>Eae</i> I	YGGCCR	?	YGG <sup>m5</sup> CCR #	Ja2,Wh1

Table I. (cont.)

Restriction enzyme	Recognition sequence	Sites cut	Sites not cut	References
<i>EagI</i>	CGGCCG	?	YGGC <sup>m5</sup> CR CGG <sup>m5</sup> CCG <sup>m5</sup> CGGC <sup>m5</sup> CG	Mc11
<i>Eam1105I</i>	GACN <sub>5</sub> GTC	GA <sup>m5</sup> CN <sub>5</sub> GT <sup>m5</sup> C	?	Fo1
<i>EarI</i>	GAAGAG	CTCTT <sup>m5</sup> C	G <sup>m6</sup> AAGAG GAAG <sup>m6</sup> AG <sup>m5</sup> CT <sup>m5</sup> CTT <sup>m5</sup> C	Fo1,Ne4
<i>EcaI</i>	GGTNACC	?	GGTN <sup>m6</sup> ACC <sup>#</sup>	Ne14
<i>EclXI</i>	CGGCCG	?	<sup>m5</sup> CGGC <sup>m5</sup> CG CGG <sup>m5</sup> CCG	Br3
<i>Ecl136II</i>	GAGCTC	?	GAGCT <sup>m5</sup> C	Q3
<i>EcoAI</i>	GAGN <sub>5</sub> GTCA <sup>b</sup>	?	G <sup>m6</sup> AGN <sub>5</sub> G <sup>m</sup> TCA <sup>#b</sup>	Bi2,Co6,Fu2
<i>EcoBI</i>	TGAN <sub>8</sub> TGCT <sup>b</sup>	?	TG <sup>m6</sup> AN <sub>8</sub> <sup>m</sup> TGCT <sup>#b</sup>	Bi2,La10,La11
<i>EcoDI</i>	TTAN <sub>7</sub> GTCY <sup>b</sup>	?	TT <sup>m6</sup> AN <sub>7</sub> GTCY <sup>#b</sup>	Na6
<i>EcoDXXI</i>	TCAN <sub>7</sub> AATC <sup>b</sup>	?	TCAN <sub>7</sub> <sup>m6</sup> AA <sup>m</sup> TC <sup>#b</sup>	Pi1
<i>EcoEI</i>	GAGN <sub>7</sub> ATGC <sup>b</sup>	?	G <sup>m6</sup> AGN <sub>7</sub> ATGC <sup>#b</sup>	Co6,Fu2
<i>EcoHI</i>	CCSGG	?	C <sup>m5</sup> CSGG <sup>#</sup>	Kr3
<i>EcoKI</i>	AACN <sub>6</sub> GTGC <sup>b</sup>	?	A <sup>m6</sup> ACN <sub>6</sub> G <sup>m</sup> TGC <sup>#b</sup>	Bi2,Bi3,Ka1
<i>EcoO109I</i>	RGGNCYY	?	RGGNC <sup>m5</sup> CY	Sc8
<i>EcoPI</i>	AGACC <sup>b</sup>	AGA <sup>hm5</sup> C <sup>hm5</sup> C	AG <sup>m6</sup> ACC <sup>#</sup>	Ba1,Ba2,Ha4,Re4
<i>EcoP15I</i>	CAGCAG <sup>b</sup>	?	CAGC <sup>m6</sup> AG <sup>#</sup>	Hu2,Me2
<i>EcoRI</i>	GAATT	GAATT <sup>hm5</sup> C	G <sup>m6</sup> AATT <sup>b</sup>	Mc11,Ne2,Ru1
		GAA <sup>hm5</sup> U <sup>hm5</sup> UC	GA <sup>m6</sup> AATT <sup>#</sup>	Br1,Br8,Du1,Ho1
			GAATT <sup>m5</sup> C <sup>b</sup>	Hu1,Ka3,Ta1
<i>EcoRII</i>	CCWGG	<sup>m5</sup> CCWGG <sup>b</sup>	<sup>m4</sup> CCWGG C <sup>m4</sup> CWGG C <sup>m5</sup> CWGG <sup>#</sup> CC <sup>m6</sup> AGG hm5C <sup>hm5</sup> CWGG	Ku1,Yo1 Bu8,Na5,Ro3 Bo7,Mc11 Bu7 Hu1,Ka3
<i>EcoRV</i>	GATATC	GATAT <sup>m5</sup> C <sup>b</sup> GATAT <sup>hm5</sup> C	G <sup>m6</sup> ATATC <sup>#</sup> GAT <sup>m6</sup> ATC	Mc11,Ne2,Wo1
<i>EcoT22I</i>	ATGCAT	?	ATG <sup>m5</sup> CAT ATGC <sup>m6</sup> AT	Fl1,Ho1
<i>Eco31I</i>	GGTCTC	?	GGT <sup>m5</sup> CTC <sup>#</sup> G <sup>m6</sup> AGACC <sup>#</sup>	Ne14
<i>Eco47I</i>	GGWCC	?	GGWC <sup>m5</sup> C	Bi7
<i>Eco47II</i>	GGNCC	?	GGNC <sup>m5</sup> C	Ja5
<i>Eco47III</i>	AGCGCT	<sup>m6</sup> AGCGCT	AG <sup>m5</sup> CGCT	Po6
<i>Eco57I</i>	CTGAAG	?	CTGA <sup>m6</sup> AG <sup>#</sup>	Ne14,Ne4
			CTTC <sup>m6</sup> AG <sup>#</sup>	Ja8,Po6
<i>Eco183II</i>	CCSGG	?	C <sup>m5</sup> CSGG <sup>#</sup>	Kr3
<i>EheI</i>	GGCGCC	?	GG <sup>m5</sup> CGCC	Co2,Ne14
			GGCG <sup>m5</sup> CC GG <sup>hm5</sup> CG <sup>hm5</sup> C <sup>hm5</sup> C	
<i>EspI</i>	GCTNAGC	GCTNAG <sup>m5</sup> C	G <sup>m5</sup> CTNAGC	Ne4
<i>Esp3I</i>	CGTCTC	?	<sup>m5</sup> CGTCTC CGT <sup>m5</sup> CTC <sup>#</sup> GAG <sup>m6</sup> ACG <sup>#</sup>	Fo1
				Bi7,Ja3
<i>Esp1396I</i>	CCAN <sub>5</sub> TGG	C <sup>m5</sup> CAN <sub>5</sub> TGG	?	Ja3
<i>Fnu4HI</i>	GCNGC	?	G <sup>m5</sup> CNGC <sup>#</sup> GCNG <sup>m5</sup> C <sup>#</sup>	Gu9,Ko3
<i>FnuDII</i>	CGCG	?	<sup>m5</sup> CGCG CG <sup>m5</sup> CG	Ga1,Ga2, Ne2,Ne6,St6
<i>FnuEI</i>	GATC	G <sup>m6</sup> ATC	?	Lu1,Ne2
<i>FokI</i>	CATCC	CAT <sup>m5</sup> CC CATC <sup>m5</sup> C <sup>b</sup>	GG <sup>m6</sup> ATG C <sup>m6</sup> ATCC CATC <sup>m6</sup> C	Po3,Po4,Sc2
<i>FseI</i>	GGCCGGCC	?	GG <sup>m5</sup> CCGG <sup>m5</sup> CC GGC <sup>m5</sup> CGGCC GG <sup>m5</sup> CCGGCC	Ne14
<i>FspI</i>	TGGCCA	?	TG <sup>m5</sup> CGCA	Ne7
<i>FsuI</i>	GGWCC	?	GGW <sup>m5</sup> C	Le1
<i>HaeII</i>	RGCAGY <sup>b</sup>	?	RG <sup>m5</sup> CGCY RGCGm5CY RG <sup>hm5</sup> CG <sup>hm5</sup> CY	Eh2,Gr5,Ka2,Ko3,Mc11,Pi5
				Ne14
<i>HaeIII</i>	GGCC	GGC <sup>m5</sup> C	GG <sup>m5</sup> CC <sup>#</sup> GG <sup>hm5</sup> C <sup>hm5</sup> C	Hu1
<i>HapII</i>	CCGG	?	C <sup>m5</sup> CGG <sup>#</sup>	Ba3,Ka2,Ko3,Ma5
<i>HgaI</i>	GACGC	?	GA <sup>m5</sup> CGC <sup>#</sup> G <sup>m5</sup> CGTC <sup>#</sup>	Ne14
				Wj7
<i>HgiAI</i>	GWGCWC	GWGCW <sup>m5</sup> C	GACG <sup>m5</sup> C	Mc11
<i>HgiBI</i>	GGWCC	?	GWG <sup>m5</sup> CWC GGWC <sup>(m5</sup> C)	Fo1,Ne2,Wh3
<i>HgiCI</i>	GGYRCC	?	GGYR <sup>m5</sup> CC <sup>#</sup>	Du2
				Er1

Table I. (cont.)

Restriction enzyme	Recognition sequence	Sites cut	Sites not cut	References
<i>HgiCII</i>	GGWCC	?	GGWC <sup>m5</sup> C	Er1
<i>HgiDI</i>	GRCGYC	?	GRCGYC	Du4
<i>HgiEI</i>	GGWCC	?	GGWC <sup>m5</sup> C	Er1
<i>HgiGI</i>	GRCGYC	?	GR <sup>m5</sup> CGY <sup>m5</sup> C	Kr4,Er1
<i>HgiJII</i>	GGYRCC	?	GGYRC <sup>m5</sup> C	Wh3
<i>HhaI</i>	GCGC	?	G <sup>m5</sup> CGC <sup>#</sup>	Eh2,Sm1
			GCG <sup>m5</sup> C	Mc11,Ko3
			G <sup>hm5</sup> CG <sup>hm5</sup> C	Hu1
<i>HhaII</i>	GANTC	?	G <sup>m6</sup> ANTC <sup>#</sup>	Ma5
<i>HincII</i>	GTYRAC	GT <sup>m5</sup> CRAC	GTYRA <sup>m5</sup> C <sup>b</sup>	Bu11
			GTYR <sup>m6</sup> AC <sup>#</sup>	Gr5,Ro7
			GTYRA <sup>hm5</sup> C	Hu1
<i>HindII</i>	GTYRAC	?	GTYR <sup>m6</sup> AC <sup>#</sup>	Ro7
			GTYRA <sup>hm5</sup> C	
<i>HindIII</i>	AAGCTT	A <sup>m6</sup> AGCTT	m <sup>6</sup> AAGCTT <sup>#</sup>	Br8,Gr5,Ne14,Ro7
		AAGC <sup>hm5</sup> U <sup>hm5</sup> U	AAG <sup>m5</sup> CTT <sup>b</sup>	Ho1,Ne2
			AAG <sup>hm5</sup> CTT	Hu1,Ka3
<i>HinfI</i>	GANTC	GANT <sup>m5</sup> C <sup>b</sup>	G <sup>m6</sup> ANTC	Ch1,Co1,Ne2,Pe1
			GANT <sup>hm5</sup> C	Hu1
<i>HinPI</i>	GCGC	?	G <sup>m5</sup> CGC	Mc11,Ne6
<i>HpaI</i>	GTAAAC	GTAAAC <sup>m5</sup> C	GTAA <sup>m6</sup> AC <sup>#</sup>	Br8,Gr5,Hu1,Yo3
			GTAA <sup>hm5</sup> C	Hu1
			G <sup>hm5</sup> U <sup>hm5</sup> UAAC	Ho1
<i>HpaII</i>	CCGG	?	m <sup>4</sup> CCGG	Be3,Bu10,Eh2,Ma5
			m <sup>5</sup> CCGG <sup>b</sup>	Ko3,Qu1,Wa5
			C <sup>m4</sup> CGG <sup>b</sup>	
			C <sup>m5</sup> CGG <sup>#</sup>	
			hm <sup>5</sup> C <sup>hm5</sup> CGG	
<i>HphI</i>	TCACC	TCAC <sup>m5</sup> C	T <sup>m5</sup> CACC <sup>#</sup>	Hu1
			TCAm <sup>m5</sup> CC	Fo1,Mc11,Ne2
			GGTG <sup>m6</sup> A	
<i>KasI</i>	GGCGCC	?	GG <sup>m5</sup> CGCC	Fo1
<i>KpnI</i>	GGTACC <sup>b</sup>	GGTA <sup>m5</sup> CC	GGT <sup>m6</sup> ACC <sup>#</sup>	Eh3,Ki4,Mc11
		GGTAC <sup>m5</sup> C	GGTA <sup>m4</sup> CC <sup>b</sup>	Ne14
<i>Kpn2I</i>	TCCGGA	TCCGG <sup>m6</sup> A	GGTA <sup>m5</sup> C <sup>m5</sup> C <sup>b</sup> GGTAC <sup>m4</sup> C	
			T <sup>m5</sup> CCGGA	Mc1,Ne14
			TC <sup>m5</sup> CGGA	Ne14
<i>KspI</i>	CCGCGG	?	m <sup>5</sup> CCGCGG	Ne14
			C <sup>m5</sup> CGCGG	Qi2
<i>MaeII</i>	ACGT	?	A <sup>m5</sup> CGT <sup>b</sup>	Mo2
<i>MamI</i>	GATN <sub>4</sub> ATC	?	G <sup>m6</sup> ATN <sub>4</sub> m6ATC	St4
<i>MboI</i>	GATC <sup>b</sup>	GAT <sup>m4</sup> C	G <sup>m6</sup> ATC <sup>#</sup>	Br5,Ge1,Mc8
		GAT <sup>m5</sup> C <sup>b</sup>	GAT <sup>hm5</sup> C	Hu1,Ro3
			GA <sup>hm5</sup> UC	Ho1
<i>MboII</i>	GAAGA	T <sup>m5</sup> CTT <sup>m5</sup> C <sup>b</sup>	GAAG <sup>m6</sup> A <sup>#</sup>	Ba3,Mc11,Mc12,Ne2
		G <sup>m6</sup> AAGA	GA <sup>m6</sup> AGA	
<i>MfII</i>	RGATCY <sup>b</sup>	?	RG <sup>m6</sup> ATCY	On1
			RGAT <sup>m5</sup> CY	
<i>MluI</i>	ACGCGT	m <sup>6</sup> ACGCGT	A <sup>m5</sup> CGCGT	Mc11,Sh1,St5,Qi3
			ACG <sup>m5</sup> CGT	Ne5
<i>Mlu9273I</i>	TCGCGA	?	T <sup>m5</sup> CGCGA	Ne14
<i>Mlu9273II</i>	GCCGGC	?	G <sup>m5</sup> CCGGC	Ne14
			GC <sup>m5</sup> CGGC	
<i>MmeII</i>	GATC	?	G <sup>m6</sup> ATC	Bo6
<i>MnlI</i>	CCTC	G <sup>m6</sup> AGG	m <sup>5</sup> CCTC	Eh3,Mc11
			m <sup>5</sup> C <sup>m5</sup> CT <sup>m5</sup> C	
<i>MphI</i>	CCWGG	?	C <sup>m5</sup> CWGG	Ro3
<i>MroI</i>	TCCGGA	TCCGG <sup>m6</sup> A	T <sup>m5</sup> CCGGA	Mc1,Ne14
			TC <sup>m5</sup> CGGA	Ne14
<i>MscI</i>	TGGCCA	?	TGGC <sup>m5</sup> CA	Fo1
<i>MspI</i>	CCGG <sup>b</sup>	m <sup>4</sup> CCGG	m <sup>5</sup> CCGG <sup>#</sup>	Eh2,Je2,Va3,Wa1,Wa5
		C <sup>m4</sup> CGG	hm <sup>5</sup> C <sup>hm5</sup> CGG	Bu10,Hu1
		C <sup>m5</sup> CGG		
<i>MstII</i>	CCTNAGG	m <sup>5</sup> CCTNAGG	CCTN <sup>m6</sup> AGG	Ne5
<i>MthTI</i>	GGCC	?	GG <sup>m5</sup> CC <sup>#</sup>	No4,No6
<i>MthZI</i>	CTAG	?	m <sup>4</sup> CTAG <sup>#</sup>	No5
<i>MvaI</i>	CCWGG	C <sup>m5</sup> CWGG <sup>b</sup>	C <sup>m4</sup> CWGG <sup>#</sup>	Bu8,Ku2,Kl2
		m <sup>5</sup> CCWGG	CC <sup>m6</sup> AGG <sup>b</sup>	Gr4,Ku1
			m <sup>4</sup> CCWGG <sup>b</sup>	
			m <sup>5</sup> C <sup>m5</sup> CWGG <sup>b</sup>	
<i>MunI</i>	CAATTG	?	CA <sup>m6</sup> ATTG <sup>#</sup>	Ne14
<i>MvnI</i>	CGCG	?	m <sup>5</sup> CGCG	St8
<i>NaeI</i>	GCCGGC	?	G <sup>m5</sup> CCGGC	Ne14
				Eh3,Kl1,Mc11,Ne5

Table I. (cont.)

Restriction enzyme	Recognition sequence	Sites cut	Sites not cut	References
<i>Nan</i> II	G <sup>m6</sup> ATC <sup>b</sup>	G <sup>m6</sup> ATC	GC <sup>m5</sup> CGGC GCCGG <sup>m5</sup> C	Pa1,Ne5
<i>Nar</i> I	GGCGCC	GGCGC <sup>m5</sup> C	GATC G <sup>m6</sup> AT <sup>m5</sup> C <sup>b</sup> GG <sup>m5</sup> CGCC GGCGC <sup>m4</sup> C GG <sup>hm5</sup> CG <sup>hm5</sup> C <sup>hm5</sup> C	GAT <sup>m5</sup> C Ko3,Mc11,Ne5 Ne14
<i>Nei</i> I	CCSGG	<sup>m5</sup> CCSGG	C <sup>m4</sup> CSGG C <sup>m5</sup> CSGG <sup>b</sup>	Br8,Ko3,Mc11 Me3
<i>Nco</i> I	CCATGG	CC <sup>m6</sup> ATGG	<sup>m4</sup> CCATGG <sup>b</sup> <sup>m5</sup> CCATGG	KI1,Ne2,Ne4
<i>Nci</i> I	AGATCT	AG <sup>m6</sup> ATCT <sup>b</sup>	?	Qi1
<i>Ncu</i> I	GAAGA	?	GAAG <sup>m6</sup> A	Mc13
<i>Nde</i> I	CATATG	<sup>m5</sup> CATATG <sup>b</sup>	CAT <sup>m6</sup> ATG <sup>#</sup>	Be4,Mc11,Re7,Sil,We3
<i>Nde</i> II	GATC	GAT <sup>m5</sup> C <sup>b</sup>	G <sup>m6</sup> ATC	Mc9
<i>Ngo</i> I <sup>b</sup>	RGGCY	?	RG <sup>m5</sup> CGCY	Ko3,Ko5
<i>Ngo</i> II <sup>b</sup>	GGCC	?	GG <sup>m5</sup> CC <sup>#</sup>	Ko3,Ko5
<i>Ngo</i> BI <sup>b</sup>	TCACC	?	GGC <sup>m5</sup> C <sup>b</sup>	Su3,Su4
<i>Ngo</i> MI	GCCGGC	?	T <sup>m5</sup> CACCA G <sup>m5</sup> CCGCC <sup>#</sup>	Pi3,Pi4 Gu4
<i>Nhe</i> I	GCTAGC	?	GC <sup>m5</sup> CGGC	Fo1
<i>Nla</i> III	CATG	?	GCTAG <sup>m5</sup> C C <sup>m6</sup> ATG <sup>#</sup> <sup>m5</sup> CATG	KI1,Mc11,Ne2 La1,Mo3 Zh2
<i>Nla</i> IV	GGNNCC	?	GGNN <sup>m4</sup> CC	Ne14
<i>Nmu</i> DI	G <sup>m6</sup> ATC <sup>b</sup>	G <sup>m6</sup> ATC	GATC	Pa1
<i>Nmu</i> EI	G <sup>m6</sup> ATC <sup>b</sup>	G <sup>m6</sup> ATC	GATC	Pa1
<i>Not</i> I	GCGGCCGC	GCGGCCG <sup>m5</sup> C	GCGG <sup>m5</sup> CCGC GCGGC <sup>m5</sup> CGC	Mc11 St5,Qi2
<i>Nru</i> I	TCGCGA	TCG <sup>m5</sup> CGA	T <sup>m5</sup> CGCGA TCGCG <sup>m6</sup> A	Ne14,Qi3 Ne2
<i>Nsi</i> I	ATGCAT	?	ATG <sup>m5</sup> CAT ATGC <sup>m6</sup> AT	Be5,Wo1
<i>Nsp</i> I	RCATGY	?	R <sup>m5</sup> CATGY RC <sup>m6</sup> ATGY	Ne14 Ne14
<i>Nsp</i> V	TTCGAA <sup>#</sup>	?	?	Ue1
<i>Nsp</i> BII	CMGCKG	C <sup>m5</sup> CGCKG	?	Ne14
<i>Pae</i> R7I	CTCGAG	?	CT <sup>m5</sup> CGAG <sup>b</sup> CTCG <sup>m6</sup> AG <sup>#</sup>	Gi3 Gh1
<i>Pf</i> MI	CCAN <sub>5</sub> TGG	?	C <sup>m4</sup> CAN <sub>5</sub> TGG C <sup>m5</sup> CAN <sub>5</sub> TGG	Ne14 St7
<i>Pfu</i> I	GATC	G <sup>m6</sup> ATC	?	Ro3
<i>Pfu</i> I	CGTACG	?	CGTA <sup>m5</sup> CG	Ne14
<i>Pme</i> I	GT <sub>TT</sub> AAAC	GT <sub>TT</sub> AAA <sup>m5</sup> C	?	Fo1
<i>Pml</i> I	CACGTG	?	CA <sup>m5</sup> CGTG	Fo1
<i>Ppu</i> AI	CGTACG	?	CGTA <sup>m5</sup> CG	Ne14
<i>Ppu</i> MI	RGGWCCY	?	RGGWC <sup>m5</sup> CT	Fo1
<i>Pst</i> I	CTGCAG	?	m <sup>5</sup> CTGCAG <sup>b</sup> C <sup>hm5</sup> UGCAC CTG <sup>m5</sup> CAG CTG <sup>m6</sup> AG <sup>#</sup>	Do1,Gr5,Mc11,Ne2 Ho1 Ne5
<i>Pvu</i> I	CGATCG	CG <sup>m6</sup> ATCG	CGAT <sup>m4</sup> CG CGAT <sup>m5</sup> CG	Br8,Bu7,Eh3
<i>Pvu</i> II	CAGCTG	?	CAG <sup>m4</sup> CTG <sup>#</sup> CAG <sup>m5</sup> CTG <sup>b</sup>	Br8,Bu9,Do1 Eh3,Ja3,Ro1
<i>Rfl</i> FI	GTCGAC	?	GTCG <sup>m6</sup> AC	Mo5
<i>Rfl</i> FII	AGTACT	?	AGT <sup>m6</sup> ACT	Mo5
<i>Rrh</i> 4273I	GTCGAC	?	GTCG <sup>m6</sup> AC	Ba6
<i>Rsa</i> I	GTAC <sup>b</sup>	GTA <sup>m5</sup> C <sup>b</sup>	GT <sup>m6</sup> AC GTA <sup>m4</sup> C <sup>#b</sup>	Eh3,Fo1,Ne14,Ne4,Ne5 Wo2
<i>Rsh</i> I	CGATCG	CG <sup>m6</sup> ATCG	?	Ly1
<i>Rsp</i> XI	TCATGA	?	TC <sup>m6</sup> ATGA TCATG <sup>m6</sup> A	Pa2
<i>Rsr</i> I	GAATT	?	G <sup>m6</sup> AATT GA <sup>m6</sup> ATT <sup>#b</sup>	Ne4 Mc11
<i>Rsr</i> II	CGGWCCG	?	m <sup>5</sup> CGGWCCG CGGW <sup>m5</sup> CCG CGGW <sup>m5</sup> CG	Ba5 Mc11,Qi3
<i>Sac</i> I	GAGCTC	G <sup>m6</sup> AGCTC GAGCT <sup>m5</sup> C	GAG <sup>m5</sup> CTC	Mc11
<i>Sac</i> II	CCCGGG	?	m <sup>5</sup> CCGCGG C <sup>m5</sup> CGCGG	Fo1 KI1,Ne2 Qi2
<i>Sal</i> I	GTCGAC	GTCGA <sup>m5</sup> C	GT <sup>m5</sup> CGAC <sup>b</sup> GTCG <sup>m6</sup> AC <sup>#</sup>	Br8,Eh2,Lu2,Qi1 Mc3,Ro4,Ro5,Va4

Table I. (cont.)

Restriction enzyme	Recognition sequence	Sites cut	Sites not cut	References
<i>SalDI</i>	TCGCGA	TCGCG <sup>m6</sup> A	G <sup>hm5</sup> UCGAC T <sup>m5</sup> CGCGA	Hol
<i>Sau3PI</i>	GCCGGC	?	G <sup>m5</sup> CCGGC <sup>#</sup>	Mc13,Ne14,Qi3
<i>Sau3AI</i>	GATC <sup>b</sup>	G <sup>m6</sup> ATC GA <sup>hm5</sup> UC	GAT <sup>m5</sup> C <sup>#b</sup> GAT <sup>m4</sup> C GAT <sup>hm5</sup> C	Ok1 Dr1,Eh2,Ja3,Mc3,Ro3,Se1 Ho1,Ne5
<i>Sau96I</i>	GGNCC	?	GGN <sup>m5</sup> CC <sup>#</sup> GGNC <sup>m5</sup> C GGN <sup>hm5</sup> C <sup>hm5</sup> C	Hu1 Ko3,Ne2,Pe1
<i>Sau3239I</i>	CTCGAG	?	CTCG <sup>m6</sup> AG <sup>#</sup>	Hu1 Ze1
<i>Sbo13I</i>	TCGCGA	TCGCG <sup>m6</sup> A	T <sup>m5</sup> CGCGA	Mc11,Ne14
<i>Scal</i>	AGTACT	AGTA <sup>m5</sup> CT	?	Wo1
<i>ScrFI</i>	CCNGG	<sup>m5</sup> CCNGG	C <sup>m5</sup> CNGG C <sup>m4</sup> CNGG	Da4,Mc11,Ne2 Ne14
<i>SfaNI</i>	GATGC	GATG <sup>m5</sup> C	G <sup>m6</sup> ATGC	Mc11,Po4
<i>SfiI</i>	GGCCN <sub>5</sub> GGCC	GG <sup>m5</sup> CCN <sub>5</sub> GG <sup>m5</sup> CC	GGC <sup>m5</sup> CN <sub>5</sub> GGCC GGCCN <sub>5</sub> GGC <sup>m5</sup> C	Mc11,Qi2 GG <sup>m4</sup> CCN <sub>5</sub> GGCC
<i>SfuI</i>	TTCGAA	TT <sup>m5</sup> CGAA	TTCG <sup>hm5</sup> AA	Ne5
<i>SfII</i>	CTGCAG	?	CTGC <sup>m6</sup> AG	Br8
<i>SgrAI</i>	CRCCGGYG	?	CRC <sup>m5</sup> CGGYG	Ta3
<i>SinI</i>	GGWCC	?	GGW <sup>m5</sup> CC <sup>#</sup>	Ka5,Ka6
<i>SmaI</i>	CCCGGG	C <sup>m5</sup> CCGGG	m <sup>4</sup> CCC <sup>m5</sup> GGG <sup>#</sup> m <sup>5</sup> CCC <sup>m5</sup> GGG <sup>b</sup> C <sup>m4</sup> CCGGG <sup>b</sup> CC <sup>m4</sup> CGGG CC <sup>m5</sup> CGGG <sup>b</sup>	Br8,Bu10,Eh2,Ga4 Ja3,Ka7,Mc3,Qu1
<i>SnaBI</i>	TACGTA	?	TA <sup>m5</sup> CGTA T <sup>m6</sup> ACGT <sup>m6</sup> A	Fo1,Ya1
<i>SnoI</i>	GTGCAC	?	GTG <sup>m5</sup> CA <sup>m5</sup> C	Ho3,Wo1
<i>SpeI</i>	ACTAGT	?	m <sup>6</sup> ACTAGT A <sup>m5</sup> CTAGT	Ho2 Wo1
<i>SphI</i>	GCATGC	GCATG <sup>m5</sup> C	GC <sup>m6</sup> ATGC G <sup>hm5</sup> CATG <sup>hm5</sup> C	Mc11,Mo3,Ne2
<i>SpI</i>	CGTACG	CGT <sup>m6</sup> ACG <sup>b</sup>	CGTA <sup>m5</sup> CG CGTA <sup>m4</sup> CG	Ne14,Ne4,Qi3
<i>SpoI</i>	TCGCGA	TCGCG <sup>m6</sup> A	T <sup>m5</sup> CGCGA TCG <sup>m5</sup> CGA	Ne14,Ne4
<i>SrfI</i>	GCCCCGGC	?	G <sup>m5</sup> CCCCGGC GC <sup>m5</sup> CCGGC GCC <sup>m5</sup> CGGGC GCCCGGG <sup>m5</sup> C	Ma11
<i>SsoI</i>	GAATTG	?	G <sup>hm6</sup> AATTG <sup>#</sup>	Ni4
<i>SsoII</i>	CCNGG	?	C <sup>m5</sup> CN <sub>5</sub> GG m <sup>5</sup> CCNGG	Ni1,Vi1 Gr4
<i>SspRFI</i>	TTCGAA	?	TTCG <sup>m6</sup> AA	Li1
<i>SstI</i>	GAGCTC	?	GAG <sup>m5</sup> CTC	Br8,Ro1
<i>SstII</i>	CCCGGG	?	GAG <sup>hm5</sup> CT <sup>hm5</sup> C m <sup>5</sup> CCCGGG	Hu1 Ne5
<i>StsI</i>	GGATG	?	C <sup>m5</sup> CGCGG GG <sup>m6</sup> ATG <sup>#</sup>	Ne5 Ki5
<i>StuI</i>	AGGCCT	?	C <sup>m6</sup> ATCC <sup>#</sup> AGG <sup>m5</sup> CCT AGGC <sup>m3</sup> CT AGGC <sup>m4</sup> CT	Ca4,Mc11 So3 Ne14
<i>StyDI</i>	CCWWGG	?	C <sup>m5</sup> CWWGG	Mi3
<i>StyLTI</i>	CAGAG	?	CAG <sup>m6</sup> AG <sup>#</sup>	Da5
<i>StyQI</i>	AACN <sub>6</sub> RTAYG <sup>b</sup>	?	AACN <sub>6</sub> R <sup>m</sup> TAYG	Na2
<i>StyR124I</i>	GAAN <sub>6</sub> RTCG <sup>b</sup>	?	GA <sup>m6</sup> AN <sub>6</sub> R <sup>m</sup> TCG <sup>#</sup>	Bi1,Pr2,Pr3
<i>StyR124/3I</i>	GAAN <sub>7</sub> RTCG <sup>b</sup>	?	GA <sup>m6</sup> AN <sub>7</sub> R <sup>m</sup> TCG <sup>#</sup>	Pr1,Pr2
<i>StySJI</i>	GAGN <sub>6</sub> GT <sup>b</sup> RC	?	GAGN <sub>6</sub> G <sup>m</sup> TRC	Ga3
<i>StySBI</i>	GAGN <sub>6</sub> RTAYG <sup>b</sup>	?	G <sup>m6</sup> AGN <sub>6</sub> R <sup>m</sup> TAYG <sup>#b</sup>	Na1,Na2
<i>StySPI</i>	AACN <sub>6</sub> GT <sup>b</sup> RC	?	A <sup>m6</sup> ACN <sub>6</sub> G <sup>m</sup> TRC <sup>#b</sup>	Na1,Na2
<i>TaqI</i>	TCGA	T <sup>m5</sup> CGA <sup>b</sup>	TCG <sup>hm6</sup> A <sup>#</sup> T <sup>hm5</sup> CGA <sup>b</sup>	Gr5,Hu1,Mc3,Va3 Ba13,Hu1
<i>TaqII</i>	GACCGA	?	G <sup>m6</sup> ACCGA	Ne4
<i>TaqIII</i>	CACCCA			
<i>TaqXI</i>	CCWGG	m <sup>5</sup> CCWGG C <sup>m5</sup> CWGG	?	Gr1
<i>TflI</i>	GAWTC	GAWT <sup>m5</sup> C	?	Fo1
<i>TflII</i>	TCGA	?	TCG <sup>m6</sup> A	Sa3,Va6
<i>Thal</i>	CGCG	?	m <sup>5</sup> CGCG	Gal,Ne14
<i>Tth111I</i>	GACN <sub>3</sub> GTC	GA <sup>m5</sup> CN <sub>3</sub> GTC GACN <sub>3</sub> GT <sup>m5</sup> C	hm <sup>5</sup> CG <sup>hm5</sup> CG ?	Hu1 Fo1

Table I. (cont.)

Restriction enzyme	Recognition sequence	Sites cut	Sites not cut	References
TthHBI	TCGA	T <sup>m5</sup> CGA	TCG <sup>m6</sup> A #	Sa3
Tsp509I	AATT	?	m <sup>6</sup> AATT	Fo1
Van91I	CCAN <sub>5</sub> TGG	?	C <sup>m5</sup> CAN <sub>5</sub> TGG	Ja3
XbaI	TCTAGA	?	TCTAG <sup>m6</sup> A #	Mc13,We1
			T <sup>m5</sup> CTAGA <sup>b</sup>	Gr5,Hu1,Ne2
XbaII	CCCAGG	?	T <sup>hm5</sup> CTAGA	
XbaI	CTCGAG	?	C <sup>m4</sup> CCGGG #	Wi6
		CT <sup>m5</sup> CGAG <sup>b</sup>	m <sup>5</sup> CTCGAG	Ne2,Ka7
XbaII	RGATCY	RG <sup>m6</sup> ATCY	CTCG <sup>m6</sup> AG	Eh3
XbaI	CCCGGG	CC <sup>m5</sup> CGGG <sup>b</sup>	RGAT <sup>m5</sup> CY <sup>b</sup>	Mc3,Va3
XbaIII	CGGCCG	?	m <sup>4</sup> CCCGGG	Br8
XbaI	GAAN <sub>4</sub> TTC	GA <sup>m6</sup> AN <sub>4</sub> TTC	m <sup>5</sup> CCCCGG	Bu10,Yo5,Yo6
XbaII	CGATCG	?	C <sup>m4</sup> CCGGG	
			CC <sup>m4</sup> CGGG	
			CGG <sup>m5</sup> CCG #	Gu9,Ne2,Tr5
			G <sup>m6</sup> AAN <sub>4</sub> TTC	Mc11,Ne2
			GAAN <sub>4</sub> T <sup>m5</sup> C <sup>b</sup>	
			CGAT <sup>m5</sup> CG	Br8,Eh2
			CG <sup>m6</sup> ATCG <sup>b</sup>	
			hm <sup>5</sup> CGAT <sup>hm5</sup> CG	Hu1,Sm4

<sup>a</sup> # denotes canonical modification MTase specificity. M = A or C, K = G or T, N = A,C,G, or T, R = A or G, Y = C or T, W = A or T, S = G or C, D = A,G or T, H = A,C or T. Sequences are in 5'-3' order. <sup>m4</sup>C = N4-methylcytosine; <sup>m5</sup>C = C5-methylcytosine; <sup>hm5</sup>C=hydroxymethylcytosine; <sup>hm5</sup>U=hydroxymethyluracil; <sup>m</sup>C = methylcytosine, in which N4 or C5-methylcytosine unspecified; <sup>m6</sup>A = N6-methyladenine. Nomenclature is according to (Sm2) and (Co4).

<sup>b</sup> AccI nicks slowly in the unmethylated strand of the hemimethylated sequence GTMKA<sup>m5</sup>C. AccI cuts slowly at hemimethylated GTMKA<sup>m5</sup>C (Ne10).

AccIII cuts slowly at T<sup>m5</sup>CCGA and TC<sup>m5</sup>CGGA (Sc10).

AflI cuts slowly at GGWC<sup>m4</sup>C.

AhaII (GRCGYC) will cut GRCGCC faster if these sites are methylated at GRCG<sup>m5</sup>CC (Ne5), but will not cut GRCGY<sup>m5</sup>C sites (Ne2,Ne5).

Asp718I cuts GT<sup>m6</sup>AC- and <sup>m5</sup>CC-modified Chlorella virus NY2A DNA but does not cut GGTAC<sup>m5</sup>CWGG overlapping dcm sites (Mu2) or <sup>m5</sup>C-substituted phage XP12 DNA. In contrast, KpnI cuts these modified substrates readily (Ne4).

AvaI nicking occurs slowly in the unmethylated strand of the hemimethylated sequence CTCG<sup>m6</sup>AG/CTCGAG (Ne5).

AvaII cuts slowly at GGWC<sup>m4</sup>C.

Bacillus species have been surveyed for G<sup>m6</sup>ATC and C<sup>m5</sup>CWGG specific methylases. Many species have G<sup>m6</sup>ATC specific methylases but none had C<sup>m5</sup>CWGG specific methylases (Di2).

BalI sites overlapping dcm sites (TGGC<sup>m5</sup>CAGG) are 50-fold slower than unmethylated sites (Gi1).

BanI gives various rate effects when its recognition sequence is <sup>m4</sup>C- or <sup>m5</sup>C-methylated at different positions.

BglI cleavage rate at certain GC<sup>m5</sup>CN<sub>5</sub>GGC, GC<sup>m4</sup>CN<sub>5</sub>GGC, and GCCN<sub>5</sub>GG<sup>m5</sup>C hemimethylated sites is extremely slow. However, <sup>m5</sup>C bi-methylated M·HaeIII-BglI sites are completely refractory to BglI (Ko3,Ne2).

BspEI cleavage slowed by TC<sup>m5</sup>CGGA (Fo1).

BsrBI cleavage slowed by GAG<sup>m5</sup>CGG (Fo1).

BssHII does not cut M·HhaI-modified DNA, in which two different cytosine positions are hemimethylated, G<sup>m5</sup>CGCGC/GCG<sup>m5</sup>CGC (Ne4).

M·BstI modifies the internal cytosine GGAT<sup>m5</sup>CC, but it is not known whether this modification is <sup>m5</sup>C or <sup>m4</sup>C (Le3).

BstEII cuts the fully <sup>m5</sup>C-substituted phage XP12 DNA (Ne5).

BstNI isoschizomers that are insensitive to C<sup>m5</sup>CWGG include AorI, ApyI, BspNI, MvaI and TaqXI (Mc4).

BsuRI nicking occurs in the unmethylated strand of the hemimethylated sequence GG<sup>m5</sup>CC/GGCC.

Cfr9I, see reference Bu10 for rate effects.

ClaI cuts slowly at hemimethylated AT<sup>m5</sup>CGAT (Ne10).

M·CreI is from the unicellular eukaryote *Chlamydomonas reinhardtii* (Sa2).

DpnI requires adenine methylation on both DNA strands. Isoschizomers of DpnI include CfuI, NanII, NmuEI, NmuDI and NsuDI (Ca1). DpnI cuts dam modified XP12 DNA (Ne6).

M·Eco dam modifies GAT<sup>m5</sup>C at a reduced rate (Ne5). Many other bacteria that modify their DNA at G<sup>m6</sup>ATC are listed in references Bal and Lo1.

EcoAI, EcoBI, EcoDI, EcoEI, EcoDXI, EcoKI and others (Bi6) are Type I restriction endonucleases. <sup>m</sup>T represents a 6-methyladenine in the complementary strand. EcoPI is a Type III restriction endonuclease (Ba1,Ba2,Ha4).

EcoP15I is a Type III restriction endonuclease (Hu2).

EcoRI cannot cut hemimethylated G<sup>m6</sup>AATT/GAATT sites. Bismethylated GA<sup>m6</sup>ATT/GA<sup>m6</sup>ATT sites are not cut by EcoRI or Rsrl (Ne5). EcoRI shows a reduced rate of cleavage at hemimethylated GAATT<sup>m5</sup>C (Tr1) and does not cut an oligonucleotide that contains GAATT<sup>m5</sup>C in both strands (Br1).

EcoRII does not cleave some DNA molecules that carry only a single site. However, oligonucleotides containing the EcoRII site can be used to transactivate sites that are resistant to cleavage (Re5). EcoRII isoschizomers that are sensitive to C<sup>m5</sup>CWGG include AtuBI, AtuII, BstGII, BinSI, EclII, EcalII, Eco27I, Eco38I and MphI (Ro3). EcoRII shows reduced rate of cleavage at hemimethylated <sup>m5</sup>CCWGG/CCWGG sites (Yo1).

EcoRV cuts the fully <sup>m5</sup>C-substituted phage XP12 DNA (Ne5).

EcoR124I and EcoR124/3I are now called StyR124I and StyR124/3I.

FokI cuts about two-fold to four-fold more slowly at CATC<sup>m5</sup>C than at unmodified sites (Ne5).

M·FokI in ref Po3 corresponds to M·FokIA in ref Po4.

HaeII shows a reduced rate of cleavage when its recognition sequence is modified at RGCG<sup>m5</sup>CY.

HaeIII nicking occurs in the unmethylated strand of the hemimethylated sequence GG<sup>m5</sup>CC/GGCC.

HinfI cuts GANT<sup>m5</sup>C, however, detectable rate differences are observed between unmethylated, hemimethylated (GANT<sup>m5</sup>C/GANTC) and bi-methylated (GANT<sup>m5</sup>C/GANT<sup>m5</sup>C) target sequences (Co1,Gr5,Ne5,Ne10). However, the rate difference between unmethylated and fully methylated HinfI sites is only about ten-fold (Hu1,Ne5,Pe1).

HincII. There is conflicting data regarding cleavage of GTYRA<sup>m5</sup>C.

HindIII cuts slowly at hemimethylated AAC<sup>m5</sup>CTT (Ne10).

HpaII nicking in the unmethylated strand of the hemimethylated sequence <sup>m5</sup>CCGG/CCGG is in dispute (Be3,Bu10,Ko3). HpaII cuts hemimethylated mCCGG fifty

times slower and fully methylated mCCGG 3000 times slower than unmethylated DNA (Ko3). See reference (Bu10) for *Hpa*II rate effects.

*Kpn*I cuts  $m^5C$ -substituted phage XP12 DNA (Ne4) but cuts slowly at hemimethylated GGT $A^{m5}C^{m5}C$ , GGT $A^{m5}CC$  and GGTAC $m^5C$  (Ne10).

*Mae*II nicks slowly in the unmethylated strand of hemimethylated A $m^5CGT/ACGT$  (Mo2).

*Mbo*I isoschizomers that are sensitive to G $m^6ATC$  include *Bss*GII, *Bsa*PI, *Bsp*74I, *Bsp*76I, *Bsp*105I, *Bst*XII, *Bst*EIII, *Bss*GII, *Cpa*I, *Cry*I, *Cvi*AI, *Cvi*II, *Cvi*HI, *Cvi*SVI, *Dpn*II, *Fnu*AI, *Fnu*CI, *Hac*I, *Meu*I, *Mkr*AI, *Mme*II, *Mno*III, *Mos*I, *Msp*67II, *Mth*I, *Mth*AI, *Nde*II, *NfI*AI, *NfI*BI, *NfI*II, *Nla*DI, *Nla*II, *Nme*CI, *Nph*I, *Nsi*AI, *Nsp*AI, *Nsl*I, *Pfl*I, *Rfu*II, *Sal*AI, *Sal*II, *Sau*782I, *Sin*MI, *Tru*II (Ro3).

*Mbo*II cuts the fully  $m^5C$ -substituted phage XP12 DNA (Ne5), although certain hemimethylated  $m^5C$ -containing substrates are reported not to be cut (Gr5).

*MfI* cuts slowly at  $m^6AGATCY$  sites (On1).

Mammalian methylase is the  $m^5CG$  methyltransferase from *Mus musculus*. (mouse) (Be7).

*Msp*I cuts the hemimethylated sequence C $m^5CGG/CCGG$  (Wa5) and C $m^4CGG/CCGG$  duplexes (Bu10). *Msp*I cuts very slowly at GGC $m^5CGG$  (Bu6). An M·*Msp*I clone methylates  $m^5CCGG$  (Wa5,Wa2). However, there is a report that *Moraxella* sp. chromosomal DNA is methylated at  $m^5C^{m5}CGG$  (Je2).

*Mva*I nicking occurs in the unmethylated strand of the hemimethylated sequence CC $m^6CCWGG/CCWGG$  and CC $m^6AGG/CCTGG$  (Ku1). *Mva*I cuts XP12 DNA very slowly at  $m^5C^{m5}CWGG$ .

*Nan*II requires adenine methylation on both DNA strands (Ca1). *Nan*II cuts *dam* methylase-modified XP12 DNA (Ne5).

*Nci*I ability to cut C $m^5CGGG$  in dispute.

*Nco*I is blocked by M·*Sec*I (CCNNGG) (Ne5).

*Ncr*I is a *Bgl*II isoschizomer from *Nocardia carnia* Beijing (Qi1).

*Nde*I cuts the fully  $m^5C$ -substituted phage XP12 DNA (Ne5).

*Nde*II cuts the fully  $m^5C$ -substituted phage XP12 DNA (Ne5).

*Ngo*. There is some confusion about naming restriction enzymes from these strains (Gu4). *Ngo*PI may be *Ngo*I *Ngo*PII, *Ngo*II and *Ngo*SI may be the same. *Ngo*PIII may be *Ngo*III.

*Ngo*II does not cut overlapping dcm sites (Su4).

*Nmu*DI requires adenine methylation on both DNA strands (Ca1).

*Nmu*EI requires adenine methylation on both DNA strands (Ca1).

*PaeR7I* cuts hemimethylated CT $m^5CGAG/CTCGAG$  sites 100-fold slower and cuts fully methylated CT $m^5CGAG/CTm^5CGAG$  2900 fold slower than unmethylated sites (Gh1). Hemi- or full methylation at  $m^6A$  completely protects against *PaeR7I* cleavage (Gh1).

*Pst*I cuts slowly at hemimethylated  $m^5CTGm^5CAG$  (Ne10).

*Pvu*II cuts slowly at hemimethylated  $m^5CAGm^5CTG$  (Ne10).

*Rsa*I cuts the fully  $m^5C$ -substituted phage XP12 DNA (Ne5), [contradicted by (Fo1) and by NEB catalog which says *Rsa*I does not cut if fully  $m^5C$ - methylated in both strands. It may be very slow at these sites. It is likely that M·*Rsa*I modifies at GTA $m^4C$ .

*Rsr*I cannot cut hemimethylated G $m^6AATT/GAATT$  sites.

*Sall* cuts slowly at hemimethylated GT $m^5CGAC$  (Ne10).

*Sau*3AI nicking occurs in the unmethylated strand of the hemimethylated sequence GAT $m^5C/GATC$  (St3). *Sau*3AI cuts at a reduced rate at  $m^6AGATC$  (On1). *Sau*3AI isoschizomers that are insensitive to G $m^6ATC$  include *Bce*243I, *Bsp*49I, *Bsp*51I, *Bsp*52I, *Bsp*54I, *Bsp*57I, *Bsp*58I, *Bsp*59I, *Bsp*60I, *Bsp*61I, *Bsp*64I, *Bsp*65I, *Bsp*66I, *Bsp*67I, *Bsp*72I, *Bsp*AI, *Bsp*91I, *Bsp*PII, *Cpf*I, *Csp*5I, *Cpel*, *Fnu*EI, *Msp*BI, *Sau*CI, *Sau*EI, *Sau*FI, *Sau*GI and *Sau*MI (Ro3).

*Sma*I nicking occurs in the unmethylated strand of the hemimethylated sequence CC $m^5CGGG/CCCGGG$  (Bu10,Wa5). *Sma*I may cut C $m^5C^{m5}CGGG$  methylated DNA (Br8,Je2) Possibly the second methylation negates the effect of CC $m^5CGGG$ . There are conflicting results regarding *Sma*I:  $m^5CCCCGGG$  is not cut when modified by M·*Aql* methyltransferase (Ka7) or at overlapping M·*Hae*III-*Sma*I sites (GG $m^5CCCCGGG$ , Ne5). Other investigators have reported that *Sma*I cuts at a reduced rate at hemimethylated  $m^5CCCCGGG$  sites (Bu10).

*Spel* cuts slowly at hemimethylated A $m^5CTAGT$  (Ne10).

*SpI* cuts GT $m^6AC$ -modified *Chlorella* virus NY2A DNA, but does not cut *Kpn*I-digested XP12 DNA (Ne4).

*Sty*QI is a Type I restriction endonucleases.  $m^6T$  represents a 6-methyladenine in the complementary strand.

*StyR124I* and *StyR124/3I* are Type I restriction endonucleases formerly called *EcoR124I* and *EcoR124/3I*.  $m^6T$  represents a 6-methyladenine in the complementary strand.

*StySBI* and *StySPI* are Type I restriction endonucleases.  $m^6T$  represents a 6-methyladenine in the complementary strand.

*StySJI* is a Type I restriction endonucleases.  $m^6T$  represents a 6-methyladenine in the complementary strand.

*Taq*I cuts very slowly at T $m^5CGA$  (Hu1). *Taq*I cuts the fully  $m^5C$  substituted phage XP12 DNA (Hu1,Ne5).

M·*Taq*I methylates T $m^5CGA$  at least 20 fold slower than unmodified TCGA (Mc7).

*Xba*I will cut T $m^5CTAGA/TCTAGA$  hemimethylated DNA at high enzyme levels (> 100U *Xba* I/ $\mu$ g), but will not cut this sequence in twenty to forty-fold overdigestions (Ne5,Ne10).

*Xho*II may cut CT $m^5CGAG$  according to the NEB catalog.

*Xho*II nicking occurs slowly in the unmethylated strand of the hemimethylated sequence RGAT $m^5CY/RGATCY$ .

*Xna*I is claimed not cut CC $m^5CGGG$  in one report (Br8). See reference Bu10 for rate effects.

*Xnn*I cuts the fully  $m^5C$  substituted phage XP12 DNA (Ne5). *Xnn*I cuts slowly at some sites in DNA methylated on *both* strands at GAAN<sub>4</sub>TT $m^5C$  (Ne5).

*Xor*II, according to the BRL-Gibco catalog, may cut CG $m^6ATCG$ .

Table II. Isoschizomer pairs that differ in their sensitivity to sequence-specific methylation

Restriction isoschizomer pairs <sup>a,b</sup>

Methylated sequence <sup>c</sup>	Cut by	Not cut by	References
$m^5CATG$	<i>Cvi</i> AII	<i>Nla</i> III	Zh2
$Cm^5CAN_5TGG$	<i>Esp</i> 1396I	<i>Pfl</i> MI, <i>Van</i> 91I	Ja3, St7
$m^4CCGG$	<i>Msp</i> I	<i>Hpa</i> II	Bu10
$Cm^5CGG$	<i>Msp</i> I	<i>Hpa</i> II, <i>Hpa</i> II	Eh2, Mc11
$Cm^4CGG$	<i>Msp</i> I	<i>Hpa</i> II	Bu10
$CCm^5CGGG$	<i>Cfr</i> 9I, <i>Xma</i> I	<i>Sma</i> I	Bu10
$m^5CCTNAGG$	<i>Mst</i> II	<i>Bsu</i> 36I	Ne5
$Cm^5CSGG$	<i>Bcn</i> I	<i>Eco</i> HI, <i>Eco</i> 1831I	Kr3
$Cm^5CWGG$	<i>Apy</i> I, <i>Bst</i> NI, <i>Mva</i> I	<i>Eco</i> RI <sup>d</sup>	Bu8
$m^5CCWGG$	<i>Bst</i> NI, <i>Eco</i> RI, <i>Mva</i> I	<i>Apy</i> I	Ke1, Ku1, Ne3, Yo1
$CGm^6ATCG$	<i>Pvu</i> I	<i>Xor</i> II	Bi3, Br8, Sm4
$GAAN_4TTm^5C$	<i>Asp</i> 700I	<i>Xnn</i> I	Ne14, Ne2
$GAGCTm^5C$	<i>Sac</i> I	<i>Ecl</i> 136II	Qi3, Fo1
$Gm^6ATC$	<i>Fnu</i> EI, <i>Sau</i> 3AI	<i>Mbo</i> I, <i>Nde</i> II	Ge1, Lu1, Mc9, Ro3
$GATm^5C$	<i>Mbo</i> I	<i>Sau</i> 3AI	Ne4
$GATm^4C$	<i>Mbo</i> I	<i>Sau</i> 3AI	Ne4
$GGCm^5C$	<i>Hae</i> III	<i>Ngo</i> I	Su4

Table II. (cont.)

Restriction isoschizomer pairs <sup>a,b</sup>			
Methylated sequence <sup>c</sup>	Cut by	Not cut by	References
GGNC <sup>m5</sup> C	<i>Ase</i> I	<i>Sau</i> 96I	Ko3
GTG <sup>m5</sup> CAC	<i>Apa</i> LI	<i>Alw</i> 44I	Ne14
GGTAC <sup>m5</sup> C	<i>Kpn</i> I	<i>Asp</i> 718I, <i>Acc</i> 65I	Mu2,Ne5
GGTA <sup>m5</sup> C <sup>m5</sup> C	<i>Kpn</i> I	<i>Asp</i> 718I	Ne4
GGWC <sup>m5</sup> C	<i>Afl</i> I	<i>Ava</i> II, <i>Eco</i> 47I	Ba3,Ja5,Wh2, Mc10,Mc11
RG <sup>m6</sup> ATCY	<i>Bst</i> YI, <i>Xba</i> II	<i>Mf</i> II	Mc9,Ne4,On1
RGAT <sup>m5</sup> CY	<i>Bst</i> YI	<i>Mf</i> II, <i>Xba</i> II	Ne4,On1
T <sup>m5</sup> CCGGA	<i>Acc</i> III	<i>Bsp</i> MI, <i>Kpn</i> 2I, <i>Mro</i> I	La2,Sc2
TC <sup>m5</sup> CGGA	<i>Acc</i> III, <i>Bsp</i> EI	<i>Bsp</i> MI, <i>Kpn</i> 2I, <i>Mro</i> I	Fo1,Sc2
TCCG <sup>m6</sup> A	<i>Bsp</i> MI, <i>Kpn</i> 2I, <i>Mro</i> I	<i>Acc</i> III	Ke3,Ne4
T <sup>hm5</sup> CGA	<i>Taq</i> I	<i>Cvi</i> SIII	Ne14,Hu1
TCGCG <sup>m6</sup> A	<i>Am</i> I, <i>Sal</i> DI, <i>Sbo</i> 13I, <i>Spo</i> I	<i>Nru</i> I	Mc11,Mc13,Ne4
TCG <sup>m5</sup> CGA	<i>Nru</i> I	<i>Spo</i> I	Ne14,Qi3
TT <sup>m5</sup> CGAA	<i>Asu</i> II, <i>Sfi</i> I	<i>Bst</i> BI	Ne5,Wo1
TC <sup>gm6</sup> AA	<i>Cbi</i> I	<i>Bst</i> BI, <i>Csp</i> 45I, <i>Ssp</i> RFI	Li1,Mu1,Ne4, Sc11,Wo1
CGGWC <sup>m5</sup> CG	<i>Csp</i> I	<i>Rsr</i> II	Qi3

  

Restriction isomethylator pairs <sup>e,f</sup>			
Methylated sequence <sup>c</sup>	Methylated by	Not methylated by	References
T <sup>m5</sup> CGA	M· <i>Cvi</i> BIII (TCG <sup>m6</sup> A)	M· <i>Taq</i> I	We2

<sup>a</sup> In each row the first column lists a methylated sequence, the second column lists an isoschizomer that cuts this sequence, and the third column lists an isoschizomer that does not cut this sequence.

<sup>b</sup> An enzyme is classified as insensitive to methylation if it cuts the methylated sequence at a rate that is at least one tenth the rate at which it cuts the unmethylated sequence. An enzyme is classified as sensitive to methylation if it is inhibited at least twenty-fold by methylation relative to the unmethylated sequence.

<sup>c</sup> See footnote 'a' of Table I.

<sup>d</sup> See footnote 'b' of Table I.

<sup>e</sup> In each row the first column lists a methylated sequence, the second column lists an isomethylator that modifies this sequence, and the third column lists an isomethylator that does not modify this sequence.

<sup>f</sup> An enzyme is classified as insensitive to methylation if it modifies the methylated sequence at a rate that is at least one tenth the rate at which it modifies the unmethylated sequence. An enzyme is classified as sensitive to methylation if it is inhibited at least twenty-fold by methylation relative to the unmethylated sequence.

Table III. DNA methyltransferases and their modification specificities

Methylase <sup>a</sup>	Specificity <sup>a</sup>	EMBL accession #	References
M· <i>Aaf</i> II	GACGTC		Lu2
M· <i>Acc</i> I	GTMK <sup>m6</sup> AC	D10671	Lu2
M· <i>Afl</i> III	CTTAAG ( <sup>m6</sup> A)		Lu2
M· <i>Ala</i> K21	GAT <sup>m5</sup> C		Sl1
M· <i>Alu</i> I	AG <sup>m5</sup> CT	Z11841	Kr1,Lu2,Zh1
M· <i>Alw</i> 26I	GT <sup>m5</sup> CTC and G <sup>m6</sup> AGAC		Bi7,Bu8 Bu8
M· <i>Apal</i>	GGG <sup>m5</sup> CCC		Gu9,Mc8
M· <i>Aql</i>	<sup>m5</sup> CYCGRG	M28051	Ka7,Ka8
M· <i>Ase</i> I	ATTAAT		Mo3
M· <i>Ase</i> II	CCSGG		Mo3
M· <i>Ava</i> I	CYCGRG		Lu2
M· <i>Ava</i> II	GGWCC		Lu2
M· <i>Avr</i> I	CYCGRG		Lu3
M· <i>Bal</i> I	TGG <sup>m5</sup> CCA		Lu2,Mc8
<i>M.Bacillus</i>	G <sup>m6</sup> ATC <sup>b</sup>		Di2
M· <i>Bam</i> HII	GGAT <sup>m4</sup> CC	X55285	Ha3,Lu2,Na3
M· <i>Bam</i> HII	G <sup>m</sup> CWGC?	M72412	Ha3
M· <i>Ban</i> I	GGYRCC ( <sup>m5</sup> C)	D00704	Lu2,Ma12,Su1
M· <i>Ban</i> II	GRGCYC		Lu2
M· <i>Ban</i> III	ATCG <sup>m6</sup> AT	P22772 (protein)	Ka12
M· <i>Bbv</i> I	G <sup>m5</sup> CAGC		Do1,Ha3,Va5
M· <i>Bbv</i> SI	G <sup>m5</sup> CWGC		Ha5,Va5
M· <i>Bbv</i>	G <sup>m6</sup> AT		Ha3
M· <i>Bbv</i>	A <sup>m6</sup> AG		Ha3
M· <i>Bcg</i> I	CG <sup>m6</sup> AN,TGC	L17341	Ra4
M· <i>Bcn</i> I	C <sup>m4</sup> CSGG		Ja4,Ja6,Ja7,Pe2,Po6
M· <i>Bep</i> I	<sup>m5</sup> CGCG	X13555	Ku3
M· <i>Bgl</i> II	GCCN <sub>5</sub> GGC ( <sup>m4</sup> C)		Lu2

Table III. (cont.)

Methylase <sup>a</sup>	Specificity <sup>a</sup>	EMBL accession #	References
M· <i>Bme</i> 216I	GGWC <sup>m</sup> C		Ma9
M· <i>BnAI</i>	GGAT <sup>m</sup> CC		Ki1
M· <i>Bse</i> CI	ATCG <sup>m</sup> AT		Ri3,Ri4
M· <i>Bsp</i> RI	GG <sup>m</sup> CC	X15758	Fe2,Ko1,Po2,Qi3,Sz4,Ve1
M· <i>Bsp</i> 106I	ATCG <sup>m</sup> AT		Pa2
M· <i>Bsp</i> 6I	GCNCG		Ja3
M· <i>Bst</i> I	GGAT <sup>m</sup> CC		Le3
M· <i>Bst</i> VI	CTCG <sup>m</sup> AG	L07642,L07643	Ba7
M· <i>Bst</i> NI	C <sup>m</sup> CWGG		Ba12
M· <i>Bst</i> YI	RGAT <sup>m</sup> CY		Va2
M· <i>Bsu</i> 15I	ATCG <sup>m</sup> AT		Re6
M· <i>Bsu</i> BI	CTGC <sup>m</sup> AG	L01541	Xu1
M· <i>Bsu</i> EII	m <sup>5</sup> CGCG		Gu1,Gu9,Ik1,Je1
M· <i>Bsu</i> FI	m <sup>5</sup> CCGG	X62104,X51515	Gu9,Ik1,Je1,Wa7
M· <i>Bsu</i> H2	GG <sup>m</sup> CC		La8
	G <sup>m</sup> CNGC		
	GDGCHC		
M· <i>Bsu</i> MI	CT <sup>m</sup> CGAG		Gu2,Gu3,Gu9,Je1,Sh1
M· <i>Bsu</i> σ3T	GG <sup>m</sup> CC	M13488	Be1,Gu7,Gu6,No2,No3
	and G <sup>m</sup> CNGC		No1,Tr1
M· <i>Bsu</i> Q11I	GG <sup>m</sup> CC	X05242	Gu6,Gu7,Gu9,No1,No2
	and G <sup>m</sup> CNGC		
M· <i>Bsu</i> Q11s	GGCC		Be1
	and GDGCHC		
M· <i>Bsu</i> QI	m <sup>5</sup> CCGG		Je2
M· <i>Bsu</i> RI	GG <sup>m</sup> CC <sup>b</sup>	X02988	Gu8,Ki2,Ki3
M· <i>Bsu</i> SPb	GG <sup>m</sup> CC	M19513,M19514	Gu6,Gu7,Gu9,Je2,Ki2
	and G <sup>m</sup> CNGC		No2,Tr1,Tr3
M· <i>Bsu</i> SPRI	GG <sup>m</sup> CC	X01670,K02124	Be1,Gu5,Gu9,No2
	and m <sup>5</sup> C <sup>m</sup> CGG		Po1,Be2,Bu1,Gu5,Gu7,Ki2,Po1
M· <i>Bsu</i> SPR19I	m <sup>5</sup> C <sup>m</sup> CGG		Je2,No2,Po1
	and C <sup>m</sup> CWGG		
M· <i>Bsu</i> SPR83I	GG <sup>m</sup> CC		Gu5
	and C <sup>m</sup> CWGG		
M· <i>Cfr</i> A	GCAN <sub>8</sub> GTGG		Da2,Da3
M· <i>Cfr</i> BI	CCWWGG (m <sup>4</sup> C)	X57945	Za2
M· <i>Cfr</i> I	YGG <sup>m</sup> CCR		Po6
M· <i>Cfr</i> 6I	CAG <sup>m</sup> CTG		Bu9
M· <i>Cfr</i> 9I	C <sup>m</sup> CCGGG	X17022	Kl2,Po6
M· <i>Cfr</i> 10I	R <sup>m</sup> CCGGY		Po6
M· <i>Cfr</i> 13I	GGN <sup>m</sup> CC		Bi5
M· <i>Clal</i>	ATCG <sup>m</sup> AT		Mc3
M· <i>Cre</i> I	T <sup>m</sup> CR		Sa2
M· <i>Cyt</i> I	G <sup>m</sup> ATC		Ri2
M· <i>Cvi</i> AI	G <sup>m</sup> ATC		Ne14,Xi1,Xi6
M· <i>Cvi</i> AI	C <sup>m</sup> ATG	M86639	Ne14,Zh2
M· <i>Cvi</i> BI	G <sup>m</sup> ANTC	M96366	Xi2
M· <i>Cvi</i> BI	G <sup>m</sup> ATC		Ne14
M· <i>Cvi</i> BII	TCG <sup>m</sup> A	X06618	Na4
M· <i>Cvi</i> JI	RG <sup>m</sup> CY	M27265	Sh3
M· <i>Cvi</i> JII	G <sup>m</sup> ANTC		Ne14
M· <i>Cvi</i> PI	m <sup>5</sup> CC		Xi4
M· <i>Cvi</i> QI	GT <sup>m</sup> AC		Xi2,Xi5
M· <i>Cvi</i> QII	G <sup>m</sup> ANTC		Ne14
M· <i>Cvi</i> QIII	C <sup>m</sup> ATG		Ne14
M· <i>Cvi</i> QIV	R <sup>m</sup> AR		Ne14
M· <i>Cvi</i> RI	TGC <sup>m</sup> A	M38173	St1
M· <i>Cvi</i> RII	GT <sup>m</sup> AC		St1
M· <i>Cvi</i> SI	TGC <sup>m</sup> A		Ne14
M· <i>Cvi</i> SII	C <sup>m</sup> ATG		Ne14
M· <i>Cvi</i> SIII	TCG <sup>m</sup> A		Ne14
M· <i>Cvi</i> SIV	G <sup>m</sup> ATC		Ne14
M· <i>Cvi</i> TI	RG <sup>m</sup> CB		Ne14
M· <i>Dde</i> I	m <sup>5</sup> CTNAG	Y00449	Ho4,Lu2,Sz3
M· <i>Dpn</i> II	G <sup>m</sup> ATC	M14339	De1,La3,La4,La5,Ma6
M· <i>Dpn</i> A	G <sup>m</sup> ATC	M14339	De1
M· <i>Eae</i> I	YGG <sup>m</sup> CCR		Ja1,Wh1
M· <i>Eag</i> I	CGGCCG		Sz2
M· <i>Eca</i> I	GGTN <sup>m</sup> ACC	X17111	Br3
M· <i>Eco</i> dam	G <sup>m</sup> ATC	V00272	Br6,Bu5,Dr1,Gi2,Ha4,He5,Ur1
M· <i>Eco</i> dcmI	C <sup>m</sup> CWGG	M32307	Bo7,Ma10,So1,Ur1
M· <i>Eco</i> dcmII	R <sup>m</sup> CCGG		Bu4,Ne8
M· <i>Eco</i> dcmIII	m <sup>5</sup> CCWGG		Ni2
M· <i>Eco</i> dcmIV	GGWC <sup>m</sup> C		Mo1,Ni2
M· <i>Eco</i> AI	G <sup>m</sup> AGN,G <sup>m</sup> TCA <sup>b</sup>	J03150	Co7,Fu2

Table III. (cont.)

Methylase <sup>a</sup>	Specificity <sup>a</sup>	EMBL accession #	References
M·EcoBI	TG <sup>m6</sup> AN <sub>n</sub> TGCT <sup>b</sup>	J01630	Go3
M·EcoD	TTAN <sub>7</sub> GTCY <sup>b</sup>	J01631	Go3
M·EcoDXXI	TCAN <sub>7</sub> ATTC <sup>b</sup>	X73984	Gu1
M·EcoEI	GAGN <sub>7</sub> ATGC <sup>b</sup>	J03162	Fu2
M·EcoKI	A <sup>m6</sup> ACN <sub>6</sub> G <sup>m</sup> TGC <sup>b</sup>	J01632,L02508	Bo3,Go3,Ka1,Lo2,Sa1
M·EcoPI	AG <sup>m6</sup> ACC <sup>b</sup>	X06287,X07312	Ba2,Hu2
M·Eco P1 dam	G <sup>m6</sup> ATC <sup>b</sup>		Co5
M·EcoP15I	CAGC <sup>m6</sup> AG	X06288,X07312	Hu2,Me2
M·EcoRI	GA <sup>m6</sup> ATTC	J01675	Du1,Gr2,Ke2,Ne2,Ne9,Ru1
M·EcoRII	C <sup>m5</sup> CWGG	X16025,X05050	Bh1,Bu3,Bu4,Ko6,Ko7,Ko8, Ma10,Sc6,So2,Yo4
M·EcoRV	G <sup>m6</sup> ATATC	X00530	Bo4,Ga5
M·EcoR124	GA <sup>m6</sup> AAN <sub>6</sub> RTCG		Pa3,Pr2,Pr3
M·EcoR124/3	GA <sup>m6</sup> AN <sub>7</sub> RTCG	X13145	Pr2,Pr3
M·EcoT1 dam	G <sup>m6</sup> ATC	J05393	Sc1,Sc7
M·EcoT2 dam	G <sup>m6</sup> AT	M22342	Br7,Ha4,Ha5,Mi1,Sc4,Sc5
M·EcoT4 dam	G <sup>m6</sup> ATC	X01416	Ha6,Ma1,Mi2,Sc3,Sc4,Sc5
M·Eco31I	GGT <sup>m5</sup> CTC and G <sup>m6</sup> AGACC		Bi7
M·Eco47II	GGNCC		Po6
M·Eco51I	CTGAAG (m <sup>6</sup> A)		Po6
M·Eco57I	CTGA <sup>m6</sup> AG and CTTC <sup>m6</sup> AG	M74821,X61122	Ja8,Ja9,Po6
M·Eco64I	GGYRCC		Po6
M·Eco72I	CACGTG (m <sup>5</sup> C)		Po6
M·Eco98I	AAGCTT		Po6
M·Eco105I	TACGTA		Po6
M·ErhI	G <sup>m6</sup> ATC		Ne5
M·Esp3I	GGT <sup>m5</sup> CTC and GAG <sup>m6</sup> ACC		Bi7,Ja3
M·eukaryote	m <sup>5</sup> CG <sup>b</sup>	X14805 X63692 L10692	Be7 (Mouse) (human) Fi2 (Arabidopsis) Lu2,Va1
M·FnuDI	GG <sup>m5</sup> CC		Lu2,Ne14
M·FnuDII	m <sup>5</sup> CGCG		Lu2
M·FnuDIII	GC <sub>2</sub> C		La6,Lo3,Lu2,Ma8,Nw1
M·FokI	GG <sup>m6</sup> ATG and C <sup>m6</sup> ATCC	J04623,M28828	
M·FspI	TGCGCA		Me1
M·FsuI	GGWCC (m <sup>5</sup> C)		Le1
M·FV3	?? <sup>m</sup> C??		Es1 (Frogvirus)
M·H2	GGCC and GCNGC and GDGCHC	M72412	La8
M·HaeII	RGC <sub>2</sub> CY		
M·HaeIII	GG <sup>m5</sup> CC <sup>b</sup>	M24625	Lu2,Ma5,Sl3
M·HapII	C <sup>m5</sup> CGG		Wa1
M·Hgal	GA <sup>m5</sup> CGC and G <sup>m5</sup> CGTC	D90363	Lu2,Nw1 Wi7
M·HgiAI	GWGCWC		Lu2
M·HgiBI	GGWCC (m <sup>5</sup> C)	X55137	Kr4,Du2
M·HgiCI	GGYRC <sup>m5</sup> C	X55138	Kr4,Er1
M·HgiCII	GGWCC (m <sup>5</sup> C)	X55139	Kr4,Er1
M·HgiDI	GRC <sub>2</sub> YC (m <sup>5</sup> C)	X55140	Kr4,Du4
M·HgiDII	GTCGAC (m <sup>5</sup> C)	X55141	Kr4,Du3
M·HgiEI	GGWCC (m <sup>5</sup> C)	X55142	Kr4
M·HgiGI	GRC <sub>2</sub> YC (m <sup>5</sup> C)	X55143	Kr4,Er1
M·Hhal	G <sup>m5</sup> CGC	J02677	Ba9,Ca3,Lu2,Sm1,Wu1,Za1
M·HhalII	G <sup>m6</sup> ANTC	K00508	Ch1,Ke1,Ma3,Ma4,Sc9,Sm1
M·HincII	GTYR <sup>m6</sup> AC	X52124	Gr5,Mc8,Ro7,Re2
M·HindII	GTYR <sup>m6</sup> AC		Lu2,Re2,Ro6,Ro7
M·HindIII	m <sup>6</sup> AAGCTT		Lu2,Ro6,Ro7
M·HinfI	G <sup>m6</sup> ANTC	M22862	Ch1,Lu2
M·HinPI	GC <sub>2</sub> C		Ba9,Lu2
M·HjaI	GATATC (m <sup>6</sup> A)		Da1
M·HpaI	GTTA <sup>m6</sup> AC	D10668	Br8,It1,Yo3
M·HpaII	C <sup>m5</sup> CGG	X51322	Lu2,Ma5,Qu1,Wi2,Yo2
M·HphI	T <sup>m5</sup> CACC		Mc8,Ne2,Ne4
M·KpnI	GGT <sup>m6</sup> ACC	X61796	Ch2,Ki4
M·KpnII	TCCGGA		Po6
M·LlaI	?	M77136	Hi1
M·MboI	G <sup>m6</sup> ATC	D13968	Mc8,Ue1
M·MboII	GAAG <sup>m6</sup> A	X56977	Mc12,Ne4,Ne2
M·MspI	m <sup>5</sup> CCGG <sup>b</sup>	X14191	Eh2,Je2,Lu2,Nw2,Wa1,Wa5
M·MstI	TGCGCA		Me1

Table III. (cont.)

Methylase <sup>a</sup>	Specificity <sup>a</sup>	EMBL accession #	References
<b>M·MthTII</b>	GG <sup>m5</sup> CC	X68366,X97222	No4,No6
<b>M·MthZII</b>	m <sup>4</sup> CTAG	X67212,X68367	No4,No5
<b>M·MunI</b>	CA <sup>m6</sup> ATTG	X76192	St8
<b>M·MvaI</b>	C <sup>m4</sup> CWGG	X16985	Bu8,Kl2,Po6
<b>M·MwoI</b>	GCN <sub>n</sub> GC (m <sup>4</sup> C)		Lu2,Lu4
<b>M·NaeI</b>	GCCGGC		Lu2,Va1
<b>M·NcoI</b>	CCATGG (m <sup>3</sup> C)		Lu2,Va1
<b>M·NdeI</b>	CAT <sup>m6</sup> ATG		Re7,Si1,We3
<b>M·NgoMI</b>	G <sup>m5</sup> CCGGC <sup>b</sup>	M86915	Pi5,St9
<b>M·NgoMVI</b>	GGNN <sup>m5</sup> CC <sup>b</sup>		Pi5
<b>M·NgoI</b>	RG <sup>m5</sup> CGCY <sup>b</sup>		Ko5,Ri1,St9,Su2
<b>M·NgoAI</b>	GG <sup>m5</sup> CC <sup>b</sup>		Pi3
<b>M·NgoII</b>	GG <sup>m5</sup> CC <sup>b</sup>	X52661,X06965	Ko5,Ri1,Su3,Su4
<b>M·NgoIII</b>	CCGCGG <sup>b</sup>		Ko5,Ri1,St9
<b>M·NgoIV</b>	G <sup>m5</sup> CCGGC <sup>b</sup>		Ch3,Ko15,Ri1,St9
<b>M·NgoV</b>	GGNN <sup>m5</sup> CC <sup>b</sup>		Ko5,Pi2,St9
<b>M·NgoVI</b>	G <sup>m6</sup> ATC <sup>b</sup>		Ko5,St9
<b>M·NgoVII</b>	G <sup>m5</sup> CSGC <sup>b</sup>		Gu4,Ko5,St9
<b>M·NgoIX</b>	GTAN <sup>m5</sup> CCTC <sup>b</sup>		St9
<b>M·NgoBI</b>	T <sup>m5</sup> CACC <sup>b</sup>		Pi3
<b>M·NgoBII</b>	GTAN <sub>n</sub> m <sup>5</sup> CTC <sup>b</sup>		Pi3
<b>M·NlaI</b>	GGCC		Mo3
<b>M·NlaIII</b>	C <sup>m6</sup> ATG	X54485	La1,Lu2,Mo3
<b>M·NlaIV</b>	GGNNCC (m <sup>5</sup> C)	U06074	Lu2
<b>M·NlaV</b>	GGNN <sup>m5</sup> CC		Mo3
<b>M·NlaX</b>	??"C"?	X54485	La1
<b>M·NspV</b>	TTCGAA	D14719	Ue2
<b>M·PaeR7I</b>	CTCG <sup>m6</sup> AG	X03274	Gi3,Th1,Th2
<b>M·PglI</b>	G <sup>m6</sup> ATC	M63469	Ba10
<b>M·PstI</b>	CTGC <sup>m6</sup> AG	K02081	Le2,Wa3,Wa4,Wa6
<b>M·PvuI</b>	CGATCG	L04163	Sm5
<b>M·PvuII</b>	CAG <sup>m4</sup> CTG	X52681,X13778	Bl1,Ta2
<b>M·Rrh4273I</b>	GTCC <sup>m6</sup> AC		Ba6,Ye1
<b>M·RsaI</b>	GT <sup>m6</sup> AC		Ne5
<b>M·RsrI</b>	GA <sup>m6</sup> ATTC	X14697,X16456	Ba5,Ka10
<b>M·SacII</b>	CCGCGG		Lu2
<b>M·SalI</b>	GTCC <sup>m6</sup> AC		Lu2,Ro4,Ro5
<b>M·SauLPI</b>	G <sup>m5</sup> CCGGC		Ok1
<b>M·Sau3AI</b>	GAT <sup>m5</sup> C	M32470	Se1
<b>M·Sau96I</b>	GGN <sup>m5</sup> CC	X53096	Lu2,Ne14,Sz1
<b>M·Sau3239I</b>	CTCG <sup>m6</sup> AG		Ze1
<b>M·ScrFI</b>	C <sup>m</sup> CNGG	M87289	Da4
<b>M·SfiI</b>	GG <sup>m4</sup> CCN <sub>n</sub> GGCC		Ba8,Ne5
<b>M·SinI</b>	GGW <sup>m5</sup> CC	J03391	Ka5,Ka6
<b>M·SmaI</b>	CC <sup>m4</sup> CGGG	X16458	He1,Po6
<b>M·SphI</b>	GCATGC		Lu2
<b>M·SsoI</b>	G <sup>m6</sup> AATTG	M97479	Ka9
<b>M·SsoII</b>	C <sup>m5</sup> CNGG	M86545	Ka9,Ni1,Ni3
<b>M·SspMQI</b>	m <sup>5</sup> CG		Nu1,Pi5,Re3
<b>M·SssI</b>	m <sup>5</sup> CG	X17195	Ko10,Re3
<b>M·StsI</b>	GG <sup>m6</sup> ATG and C <sup>m6</sup> ATCC	D11101	Ki5
<b>M·StyLTI</b>	CAG <sup>m6</sup> AG	M90544	Da5
<b>M·StyR124</b>	GAAN <sub>n</sub> RTCG (m <sup>6</sup> A)		Pr2,Pr3
<b>M·StyR124/3</b>	GAAN <sub>n</sub> RTCG (m <sup>6</sup> A)		Pr2,Pr3
<b>M·StySBI</b>	G <sup>m6</sup> AGN <sub>n</sub> R <sup>m</sup> TYG <sup>b</sup>		Fu1,Fu3,Ga3,Na1,Na2
<b>M·StySPI</b>	A <sup>m6</sup> ACN <sub>n</sub> G <sup>m</sup> TRC <sup>b</sup>		Fu1,Fu3,Na1,Na2
<b>M·StySQ</b>	A <sup>m6</sup> ACN <sub>n</sub> R <sup>m</sup> TAYG <sup>b</sup>		Fu1,Fu3
<b>M·StySJ</b>	G <sup>m6</sup> AGN <sub>n</sub> G <sup>m</sup> TRC <sup>b</sup>		Ga3
<b>M·Taql</b>	TCG <sup>m6</sup> A	M76680,M76681 Y00499	Lu2,Mc3,Sa3,Si2 Ba13
<b>M·ThhHBI</b>	TCG <sup>m6</sup> A	M74795	Ba11,Mc3,Sa3 Sa3,Va6
<b>M·TflI</b>	TCG <sup>m6</sup> A		Ca2,Go1
<b>M·Tetrahymena</b>	??"A"?		De2
<b>M·VspI</b>	ATTAAT (m <sup>6</sup> A)	X68658	Lu2,Mc13,Va1
<b>M·XbaI</b>	TCTAG <sup>m6</sup> A		Wi6
<b>M·XcyI</b>	C <sup>m4</sup> CCGGG	M98768	Ba8
<b>M·XmaI</b>	CCCGGG (m <sup>4</sup> C)		Gu9,Mc8,Tr5
<b>M·XmaIII</b>	CGG <sup>m4</sup> CCG		Fe1
<b>M·XmnI</b>	GAAN <sub>4</sub> TTC		

<sup>a</sup> See footnote 'a' of Table I.<sup>b</sup> See footnote 'b' of Table I.

Cloned methylases are shown in bold face type.