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The effect of site-specific methylation on restriction-modification enzymes

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INTRODUCTION

Previous tabulations of restriction endonuclease sensitivities to site-specific DNA methylation have shown that these endonucleases cannot cut particular DNA recognition sequences which have been methylated at ^{4}mC, ^{5}mC or ^{6}mA (54,65,69,71,72).

Since our previous tabulation in this journal (72) the major new additions are extensive data on ^{4}mC. We have altered our notation to incorporate the ^{4}mC data and added a number of footnotes. Fine structural details of cleavage reactions, rate differences on hemi- and bi-methylated substrates, and experimental discrepancies are noted where such data is available.

Knowledge of the sensitivity of restriction endonucleases to prior methylation is useful in a number of experimental situations: in studies of cellular DNA methylation (^{5}mC, ^{5}mCNG, G^{6}mATC, or methylated islands) (9,70,75), in the resolution of anomalous gel banding patterns in restriction mapping experiments (2,5), in the selection of non-restricting strains for genetic constructions (14,88) and in a variety of *in vitro* DNA manipulations (59,67,78).

In addition to an updated tabulation of restriction endonuclease methylation sensitivities, we outline below selected applications and practical considerations related to the effects of site-specific DNA methylation on restriction and modification enzymes.

Isoschizomer Pairs

A number of isoschizomer pairs are now available which differ in their sensitivity to site-specific methylation. Such endonuclease pairs are useful for studying the level and distribution of site-specific methylation in cellular DNA; for example, ^{5}mCpG in mammals, ^{5}mCpG and

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^{5}mCpNpG in plants or G^{6}mATC in enterobacteria (9,70,112):

Methylated Sequence	not cut by	cut by
C^{5}mCGG	Hpa II	Msp I
CC^{5}mCGGG	Sma I	Xma I
C^{5}mCWGG	Eco RII	Bst NI (Mva I)
TCCGG^{6}mA	Acc III	Bsp MII
G^{6}mATC	Mbo I	Sau 3A and Dpn I
RG^{6}mATCY	Mfl I (Miv AV)	Xho II

Other enzymes that are *not* sensitive to certain site-specific methylations are particularly useful. In some cases, as in physical mapping of heavily methylated plant DNA, it is desirable to choose restriction endonucleases which are *insensitive* to ^{5}mCG and ^{5}mCNG. Several endonucleases may be especially useful in this regard: Bcl I, BstE II, BstN I, Dra I, EcoR V, Hin c II, Hpa I, Kpn I, Mbo II, Nde I, Rsa I, Ssp I, Taq I and Xmn I. Undoubtedly, many other endonucleases will be useful when more data on their methylation sensitivity is known.

Rate Effects

Much data has accumulated recently on rate effects of site-specific methylation on restriction endonuclease cleavage. As a practical matter, we have observed that site-specific methylation inhibits duplex DNA cleavage by most restriction endonucleases in ten- to twenty-fold overdigestions (34,77). Therefore, *most* restriction endonucleases exhibit "all-or-none" effects with respect to methylation inhibition under commonly used reaction conditions (54,71,72,77,78). Furthermore, hemi-methylation is usually sufficient to block restriction endonucleases from double-stranded DNA cleavage (34,77). Nevertheless, rate effects or nicking of one strand at certain methylated target sites are observed with a number of enzymes, such as Aha II, Ava I, Hin f I, Bal I, Ban I, Bgl I, Bgl II, Eco R I, Hae II, Kpn I, Mfl I, Msp I, Sau 3A, Taq I and Xmn I. For instance:

Ava I (CYCGRG) will cleave very slowly when its recognition sequence is methylated at a wobble position CTCG^{6}mAG but cleaves normally at C^{5}mCCGGG (77,80). Similarly, Mfl I (RGATCY) cuts more slowly at ^{6}mAGATCY sites (83).

Aha II (GRCGYC) will cut GRCGCC *faster* if these sites are methylated at GRCG^{5}mCC (66).

Bal I (TGGCCA) cuts at a 50-fold slower rate at TGGC^{5}mCA (31).

Ban I (GGYRCC), Bgl I (GCCN$_5$GGC) and Hae II (RGCGCY) give various

rate effects when their recognition sequences are methylated at different sites (53,80).

Eco R I shows a reduce rate of cleavage at hemi-methylated GAAT5mC and does not cut an oligonucleotide that contains GAATT5mC in both strands (11).

Hin fI cuts unmethylated GANTC faster than hemimethylated GANT5mC/GANTC, which is cut faster than GAT5mC/GANT5mC. However, the rate difference between unmethylated and fully methylated Hin fI sites is only about ten-fold (39,79). Xmn I cuts slowly at some sites in DNA methylated on both strands at $GAAN_4TT$5mC (79).

In a few cases a particular methylation can inhibit cleavage on only one strand of a hemi-methylated DNA duplex. Taq I can cut the unmethylated strand of TCG6mA/TCGA duplexes; and Sau3A I can cut the unmethylated strand of GAT5mC/GATC duplexes (2,104). Msp I cuts the unmethylated strand of C5mCGG/CCGG duplexes (114). Acc I cuts the unmethylated strand of GTMK5mC and Xho II cuts the unmethylated strand of RGAT5mCY (87). Further differences in strand preference and details of the reaction mechanisms of Msp I, Hpa II, and Mno I isoschizomers have been described (3). Bgl II, Bsu RI, Hae III and Mbo I have also been reported to show strand preferences in cutting hemi-methylated substrates (12,36,83).

In at least four cases, modified flanking sequences result in altered restriction endonuclease cleavage rates. Msp I fails to cut at GGC5mCGG (15,51). Sau 3A I cuts at a reduced rate at 6mAGATC (83). Fnu 4H I and Bsu R I exhibit drastically reduced cleavage rates when flanked by modified thymine residues (117).

Effect of Site-specific Methylation on Other DNA Binding Proteins

Many Type II restriction endonucleases are sensitive to site-specific methylation at more than one position (see Table). Such sensitivity to a number of different site-specific DNA methylations is clearly *not* limited to restriction endonucleases, but is a property of DNA binding proteins in general (see 102,118). We and others have recently demonstrated that site-specific methylation at *'non-canonical'* sites will block certain Type II DNA methylases. This data, which is not presented in the Table, can be summarized as follows:

Methylase	Blocked by Prior Methylation at	Not Blocked by Prior Methylation at
M.Hpa II (C5mCGG)	5mCCGG	(66,67)

M.Msp I (^{5}mCCGG)	C^{5}mCGG		(66)
M.Eco RII (C^{5}mCWGG)	C^{4}mCWGG		(16)
M.Bam HI (GGAT^{5}mCC)	GGATC^{5}mC	GG^{6}mATCC	(66)
M.Eco RI (GA^{6}mATTC)	G^{6}mAATTC	GAATT^{5}mC	(11)
E.coli dam (G^{6}mATC)		GAT^{5}mC	(66)
M.Mbo II (GAAG^{6}mA)		T^{5}mCTT^{5}mC	(66)
M.Hha II (G^{6}mANTC)		GANT^{5}mC	(66)
M.Taq I (TCG^{6}mA)		T^{5}mCGA	(66)
M.Mva I*(C^{4}mCWGG)		C^{5}mCWGG	(16)

Megabase Mapping: Double Methylation Reactions, Dpn I Cleavages, and Cross-Protections

Modification methylases can differ from their corresponding restriction endonucleases in their sensitivity to site-specific methylation. This difference in *non-canonical* methylation sensitivity is not surprising, since methylases and endonucleases from the same restriction system show little or no protein sequence homology. It is possible to take practical advantage of such differences in the methylation sensitivity of methylase/endonuclease pairs. For example, M.Bam HI will not methylate GGATC^{5}mC, but Bam HI will cut this methylated sequence. On this basis we were able to show that methylation of DNA with M.Hpa II (C^{5}mCGG), followed by M.Bam HI (GGAT^{5}mCC), will allow cutting of DNA by Bam HI only at the ten base pair sequence CCGGATCCGG. M.Hpa II methylation blocks overlapping M.Bam HI methylation, while permitting Bam HI cleavage at these site (66,67).

The lack of ^{5}mC inhibition of two adenine methylases, M.Mbo II (GAAG^{6}mA) and M.Taq I (TCG^{6}mA) , and of Dpn I (G^{6}mATC) endonuclease, has practical consequences. Highly selective DNA cleavage schemes involving M.Taq I-Dpn I (at TCG^{6}mATCGA) and M.Mbo II-Dpn I (at GAAG^{6}mATCTTC) are possible on *methylated* chromosomal DNAs (73,74). Cross-protection of a subset of restriction endonuclease cleavage sites by overlapping methylation has been described (77,78). This strategy has produced over 50 new cleavage specificities and many more are possible (42).

Methylation-Dependent Restriction Systems in E.coli

It has only recently been recognized that E.coli K contains at least three different methylation-dependent restriction systems which distinguish various methylated target sequences: mar (^{6}mA), mcr A (^{5}mCG), and mcr B (Pu^{5}mC) (14,38,88,87). Therefore, non-restricting

strains of E.coli (87,88) are to be preferred for transformation of methylated DNA.

A knowledge of the specificities of methylation-dependent restriction systems and of the methylation state of the DNA to be transformed will also be of use in other species that carry methyl-dependent restriction systems, such as some Streptococcus (Dpn I), Neisseria (Nsu DI and Nan II), Flavobacterium (Fsa I) and Acholeplasma laidlawii (JA1) (see 14,17 and 55 for references).

α denotes a known modification methylase 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
^{4}mC=4-methylcytosine, ^{5}mC=5-methylcytosine, ^{6}mA=6-methyladenine
Nomenclature is in accordance with (99) and (18)

Restriction Enzyme	Recognition Sequence	Sites cut	Sites not cut	References
Mnl I	CCTC(r)			26
Alu I	AGCT	?	^{6}mAGCT AG^{5}mCT α	34,77,79
Bsu F I	CCGG	?	^{5}mCCGG α	48
Hap II	CCGG	?	C^{5}mCGG α	24,112
Hpa II	CCGG	?	C^{5}mCGG α ^{5}mCCGG (a) ^{4}mCCGG C^{4}mCGG	24,60,62,82,86,114
Msp I	CCGG	C^{5}mCGG (b)	^{5}mCCGG α (a) (c)	24,47,106,112,114
Tha I	CGCG	?	^{5}mCGCG CG^{5}mCG	103
Fnu D II	CGCG		^{5}mCGCG CG^{5}mCG	77,78,103
Bst E III	GATC (d)	?	G^{6}mATC	75,90
Dpn I	G^{6}mATC (x)	G^{6}mATC G^{6}mAT^{5}mC		55,111
Fnu E I	GATC	G^{6}mATC	?	58,77
Mbo I	GATC (d)	GAT^{5}mC	G^{6}mATC α	14,29,64,90
Pfa I	GATC	G^{6}mATC	?	90,108
Sau 3A	GATC (d)	G^{6}mATC	GAT^{5}mC (a)	21,24,46,68,90
Hha I	GCGC	?	G^{5}mCGC α GCG^{5}mC (w)	24,63,100
Hin P I	GCGC	?	G^{5}mCGC	78,79
Bsu R I	GGCC	?	GG^{5}mCC α (a)	35
Hae III	GGCC	GGC^{5}mC	GG^{5}mCC α (a)	2,53,60,62
Ngo II	GGCC	?	GG^{5}mCC α	52,53
Rsa I	GTAC (e)	GTA^{5}mC(f)		26,79
Taq I	TCGA	T^{5}mCGA	TCG^{6}mA α	34,68,106
Tth I	TCGA	T^{5}mCGA	TCG^{6}mA α	95
Tfl I	TCGA	?	TCG^{6}mA α	95

Restriction Enzyme	Recognition Sequence	Sites cut	Sites not cut	References
Scr F I	CCNGG	^{5}mCCNGG	C^{5}mCNGG	77,79
Dde I	CTNAG	?	^{5}mCTNAG	77
Hinf I	GANTC	GANT^{5}mC(f,g)	G^{5}mANTC	77,80
Fnu 4H I	GCNGC	?	G^{5}mCNGC	53,105
			GCNG^{5}mC	
Sau 96 I	GGNCC	?	GGN^{5}mCC	53,64,77,80
			GGCC^{5}mC	
Aac I	CCWGG	C^{5}mCWGG	?	13
Apy I	CCWGG	C^{5}mCWGG	^{5}mCCWGG	19,65,89,90
Bst N I	CCWGG (h)	C^{5}mCWGG	?	34,39,65,90
		^{5}mCCWGG (i)		
		^{5}mC^{5}mCWGG (f)		
Eco R II	CCWGG (h)	^{5}mCCWGG	C^{5}mCWGG α	10,16,64,65,76,90,98
			C^{4}mCWGG	
Mph I	CCWGG (h)	?	C^{5}mCWGG	49,90
Mva I	CCWGG	?	C^{4}mCWGG α	16
Taq X I	CCWGG	^{5}mCCWGG	C^{5}mCWGG	33
Bcn I	CCSGG	^{5}mCCSGG	C^{5}mCSGG α	43,44,46
			C^{4}mCSGG α	
Nci I	CCSGG	^{5}mCCSGG	C^{5}mCSGG (j)	13,65,87
			C^{4}mCSGG	
Bbv I	GCWGC	?	G^{5}mCWGC α	20,36,110
Ava II	GGWCC	?	GGWC^{5}mC	2,53,63,66,79
			GGW^{5}mCC	
Eco 47 I	GGWCC	?	GGWC^{5}mC	45
Eco P I	AGACC (k)	?	AG^{6}mACC α	1,37
Bsp MI	ACCTGC	?	^{5}mC	66
Fok I	CATCC	?	CATC^{5}mC	79
Mbo II	GAAGA	T^{5}mCTT^{5}mC(f,l)	GAAG^{6}mA α	2,74,77,79
Hga I	GACGC (e)	?	GACG^{5}mC	79
Sfa N I	GATGC	GATG^{5}mC	?	79
Bin I	GGATC	?	GG^{6}mATC	8
Hph I	TCACC	?	T^{5}mCACC α	77,79
			GGTG^{6}mA	
Bsp I 1286	GDGCHC	?	GDG^{5}mCHC	77
Ava I	CYCGRG	C^{5}mCCGGG	CY^{5}mCGRG	7,24,48,50,65,77
			CmTCG^{6}mAG (y)	
HgiJ II	GGYRCC		GGYRC^{5}mC	119
Aos II	GRCGYC	?	GR^{5}mCGYC	24,34,106
Aha II	GRCGYC	?	GR^{5}mCGYC	77
			GRCGY^{5}mC	
Ban II	GRGCYC	?	GRG^{5}mCYC	77
Acc I	GTMKAC	?	GTMK^{6}mAC	68, 87
			GTMKA^{5}mC(a)	
Hin C II	GTYRAC	GTYRA^{5}mC	GTYR^{6}mAC α	34,93
Hgi A I	GWGCWC	?	GWG^{5}mCWC	77,119

Restriction Enzyme	Recognition Sequence	Sites cut	Sites not cut	References
Hae II	RGCGCY (e)	?	RG^{5}mCGCY	24,34,53,79
Ngo I	RGCGCY	?	RG^{5}mCGCY α	52,53
Xho II	RGATCY	RG^{6}mATCY	RGAT^{5}mCY(a)	13
Miv A V	RGATCY		RG^{6}mATCY RGAT^{5}mCY	72,79
Mfl I	RGATCY		RG^{6}mATCY RGAT^{5}mCY RGAT^{4}mCY	83
Eae I	YGGCCR	?	YGGC^{5}mCR YGG^{5}mCCR α	42,116
Hind III	AAGCTT	?	^{6}mAAGCTT α AAG^{5}mCTT	13,34,93
Mlu I	ACGCGT	^{6}mACGCGT	?	79
Bgl II	AGATCT (e)	AG^{6}mATCT	AGAT^{5}mCT	6,13,21,23,26,84
Stu I	AGGCCT	?	AGG^{5}mCCT AGGC^{5}mCT (n)	79
Cla I	ATCGAT	?	ATCG^{6}mAT α AT^{5}mCGAT	68
Pvu II	CAGCTG	?	CAG^{5}mCTG CAG^{4}mCTG α	13,20,26,46,91
Nde I	CATATG	^{5}mCATATG (f)	?	79
Nco I	CCATGG	?	^{5}mCCATGG (m)	77
Sma I	CCCGGG	^{5}mCCCGGG C^{5}mCCGG	CC^{5}mCGGG (j)α CCm^{4}CGGG C^{4}mCCGGG ^{4}mCCCGGG	13,24,28,46,82,86
Cfr q I	CCCGGG	C^{5}mCCGGG ^{5}mCCCGGG	CC^{4}mCGGG C^{4}mCCGGG ^{4}mCCCGGG	82
Xma I	CCCGGG	CC^{5}mCGGG (p)	C^{5}mCCGGG	121,122
Sac II	CCGCGG	?	^{5}mCCGCGG	77
Pvu I	CGATCG (e)	CG^{6}mATCG	CGAT^{5}mCG	13,26
Xor II	CGATCG	CG^{6}mATCG	CGAT^{5}mCG	13,24
Eag I	CGGCGG	?	CGG^{5}mCCG ^{5}mCGGC^{5}mCG	66
Xma III	CGGCGG	?	CGG^{5}mCCG α	105
Bsu M I	CTCGAG	?	CT^{5}mCGAG α	48
Pae R 7	CTCGAG	?	CTCG^{6}mAG α	30
Xho I	CTCGAG	?	CT^{5}mCGAG CTCG^{6}mAG	13,24,26,68,106
Pst I	CTGCAG	?	CTGC^{6}mAG α ^{5}mCTGCAG	20,34,77,79,113
Sfl I	CTGCAG	?	CTGC^{6}mAG	13
Eco R I	GAATTC		GA^{6}mATTC α G^{6}mAATTC (q) GAATT^{5}mC	11,13,22,25,77,79,94
Rsr I	GAATTC		G^{6}mAATTC (q) GA^{6}mATTC	79
Sac I	GAGCTC	G^{6}mAGCTC	GAG^{5}mCTC	79
Sst I	GAGCTC	?	GAG^{5}mCTC	13,91

Restriction Enzyme	Recognition Sequence	Sites cut	Sites not cut	References
Eco R V	GATATC	GATAT^{5}mC(f)	G^{6}mATATC	77,79
Nae I	GCCGGC (e)	?	G^{5}mCCGGC GCCGG^{5}mC	26,77,79
Sph I	GCATGC	GCATG^{5}mC	?	77
Nhe I	GCTAGC	?	5mC	77,79
Bam H I	GGATCC	GGATC^{5}mC GG^{6}mATCC	GGAT^{5}mCC	13,21,36,62
Nar I	GGCGCC	GGCGC^{5}mC	GG^{5}mCGCC	53,65,79
Kpn I	GGTACC (e)	GGTA^{5}mCC (r) GGTAC^{5}mC GGTA^{5}mC^{5}mC (f)	?	26,79
Apa I	GGGCCC	?	GGG^{5}mCCC α	105
Sal I	GTCGAC	?	GTCG^{6}mAC GT^{5}mCGAC	13,24,68,106
Hpa I	GTTAAC	GTTAA^{5}mC	GTTA^{6}mAC α	13,34,39,120
Acc III	TCCGGA	?	TCCGG^{6}mA	97
Bsp M II	TCCGGA	TCCGG^{6}mA	?	97
Nru I	TCGCGA	?	TCGCC^{6}mA	77
Xba I	TCTAGA	?	T^{5}mCTAGA TCTAG^{6}mA	34,39,77
Atu C I	TGATCA	?	TG^{6}mATCA	90,98
Bcl I	TGATCA (e)	TGAT^{5}mCA	TG^{6}mATCA	2,6,13,26,90
Bst G I	TGATCA		TG^{6}mATCA	90
Cpe I	TGATCA	?	TG^{6}mATCA	27,90
Bal I	TGGCCA	?	TGG^{5}mCCA α TGGC^{5}mCA (s)	31,105
Bst X I	CCAN$_{6}$TGG	?	^{5}mCCAN$_{6}$TGG CC^{6}mAN$_{6}$TGG	77
Mst II	CCTNAGG	^{5}mCCTNAGG	?	79
Xmn I	GAAN$_{4}$TTC	GA^{6}mAN$_{4}$TTC GAAN$_{4}$TT^{5}mC (u)	G^{6}mAAN$_{4}$TTC	77,79
Bgl I	GCCN$_{5}$GGC	GC^{5}mCN$_{5}$GGC G^{5}mCCN$_{5}$GGC	GCCN$_{5}$GG^{5}mC (t)	53,77,79
Bst E II	GGTNACC	GGTNA^{5}mC^{5}mC	?	39
Eco K	AACN$_{6}$GTGC (v)	?	A^{6}mACN$_{6}$GmTGC (y) α	4
Eco A	GAGN$_{7}$GTCA (v)	?	G^{6}mAGN$_{7}$GmTCA (y) α	4
Eco B	TGAN$_{8}$TGCT (v)	?	TG^{6}mAN$_{8}$mTGCT (y) α	4,56,57
Rsr II	CGGWGGC		CGGW^{5}mCCG ^{5}mCGGWC^{5}mCG	66
Not I	GCGGCCGC	GCGGCCG^{5}mC	GCGG^{5}mCCGC	79
Sfi I	GGCCN$_{5}$GGCC	GG^{5}mCCN$_{5}$GG^{5}mCC (z) GGCCN$_{5}$GGC^{5}mC	?	79

Notes

a) Nicking occurs in the unmethylated strand of the hemimethylated sequence. For Hpa II see (114), for Sau 3A see (2,83,104), for Msp I and Hae III see (36), for Bsu RI see (12).
b) Msp I fails to cut GGC^{5}mCGG (15,51).
c) An M.Msp I clone methylates ^{5}mCCGG (114,115). However, there is a report that Msp chromosomal DNA is methylated at ^{5}mC^{5}mCGG (47).
d) Mbo I isoschizomers that are sensitive to G^{6}mATC include Bss G II, Bsa P I, Bst X II, Bst E III, Cpa I, Dpn II, Fnu A II, Fnu C I, Mno III, Mos I, Nde II, Nfl I, Nla II, Nsu I, Sin M I (90). PVCV-I (107) and Sau 3A I isoschizomers that are insensitive to GmATC include Bsr P II, Cpf I, Fnu E I, Mth I, Nsi A I, Pfa I (90).
e) From genomic base composition: M.Mnl I and M.Kpn I may be ^{5}mC or ^{6}mA specific methylases; M.Rsa I, M.Hga I, M.Pvu II, M.Pvu I and M.Xho I may be ^{4}mC or ^{6}mA specific; M.Hae II and M.Nae I may be ^{5}mC specific; M.Bgl II may be ^{4}mC specific and M.Bcl I may be ^{6}mA specific (17a).
f) Unpublished observations show cutting of phage XP12 DNA (79).
g) Hin f I cuts GANT^{5}mC however, detectable rate differences are observed between unmethylated, hemimethylated (GANT^{5}mC/CTNAG) and bi-methylated (GANT^{5}mC/m^{5}CTNAG) target sequences. Hin f I does cut phage XP12 DNA, although at a reduced rate (34, 79).
h) Isoschizomers of Eco R II that are sensitive to C^{5}mCXGG include Atu B I, Atu II, Bst G II, Bin S I, Cfr 5 I, Cfr II I, Ecl II, Eca II, Eco 27 I, Eco 38 I and Mph I (90).
Bst N I isoschizomers that are insensitive to C^{5}mCXGG include Aor I, Apy I, Mva I and Taq XI (70).
i) Bst N I cuts C^{5}mCWGG, ^{5}mCCWGG and ^{5}mC^{5}mCWGG. M.Bst N I may be a N-4 cytosine methylase (65).
j) Sma I and Nci I may cut ^{5}mC^{5}mCGG methylated DNA (13,47). Possibly the second methylation negates the effect of C^{5}mCGG. m^{5}CCZGG is cut by Nci I (53), but M.Bcn I modified plasmid DNA (C^{4}mCZGG) is not cut by Nci I (87).
k) Type III restriction endonuclease (1,37).
l) Mbo II does cut XP12 phage DNA (79) although certain hemimethylated ^{5}mC-containing substrates are not cut (34).
m) Nco I is blocked by M.Sec I (CCNNGG) (79).
n) Stu I does not cut at overlapping AGGC^{5}mCTGG Stu I-dcm^{+} sites (79).
p) There is a report that Xma I does not cut CC^{5}mCGGG (13).
q) Hemi-methylated G^{6}mAATTC/ GAATTC sites cannot be cut by Eco RI or Rsr I: Bimethylated GA^{6}mATTC/GA^{6}mATTC sites are not cut by Eco RI or Rsr I (79). Bimethylated G^{6}mAATTC/G^{6}mAATTC sites have not been tested with Rsr I.
r) Experimental results differ on Kpn I sensitivity to hemimethylated GGTA^{5}mCC and GTAC^{5}mC sites (53,77,79,85). The simplest explanation to resolve discrepancies is that rate effects are observed at certain m^{5}C methylated KpnI sites, especially at low enzyme-to-substrate ratios.
s) Overlapping (TGGC^{5}mCAGG) dcm-Bal I sites are 50-fold slower than unmethylated sites (31).
t) Different rates of Bgl I cleavage are observed at certain hemi-methylated ^{5}mC sites (overlapping M.Msp I - Bgl I and Hpa II - Bgl I sites). Bi-methylated ^{5}mC M.Hae III - Bgl I sites are completely refractory to Bgl I (53,77).
u) Xmn I cuts slowly at some XP12 phage sites (79).
v) Type I restriction endonuclease.
w) There is a report that Hha I does not cut GCGm^{5}mC (53).
x) Dpn I requires adenine methylation on both DNA strains. Isoschizomers of Dpn I include Cfu I (28), Nmu E I, Nmu D I and Nsu D I (17).
y) mT represents a 6-methyladenine in the complementary strand.
z) Bimethylated substrate.

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