## **Enzyme Information**

Enzyme	Recognition Sequence	Sites cut	Sites not cut	Enzyme	Recognition Sequence	Sites cut	Sites not cut
EcoR I	GAATTC	GAATTh <sup>m5</sup> C	G <sup>m6</sup> AATTC <sup>b</sup> GA <sup>m6</sup> ATTC#	Hph I	TCACC	TCAC <sup>m5</sup> C	T <sup>m5</sup> CACC# GGTG <sup>m6</sup> A
EcoR II	CCWGG	<sup>m5</sup> CCWGGb	GAATT <sup>m5</sup> C <sup>b</sup> m4CCWGG Cm4CWGG Cm5CWGG#	Kpn I	GGTACC <sup>b</sup>	GGTA <sup>™5</sup> CC GGTAC <sup>™5</sup> C GGTA <sup>™5</sup> C <sup>™5</sup> C <sup>b</sup> GGT <sup>™6</sup> ACC	GGT™¢A™5CC GGTAC™4C
			CC <sup>™6</sup> AGG h™⁵Ch™⁵CWGG	Kpn2 I	TCCGGA	TCCGG <sup>m6</sup> A	T <sup>m5</sup> CCGGA TC <sup>m5</sup> CGGA
EcoR V	GATATC	GATAT <sup>m5</sup> C <sup>b</sup>	G <sup>m6</sup> ATATC# GAT <sup>m6</sup> ATC	Ksp I	CCGCGG	?	m5CCGCGG Cm5CGCGG
EcoR 124	GAAN6RTCG <sup>b</sup>	?	GA™AN6RTCG GAAN6RmTCG	Mae II Mam I	ACGT GATN4ATC	?	A <sup>m5</sup> CGTb G <sup>m6</sup> ATN4 <sup>m6</sup> ATC
EcoR 124/3 Ehe I	GAAN7RTCG <sup>b</sup> GGCGCC	?	™A GG™5CGCC	Mbo I	GATC <sup>b</sup>	GAT <sup>m4</sup> C GAT <sup>m5</sup> C <sup>b</sup>	G <sup>m6</sup> ATC# GATh <sup>m5</sup> C
Esp I	GCTNAGC	GCTNAG <sup>m5</sup> C	G <sup>m5</sup> CTNAGC	Mbo II	GAAGA	T <sup>m5</sup> CTT <sup>m5</sup> C <sup>b</sup>	
Fnu4H I	GCNGC	?	G <sup>m5</sup> CNGC GCNG <sup>m5</sup> C	MfI I	REGATCY <sup>b</sup>	G <sup>m6</sup> AAGA ?	GAAG <sup>m6</sup> A# RG <sup>m6</sup> ATCY
FnuD II	CGCG	?	™CGCG CG™CG				RGAT™CY RGAT™CY
Fok I	CATCC	CAT <sup>m5</sup> CC	GG <sup>m6</sup> ATG	Mlu I	ACGCGT	m6ACGCGT	A™CGCGT
		CATC <sup>m5</sup> C <sup>b</sup>	C <sup>m6</sup> ATCC	Mme II	GATC	?	G <sup>m6</sup> ATC
Fse I	GGCCGGCC	?	CATC <sup>m4</sup> C GG <sup>m5</sup> CCGG <sup>m5</sup> CC	MnI I	CCTCb	?	<sup>m5</sup> CCTC <sup>m5</sup> C <sup>m5</sup> CT <sup>m5</sup> C
			GGC™5CGGCC GG™5CCGGCC	Mro I	TCCGGA	TCCGG <sup>m6</sup> A	T™5CCGGA TC™5CGGA
Fsp I	TGCGCA	?	TG <sup>m5</sup> CGCA	Mse I	TTAA	TTm6AA	?
Hae II	RGCGCY⁵	?	RG <sup>™5</sup> CGCY RGh <sup>™5</sup> CGh <sup>™5</sup> CY	Msp I	CCGG <sup>b</sup>	™⁴CCGG C™⁴CGG	<sup>™5</sup> CCGG# h <sup>™5</sup> Ch <sup>™5</sup> CGG
Hae III	GGCC	GGC <sup>m5</sup> C	GG™CC# GGh™Ch™C	Mst II	CCTNAGG	C <sup>m5</sup> CGG <sup>m5</sup> CCTNAGG	?
Hap II	CCGG	?	C <sup>m5</sup> CGG#	Mun I	CAATTG	?	CA <sup>m6</sup> ATTG
Hga I	GACGC	?	GA <sup>m5</sup> CGC GACG <sup>m5</sup> C	Mva I	CCWGG	C <sup>m5</sup> CWGG <sup>b</sup> <sup>m5</sup> CCWGG	C <sup>m4</sup> CWGG#
HgiA I	GRGCYC	GRGCY™5C	GRG™CYC			CC <sup>m6</sup> AGG <sup>b</sup>	m5Cm5CWGGb
HgiC I	GGYRCC	?	GGYRC <sup>m5</sup> C	Mvn I	CGCG	?	m5CGCG
HgiC II	GGWCC	?	GGWC <sup>m5</sup> C	Nae I	GCCGGC⁵	?	G <sup>m5</sup> CCGGC
HgiE I	GGWCC	?	GGWC <sup>m5</sup> C				GC™5CGGC
HgiJ II	GGYRCC	?	GGYRC <sup>m5</sup> C				GCCGG <sup>m5</sup> C
Hha I	GCGC	?	G <sup>m5</sup> CGC# GCG <sup>m5</sup> C	Nar I	GGCGCC	GGCGC™5C	GG™5CGCC GGCGC™4C
Hha II	GANTC	?	Gh <sup>™</sup> CGh <sup>™</sup> C G <sup>™</sup> ANTC#	Nci I	CCSGG	™5CCSGG	C™⁴CSGG C™⁵CSGGb
Hinc II	GTYRAC	GTYRA <sup>m5</sup> C	GTYR <sup>m6</sup> AC GTYRAh <sup>m5</sup> C	Nco I	CCATGG	CC <sup>m6</sup> ATGG	m4CCATGGb m5CCATGG
Hind II	GTYRAC	?	GTYR <sup>m6</sup> AC#	Nde I	CATATG	m5CATATGb	m6A
Hind III	AAGCTT	A <sup>m6</sup> AGCTT#	m6AAGCTT#	Nde II	GATC	GATATO GATATO	G <sup>m6</sup> ATC
. /	, , , , , , , , , , , , , , , , , , , ,	7. 7.001111	AAG <sup>m5</sup> CTT	Nhe I	GCTAGC	?	GCTAG <sup>m5</sup> C
			AAGh <sup>m5</sup> CTT	NIa III	CATG	?	C <sup>m6</sup> ATG#
Hinf I	GANTC	GANT <sup>m5</sup> C <sup>b</sup>	G <sup>m6</sup> ANTC GANTh <sup>m5</sup> C	Not I	GCGGCCGC	GCGGCCG <sup>m5</sup> C	GCGG <sup>™5</sup> CCGC GCGGC™5CGC
HinP I	GCGC	?	G <sup>m5</sup> CGC	Nru I	TCGCGA	TCG <sup>m5</sup> CGA	T <sup>m5</sup> CGCGA
Hpa I	GTTAAC	GTTAA <sup>m5</sup> C	GTTA <sup>m6</sup> AC# GTTAAh <sup>m5</sup> C	Nsi I	ATGCAT	?	TCGCG <sup>m6</sup> A ATGC <sup>m6</sup> AT
Hpa II	CCGG	?	m4CCGG				ATG <sup>m5</sup> CAT
			m5CCGGb	Nsp I	RCATGY	?	RC <sup>m6</sup> ATGY
			C <sup>m4</sup> CGG <sup>b</sup>	NSpB II	CMGCKG	C™5CGCKG	?
			C™CGG# h™Ch™CGG	PfIM I	CCAN5TGG	?	C™CAN5TGG C™CAN5TGG

a. # denotes canonical modification mTase specificity.

b. See notes section of reference 2.

\*Dpn I and its isoschizomers require the presence of 6-methyladenosine within the recognition sequence GATC

Recognition sequences given use the standard abbreviations [Eur. J. Biochem (1985) [50:15] to represent ambiguity:

R=G or A
M=A or C
S=G or C
H=A or C or T
V=G or C or A

N=A or C or G or T

Y=C or T K=G or T W=A or T B=G or T or C D=G or A or T  $^{m4}$ C = N4-methylcytosine  $^{m5}$ C = C5-methylcytosine

hm5C = hydroxymethylcytosine mC = methylcytosine

N<sub>4</sub> or C<sub>5</sub>-methylcytosine unspecified

 $^{m6}A = N_6$ -methyladenine Sequences are in 5' - 3' order.

## **Enzyme Information, continued**

Enzyme	Recognition Sequence	Sites cut	Sites not cut	Enzyme	Recognition Sequence	Sites cut	Sites not cut
Pfu I	CGTACG	?	CGTA <sup>m5</sup> CG	SnaB I	TACGTA	?	TA <sup>m5</sup> CGTA
PaeR7 I	CTCGAG	?	CTCG <sup>m6</sup> AG#				T <sup>m6</sup> ACGT <sup>m6</sup> A
			CT <sup>m5</sup> CGAG	Sno I	GTGCAC	?	GTG <sup>m5</sup> CA <sup>m5</sup> C
Pml I	CACGTG	?	CA <sup>m5</sup> CGTG	Spe I	ACTAGT	?	m6ACTAGT
PpuAl	CGTACG	?	CGTA <sup>m5</sup> CG				A <sup>m5</sup> CTAGT
PspA I	CCCGGG	C™5CCGGG CC™5CGGG		Sph I	GCATGC	GCATG <sup>m5</sup> C	
			m5CCCGGG			Gh <sup>m5</sup> CATGh <sup>m5</sup> C	GC <sup>m6</sup> ATGC
Pst I	CTGCAG	?	m5CTGCAG	Spl I	CGTAGC	CGTm6ACG	?
Pvu I			CTGC <sup>m6</sup> AG#	Spo I	TCGCGA	TCGCG <sup>m6</sup> A	Tm5CGCGA
	CGATCGb	CG <sup>m6</sup> ATCG	CGAT <sup>m4</sup> CG				TCG <sup>m5</sup> CGA
Pvu II	CAGCTG	CGAT™5CG ?	CAG <sup>m4</sup> CTG#	Srf I	GCCC/GGGC	GCCC/GGG <sup>m5</sup> C	Gm5CCC/GGGC
						GC <sup>m5</sup> CC/GGGC	
			CAG <sup>m5</sup> CTG				GCC <sup>m5</sup> C/GGGC
Rsa I	GTACb	GTA <sup>m5</sup> C <sup>b</sup>	GT <sup>m6</sup> AC	Ssp I	AATATT	m6AATATT	?
Rsr I	GAATTC	?	G <sup>m6</sup> AATTC GA <sup>m6</sup> ATTC# <sup>b</sup>	Sst I	GAGCTC	?	GAG <sup>m5</sup> CTC GAGh <sup>m5</sup> CTh <sup>m5</sup> C
Rsr II	CGGWCCG	?	™CGGWCCG CGGW™CCG CGGWC™CG	Stu I	AGGCCT	?	AGGm5CCT AGGCm5CT AGGCm4CT
Sac I	GAGCTC	G <sup>m6</sup> AGCTC	GAG <sup>m5</sup> CTC	StySP I	AACN6GTRC <sup>b</sup>	? A	<sup>n6</sup> ACN6GmTRC# <sup>b</sup>
Sac II	CCGCGG	?	m5CCGCGG	Tag I	TCGA	T <sup>m5</sup> CGA <sup>b</sup>	
Sal I	GTCGAC	GTCGA <sup>m5</sup> C	GT™5CGAC	Taq II		Th <sup>m5</sup> CGA <sup>b</sup>	TCG <sup>m6</sup> A#
			GTCG <sup>m6</sup> AC#		GACCGA		
Sau3A I	GATC <sup>b</sup>	G <sup>™6</sup> ATC	GAT <sup>m5</sup> C# <sup>b</sup>		CACCCA	?	G <sup>m6</sup> ACCGA
				Tfi I	GAWTC	GAWT <sup>m5</sup> C	?
			GATh <sup>m5</sup> C	Tha I	CGCG	<sup>m5</sup> CGCG	m5CGCG
Sau96 I	GGNCC	?	GGN <sup>m5</sup> CC#	Xba I	TCTAGA	?	h™CGh™CG
			GGNC <sup>m5</sup> C				TCTAGm6A#
			GGNh <sup>m5</sup> Ch <sup>m5</sup> C				T <sup>m5</sup> CTAGA
Sca I	AGTACT	AGTA <sup>m5</sup> CT	?				Th <sup>m5</sup> CTAGA
ScrF I	CCNGG	<sup>m5</sup> CCNGG	C <sup>m5</sup> CNGG	Xho I	CTCGAG <sup>b</sup>	?	CT <sup>m5</sup> CGAG
			C <sup>m4</sup> CNGG				CTCG <sup>m6</sup> AG
SfaN I	GATGC	GATG™⁵C	G <sup>m6</sup> ATGC				m5CTCGAG
Sfi I	GGCCN5GGCC	GG <sup>m5</sup> CCN5GG <sup>m5</sup> CC <sup>b</sup>		Xho II	RGATCY	RG™6ATCY	RGAT <sup>m5</sup> CY <sup>b</sup>
		GGCCN5GGC <sup>m5</sup> C	GGC <sup>™</sup> CN5GGCC	Xma I	CCCGGG	CC™⁵CGGG⁵	™4CCCGGG ™5CCCGGG
Sfl I	CTGCAG	?	CTGC <sup>m6</sup> AG				C <sup>m4</sup> CCGGG
SgrA I	CRCCGGYG	?	CRC <sup>m5</sup> CGGYG				CC <sup>m4</sup> CGGG
Sin I	GGWCC	?	GGW <sup>m5</sup> CC#	Xma III	CGGCCG	?	CGGm5CCG
Sma I	CCCGGG	C <sup>m5</sup> CCGGG	™4CCCGGG ™5CCCGGGb	Xmn I	GAAN4TTC	GA <sup>m6</sup> AN4TTC	G™AAN4TTC GAAN4TT™5Cb
			Cm4CCGGGb	Xor II	CGATCG	CG <sup>m6</sup> ATCG	CGAT <sup>m5</sup> CG

## LEGEND

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b. See notes section of reference 2.

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 Y=C or T

 M=A or C
 K=G or T

 S=G or C
 W=A or T

 H=A or C or T
 B=G or T or C

 V=G or C or A
 D=G or A or T

 N=A or C or G or T

 $^{m4}$ C = N4-methylcytosine  $^{m5}$ C = C5-methylcytosine

 $^{hm5}$ C = hydroxymethylcytosine  $^{m}$ C = methylcytosine

 $N_4$  or  $C_5$ -methylcytosine unspecified  $^{m6}A = N_6$ -methyladenine Sequences are in 5' - 3' order.

\*Dpn I and its isoschizomers require the presence of 6-methyladenosine within the recognition sequence GATC

## REFERENCE

1. Roberts, R.J. and Macelis, D. (1991) Nucleic Acids Res. 19: 2077-2109.

2. Nelson, M. and McClelland, M. (1991) Nucleic Acids Res. 19:2045-2071