

## Restriction Enzyme Sensitivity to CpG Methylation

Many prokaryotes and eukaryotes contain enzymes known as DNA methyltransferases, which modify DNA by methylating cytosine in the CpG sequence. While these enzymes are known to play a role in protecting host DNA from cleavage by corresponding restriction endonucleases in prokaryotes, their role in eukaryotes is not clear. However, several restriction enzymes will be unable to cleave eukaryotic DNA at sites that have been methylated by CpG methylation. These Takara enzymes are listed below. Methylation may occur at CpG sequences either within the recognition site (indicated by boldface color type), or overlapping it (indicated by boldface type).

Enzyme	Site
<i>Aat</i> II	GAC <b>GTC</b>
<i>Acc</i> I	GTM <b>KAC</b>
<i>Acc</i> II	<b>CGC</b> G
<i>Afa</i> I	GT <b>AC</b>
<i>Aor</i> 13H I	T <b>CCGGA</b>
<i>Aor</i> 51H I	AG <b>CGCT</b>
<i>Apa</i> I	GGG <b>CCC</b>
<i>Apa</i> L I	GTG <b>CAC</b>
<i>Ava</i> II	GGW <b>CC</b>
<i>Avi</i> II	TG <b>CGCA</b>
<i>Bgl</i> I	GCCN <sub>3</sub> <b>GGC</b>
<i>Bmg</i> T1201	CCW <b>GG</b>
<i>Bsp</i> T104 I	TT <b>CGAA</b>
<i>Bss</i> H II	<b>GCGCGC</b>
<i>Bst</i> 1107 I	GTATA <b>CG</b>
<i>Cfr</i> 10 I	R <b>CCGGY</b>

Enzyme	Site
<i>Cfr</i> 13 I	GGN <b>CC</b>
<i>Cla</i> I	AT <b>CGAT</b>
<i>Cpo</i> I	<b>CGGWCCG</b>
<i>Eae</i> I	YGG <b>CCR</b> <sup>1</sup>
<i>Eco</i> 52 I	<b>CCGGCCG</b>
<i>Eco</i> R I	GAAT <b>TCG</b>
<i>Fse</i> I	GG <b>CCGGCC</b>
<i>Hae</i> II	R <b>GCGY</b>
<i>Hap</i> II	<b>CCGG</b>
<i>Hha</i> I	<b>GCG</b>
<i>Hin</i> 1 I	GR <b>CGYC</b>
<i>Mlu</i> I	<b>ACGGT</b>
<i>Nae</i> I	G <b>CCGGC</b>
<i>Nhe</i> I	GCTA <b>GC</b>
<i>Not</i> I	<b>GCGCCGC</b>
<i>Nru</i> I	T <b>CCGGA</b>
<i>Pma</i> C I	CA <b>CGTG</b>

Enzyme	Site
<i>Psh</i> A I	GACN <sub>4</sub> <b>GTC</b> <sup>2</sup>
<i>Psp</i> 1406 I	AA <b>CGTT</b>
<i>Pvu</i> I	<b>CGATCG</b>
<i>Sac</i> II	CCG <b>CGG</b>
<i>Sal</i> I	GT <b>CGAC</b>
<i>Sau</i> 3A I	GAT <b>C</b>
<i>Sfi</i> I	GGCCN <sub>3</sub> <b>GGCC</b> <sup>3</sup>
<i>Sma</i> I	<b>CCCGGG</b>
<i>Sna</i> B I	TAC <b>GTA</b>
<i>Vpa</i> K11B I	GGW <b>CCG</b>
<i>Xho</i> I	CT <b>CGAG</b>

<sup>1</sup>only when R = G

<sup>2</sup>methylation at multiple sites required to block cleavage

<sup>3</sup>internal site methylated only when the adjacent N = G

## Restriction Enzyme Sensitivity to *dam* and *dcm* Methylation

DNA prepared from most common strains of *E. coli* may prove resistant to cleavage by certain restriction enzymes. This can often be caused by site-specific methylation. Two types of methylases are commonly encountered: the *dam* methylase, which methylates the N6 position of the adenine residues in the sequence GATC, and the *dcm* methylase, which methylates the internal cytosine residues in the sequences **CCWGG** at the C5 position. The sensitivity of Takara's enzymes to these types of methylation is listed below.

Enzymes Blocked by Overlapping <i>dam</i> Methylation	
Enzyme	Site
<i>Acc</i> III	TCCGG <b>ATC</b>
<i>Cla</i> I	ATCGA <b>T(C)</b>
<i>Fba</i> I	TGAT <b>CA</b>
<i>Mbo</i> I	<b>GATC</b>
<i>Mbo</i> II	GAAG <b>A(TC)</b>
<i>Mfr</i> I	RGAT <b>CY</b>
<i>Nru</i> I	TCGG <b>CA(TC)</b>
<i>Tth</i> HB8 I	T <b>CGA(TC)</b>
<i>Xba</i> I	TCTAG <b>A(TC)</b>

Enzymes Blocked by Overlapping <i>dcm</i> Methylation	
Enzyme	Site
<i>Apa</i> I	GGG <b>CCC(WGG)</b>
<i>Ava</i> II	GGW <b>CC(WGG)</b>
<i>Bal</i> I	TGG <b>CA(GG)</b>
<i>Bmg</i> T 1201	<b>CCWGG</b>
<i>Eae</i> I	YGG <b>CA(GG)</b>
<i>Eco</i> O109 I	RGGN <b>CT(GG)</b>
<i>Sfi</i> I	GGCCN <sub>3</sub> <b>GGCC(WGG)</b> or GGCC <b>WGGN<sub>2</sub>GGCC</b>
<i>Stu</i> I	AGG <b>CT(GG)</b>
<i>Van</i> 91 I	<b>CCAGGN<sub>3</sub>TGG</b>
<i>Vpa</i> K11B I	GGW <b>CCWGG</b>

Enzymes NOT Blocked by Overlapping <i>dam</i> Methylation	
Enzyme	Site
<i>Bam</i> H I	GGAT <b>CC</b>
<i>Bgl</i> II	AGAT <b>CT</b>
<i>Pvu</i> I	CGAT <b>CG</b>
<i>Sau</i> 3A I	GAT <b>C</b>

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

Enzymes NOT blocked by overlapping <i>dcm</i> methylation	
Enzyme	Site
<i>Bam</i> H I	GGAT <b>CC</b>
<i>Bst</i> P I	GGTN <b>ACC</b>
<i>Bst</i> X I	CCAN4 <b>CTGG</b>
<i>Eco</i> O65 I	GGTN <b>ACC</b>
<i>Hae</i> III	GG <b>CC</b>
<i>Hin</i> 1 I	GR <b>CGYC</b>
<i>Kpn</i> I	GGT <b>ACC</b>
<i>Mva</i> I	<b>CCWGG</b>