Effect of Site-Specific Methylation on OPTIZYME™ Restriction Enzymes

DNA methylation is the process of transferring a methyl group from a donor molecule to either a cytosine or an adenine by DNA methyltransferases. The methylase encoded by the *dam* gene of *E. Coli* cells methylates the N6-position of adenine residues within the GATC sequence (1). The methylase encoded by the *dcm* gene of *E. Coli* cells methylates the C5-position of internal cysteine residues within the CCWGG sequence (2,3). CpG methyltransferases are found in higher eukaryotes and transfer a methyl group to the C5 position of cytosine residues. The effects of CpG methylation are mainly a concern when digesting genomic DNA from eukaryotic cells. The effects of CpG methyltransferases are not retained when the DNA is cloned into a bacterial host.

Methylation should be considered when performing DNA digests with restriction endonucleases because cleavage can be either blocked or impaired when a particular base in the recognition site is methylated. Restriction sites that are blocked by methylation can be unmethylated by cloning the DNA into a dam- or dcm- E. Coli strain.

The table below summarizes the effects of Dam, Dcm and CpG methylation on restriction enzyme sensitivity. The information below should be used as a guide, rather than a definite indicator, of methylation effects on restriction enzyme activity.

Part Number	OPTIZYME* Restriction Enzyme	Recognition Sequence	Dam (G^mATC)	Dcm (C^mCWGG)	CpG (^mCG)
BP8069	AarI	CACCTGC $(N)_4$↓	□	□	◊ ol
BP8041	AatII	GACGT↓C	□	□	●
BP8075	AloI	↓$(N)_7$GAAC$(N)_6$TTC $(N)_{12\text{-}13}$↓	N/A	□	◊ ol
BP8015	AluI	AG↓CT	□	□	□
BP8059	Alw44I (ApaLI)	G↓TGCAC	□	□	⁘ ol
BP8025	ApaI	GGGCC↓C	□	◊ ol	◊ ol
BP8035	AvaI (Eco88I)	C↓YCGRG	□	□	◊ ol
BP8043	AvaII (Eco47I)	G↓GWCC	□	⁘ ol	⁘ ol
BP8039	BalI (MscI)	TGG↓CCA	□	⁘ ol	□
BP8005	BamHI	G↓GATCC	□	□	□
BP8053	BclI	T↓GATCA	●	□	□
BP8046	BglI	GCCNNNN↓NGGC	□	□	◊ ol
BP8014	BglII	A↓GATCT	□	□	□
BP8072	BpiI (BbsI)	GAAGAC $(N)_2$↓	□	□	□
BP8071	Bsh1236I (BstUI)	CG↓CG	□	□	●
BP8078	BshTI (AgeI)	A↓CCGGT	□	□	●
BP8036	BssHII (PauI)	G↓CGCGC	□	□	●
BP8038	BstEII (Eco91I)	G↓GTNACC	□	□	□
BP8081	Cfr9I (XmaI)	C↓CCGGG	□	□	◊ ol
BP8024	ClaI	AT↓CGAT	⁘ ol	□	●
BP8068	Csp6I (CviQI)	G↓TAC	□	□	□
BP8060	DdeI (HpyF31)	C↓TNAG	□	□	□
BP8009	DpnI	G m6A↓TC	□	□	⁘ ol
BP8026	DraI	TTT↓AAA	□	□	□
BP8080	Ecl136II	GAG↓CTC	□	□	◊ scol
BP8066	Eco57I (AcuI)	CTGAAG $(N)_{16}$↓	□	□	□
BP8003	EcoRI	G↓AATTC	□	□	⁘ scol
BP8054	EcoRI (HC)	G↓AATTC	□	□	⁘ scol
BP8012	EcoRV (Eco32I)	GAT↓ATC	□	□	□
BP8070	Esp3I (BsmBI)	CGTCTC $(N)_1$↓	□	□	●
BP8002	HaeIII (BsuRI)	GG↓CC	□	□	□
BP8034	HincII (HindII)	GTY↓RAC	□	□	◊ scol
BP8006	HindIII	A↓AGCTT	□	□	□
BP8051	HinfI	G↓ANTC	□	□	◊ scol
BP8049	HpaI (KspAI)	GTT↓AAC	□	□	◊ scol

Part Number	OPTIZYME* Restriction Enzyme	Recognition Sequence	Dam (G^mATC)	Dcm (C^mCWGG)	CpG (^mCG)
BP8032	HpaII (MspI)	C↓CGG	□	□	●
BP8079	Hpy8I (MjaIV)	GTN↓NAC	□	□	◊ scol
BP8083	KpnI	GGTAC↓C	□	□	□
BP8067	LguI (SapI)	GCTCTTC $(N)_1$↓	□	□	□
BP8021	MluI	A↓CGCGT	□	□	●
BP8048	MspI (HpaII)	C↓CGG	□	□	□
BP8057	NaeI	GCC↓GGC	□	□	●
BP8017	NcoI	C↓CATGG	□	□	□
BP8020	NdeI	CA↓TATG	□	□	□
BP8019	NheI	G↓CTAGC	□	□	◊ scol
BP8004	NotI	GC↓GGCCGC	□	□	●
BP8058	NsiI (Mph1103I)	ATGCA↓T	□	□	□
BP8073	PasI	CC↓CWGGG	□	□	□
BP8077	PfoI	T↓CCNGGA	◊ scol	⁘ scol	⁘ scol
BP8001	PstI	CTGCA↓G	□	□	□
BP8050	PvuI	CGAT↓CG	□	□	●
BP8022	PvuII	CAG↓CTG	□	□	□
BP8000	RsaI	GT↓AC	□	□	◊ scol
BP8016	SacI	GAGCT↓C	□	□	□
BP8023	SacII (Cfr42I)	CCGC↓GG	□	□	●
BP8013	SalI	G↓TCGAC	□	□	●
BP8030	Sau3AI (Bsp143I)	↓GATC	□	□	⁘ ol
BP8037	ScaI	AGT↓ACT	□	□	□
BP8076	SfaAI (AsiSI)	GCGAT↓CGC	□	□	●
BP8011	SmaI	CCC↓GGG	□	□	●
BP8018	SpeI (BcuI)	A↓CTAGT	□	□	□
BP8029	SphI (PaeI)	GCATG↓C	□	□	□
BP8027	StuI (Eco147I)	AGG↓CCT	□	⁘ ol	□
BP8007	TaqI	T↓CGA	⁘ ol	□	□
BP8064	Tru9I (MseI)	T↓TAA	□	□	□
BP8055	VspI (AseI)	AT↓TAAT	□	□	□
BP8008	XbaI	T↓CTAGA	⁘ ol	□	□
BP8010	XhoI	C↓TCGAG	□	□	◊
BP8082	XmaJI (AvrII)	C↓CTAGG	□	□	□
BP8052	XmnI (PdmI)	GAANN↓NNTTC	□	□	◊ scol

Legend

□	Not Sensitive
●	Blocked
⁘ ol	Blocked by Overlapping
⁘ scol	Blocked by Some Combinations of Overlapping
◊	Impaired
◊ ol	Impaired by Overlapping
◊ scol	Impaired by Some Combinations of Overlapping
N/A	Effect not determined

Single Letter Code

R = G or A
Y = C or T
W = A or T
N = G, A, T or C

1. Hattman, S., et. al. Sequence specificity of the P1 modification methylase (M.EcoP1) and the DNA methylase (M.Ecodam) controlled by the Escherichia coli dam gene, J. Mol. Bio., 126, 367-380, 1978.

2. May, M.S., Hattman, S. Analysis of bacteriophage deoxyribonucleic acid sequences methylated by host- and R-factor-controlled enzymes, J. Bacterio., 123, 768-770, 1975.

3. Buryanov, Ya. I., et. al. Site specificity and chromatogenic properties of E. Coli K-12 and EcoRII DNA cytosine methylases, FEBS Letters, 88, 251-254, 1978.