DNA METHYLATION IN *E. COLI:* EFFECT ON RESTRICTION ENZYMES

1. The problem. Many restriction enzymes are blocked (fully or partially) by methylation. A few enzymes require methylation (such as *Dpn*I)

2. Three methylation systems. Most laboratory strains of *E. coli* contain three site-specific DNA methylases that can block digestion with restriction enzymes. Avoid designing restriction sites in primers or genes that may become methylated. Problems usually occur due to bases flanking the restriction site.

Dam methylase: methylates A in GATC

Dcm methylase: methylates C in CCAGG and CCTGG

EcoKI methylase: methylates A in AAC(N_6)GTGC and AAC(N_6)GTGC

3. Sources of information. Information about the sensitivity of restriction enzymes to methylation can be found on the internet, including at http://rebase.neb.com. The following table from NEB shows information about Dam and Dcm sensitivity.

Dam Methylation: G^{m}ATC

Blocked by Overlapping Dam	
AlwI	**GGATC**
BcgI[1]	**CGATCNNNNTGC**
BclI	**TGATCA**
BsaBI	**GATCNNNATC**
BspDI	**ATCGAT**C
BspEI	**TCCGGA**TC
BspHI	**TCATGA**TC
ClaI	**ATCGAT**C
DpnII	**GATC**
HphI	**GGTGA**TC
Hpy188I	**TCNGA**TC
Hpy188III	**TCNNGA**TC
MboI	**GATC**
MboII	**GAAGA**TC
NruI	**TCGCGA**TC
TaqI	**TCGA**TC
XbaI	**TCTAGA**TC

Not Blocked by Overlapping Dam			
AsiSI	BglII	BstYI	Sau3AI
BamHI	BsaWI	PvuI	

Dcm Methylation: C^{m}CWGG

Blocked by Overlapping Dcm	
Acc65I[2]	**GGTACC**WGG
AlwNI	**CAGNNCCTG**G
ApaI	**GGGCCC**WGG
AvaII	**GGWCC**WGG
BanI	**GGYRCC**WGG
BsaI	**GAGACC**WGG
BsaHI[2]	**GRCGCC**WGG
BslI[2]	**CCWGGNNNNGG**
BsmFI	**GGGAC**T
BssKI	**CCWGG**
BstXI	**CCAGGNNNNTGG**
EaeI	**YGGCCA**GG
EcoO109I	**RGGNCCT**GG
MscI	**TGGCCA**GG
NlaIV	**GGNNCC**WGG
PflMI	**CCAGGNNNTGG**
PpuMI	**RGGWCCT**GG
PspGI	**CCWGG**
PspOMI	**GGGCCC**WGG
Sau96I	**GGNCC**WGG
ScrFI	**CCWGG**
SexAI	**ACCWGGT**
SfiI[1]	**GGCCWGGNNGGCC**
SfiI	**GGCCNNNNNGGCC**WGG
SfoI	**GGCGCC**WGG
StuI	**AGGCCT**GG

Not Blocked by Overlapping Dcm			
BamHI	BsaJI	DraIII	HphI
BanII	Bsp1286I	FokI	KasI
BciVI	BstEII	FseI	KpnI
BglI	BstNI	HaeIII	NarI
BpmI			

At rebase.neb.com, you can get specific information about enzymes of interest. This includes information, including EcoKI methylase effects. For example:

SpeI ACTAGT MS	Dam m6 G A T C C T A G m6	Dcm m5 C C W G G G G W C C m5	CpG m5 C G G C m5	EcoBI m6 T G A N N N N N N N N T G C T A C T N N N N N N N N A C G A m6	EcoKI m6 A A C N N N N N N G T G C T T G N N N N N N C A C G m6
overlaps?	n	n	n	y	y
sensitivity?	-	-	-	blocked	blocked

4. Cloning strains of *E. coli.* Most cloning strains are Dam$^+$, Dcm$^+$, EcoKI$^+$ (most are derived from E. coli K12, which lack EcoB1). Methylation contributes to post-replication DNA repair, that is why we usually work with *E. coli* that has methylases.

The lab has a Dam-, Dcm- strain (2198) that can be used as needed; strains are also available from NEB (# E4109S, ER2925) and Invitrogen (INV110, derivative of JM110). SURE and XL10 cells are Dam$^+$, Dcm$^+$, EcoKI-minus. Note that some methylation systems are not 100% efficient, so you might get some digestion from regular *E. coli.*

The genotype of DH5-alpha is: F– Φ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17 (rK–, mK+) phoA supE44 λ– thi-1 gyrA96 relA1; the hsdR17 is in the EcoK restriction enzyme (AACNNNNNNGTGC) but the corresponding methylase gene (hsdM) is present.
Those other genotypes are:

- ΔlacZYA: deletion of lac operon (the U169 mutation)
- F– Φ80lacZΔM15: low-copy plasmid, for making single-stranded DNA with M13 phage, carries lacI and lacZΔM15 gene.
- recA: mutation in gene for protein that stimulates replication.
- endA: mutation in non-specific endonuclease, resulting in improved plasmid preps
- gyrA96: resistance to nalidixic acid.
- relA: uncouples transcription and translation from amino acid levels.

5. What about DNA from a PCR reaction? DNA amplified by PCR is not subject to methylation, and should therefore be digestible, unlike DNA from *E. coli.*

6. What about cloning methylated DNA into *E. coli?* There are two systems used to digest cytosine-methylated DNA, the mcrA and mcrB systems. Mammals, higher plants & many prokaryotes contain methylcytosine in their genomic DNA, so their DNA must be cloned into a mcrA-, mcrB- strain. In most cases, you only need to worry about this when going between species, not between *E. coli* strains.