DNA Methylation & Restriction Digests

Two common types of methylation that can block cutting at a restriction site are Dam and Dcm methylation. Both arise from replicating DNA in a strain of *E. coli* that has functional Dam and Dcm methylation systems.

Sites that are Blocked by Dam/Dcm Methylation

Restriction by some enzymes can be inhibited due to methylation caused by the common *E. coli* methyltransferases. Dam methyltransferase causes methylation of the adenine in the sequence GATC while Dcm methyltransferase causes methylation of the first cytosine in the sequence CC(A/T)GG. Any restriction enzyme whose site contains either of these sequences may be affected by the relevant methylation. For example, the site for AlwI (GGATC4/5) contains the recognition site for Dam methyltransferase, GATC. If the DNA was produced in a methylating *E. coli* strain, the adenine would be methylated and cleavage by AlwI would be blocked. This can be avoided by cloning the DNA into a *dam*$^-$ strain such as GM2613 or our *dam*$^-$/*dcm*$^-$ Competent *E. coli* (NEB #C2925). These strains do not have a functional Dam methyltransferase.

Dam/Dcm Overlapping Sites

Restriction sites can also be blocked if an overlapping site is present. In this case, part of the Dam or Dcm sequence is generated by the restriction enzyme sequence followed by the flanking sequence. For example, the site for ClaI (AT/CGAT) contains GAT. If it is followed by a C, the A can be methylated, and cleavage will be blocked. On the other hand BamHI is not Dam/Dcm sensitive; the BamHI site contains GATC but cleavage by this enzyme is not blocked even when the A is methylated. The same principles apply for Dcm methylation but the enzyme sites affected would contain the sequence CC(A/T)GG.

For more information regarding methylation sensitivity please refer to www.neb.com.

Key Points to Consider

- Mammalian genomic DNA is not Dam or Dcm methylated. Inhibition of enzyme activity by Dam and Dcm methylation is not an issue when digesting mammalian DNA.
- Mammalian and plant DNA that has been cloned into a methylating *E. coli* strain will be Dam/Dcm methylated. Most commonly used laboratory *E. coli* strains methylate DNA.
- Mammalian and plant genomic DNA are CpG methylated. Some enzymes are inhibited by CpG methylation. More information on CpG methylation can be found on our website.
- Most bacterial DNA (including *E. coli* DNA) is not CpG methylated. Inhibition of enzyme activity by CpG methylation is not an issue for most DNA prepared from common *E. coli* strains.
- DNA amplified by PCR does not contain any methylated bases.
- Some enzyme recognition sequences have overlapping methylation sites. These sites are formed partially by the recognition sequence of an enzyme and partially by flanking DNA.
- To avoid Dam/Dcm methylation when subcloning in bacteria, choose NEB's *dam*$^-$/*dcm*$^-$ Competent *E. coli* for propagation (NEB #C2925).

Dam/Dcm Sensitive Restriction Enzymes available from NEB

The following enzymes are blocked or impaired by Dam or Dcm methylation. In order to achieve cleavage with these enzymes, the DNA should be passed through a *dam*$^-$/*dcm*$^-$ strain.

Acc651	BssKI	PflMI
AlwI	BstXI	PhoI
AlwNI	ClaI	PpuMI
ApaI	DpnII	PspGI
AvaII	EaeI	PspOMI
BanI	EcoO109I	Sau96I
BcgI	FokI	ScrFI
Bc1I	FspI	SexAI
BsaI/BsaI-HF®	HphI	SfiI
BsaBI	Hpy188I	SfoI
BsaHI	Hpy1881II	StuI
Bs1I	MboI	StyD41
BsmFI	MboII	TaqIα
BspDI	MscI	XbaI
BspEI	NlaIV	
BspHI	NruI	

Methylation sensitivity information can also be found on REBASE (Restriction Enzyme Database), a comprehensive database of information about restriction enzymes and related proteins. For more information, see www.neb.com.

www.neb.com
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