The restriction enzyme AlwNI is blocked by overlapping methylation

M. Bourbonnière¹ and J. Nalbantoglu¹,²,³
Departments of ¹Neurology and Neurosurgery, ²Medicine and ³McGill Center for Studies in Aging, McGill University, 3801 University Street, Montreal, Quebec H3B 2A4 Canada

Submitted July 17, 1991

Most strains of E. coli contain two sequence specific methylases, Dam and Dcm. The adenine residue of the sequence GATC is methylated at the N⁶ position by the Dam methylase whereas the Dcm methylase recognizes the sequences CC(A/T)GG methylating the internal cytosine at the C⁵ position.

The commercially available restriction enzyme AlwNI (New England Biolabs) has a recognition sequence CAGN₂CTG and is a useful enzyme for creating deletions. During our study of the 5' flanking sequence of the amyloid precursor protein (APP) gene, we cloned a 3.8 kb BamHI fragment (1) into pUC18 for subsequent deletional analyses. AlwNI digestion of recombinant plasmid which had been transformed into JM105 always resulted in a partial digest where only 4 out of 5 theoretical restriction enzyme sites were cut (Figure 1, lane 2). In addition to the expected fragments of 2179 bp, 213 bp and 190 bp, a band of 3902 bp was also generated. This large fragment contained an internal AlwNI recognition site of the following sequence ggcgCAGCCCCTGgca (1). Since the underlined sequence was a potential dam methylation site, the plasmid was transformed into the dcm⁻ bacterial strain GM272 (genotype: F⁻ dam-3 dcm-6 hsdS21 methyl lacY1 or ZA galK2 galT2 mtl-2 tonA2 or A31 tsx-1 or -78 supE44 (thi-1)) (2). AlwNI digestion of this preparation of plasmid yielded two fragments of 2612 bp and 1290 bp (Figure 1, lane 3) instead of the 3902 bp fragment. The restriction enzyme AlwNI can therefore be blocked by overlapping dcm methylation of its recognition site.

REFERENCES
2. Marinus, M. G. (private communication).

Figure 1. Digestion of APP recombinant plasmid with AlwNI. Lane 1: 1 kb ladder (BRL); lane 2: preparation of plasmid from JM105 (dcm⁻); lane 3: preparation of plasmid from GM272 (dcm⁻).