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Cleavage of DNA by *Hae*II is inhibited by the presence of 5-methylcytosine at the second cytosine within the recognition sequence

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We have cloned from Neisseria gonorrhoea a methylase (M•NgoMV, unpublished observations) that recognizes the DNA sequence GGNNCC. This enzyme methylates the first cytosine in its methylation sequence (1). When we digested the recombinant plasmid pBR322::M•NgoMV with HaeII we noticed that some HaeII sites in the pBR322 DNA were not cut (Figure 1, lane 1), while they were cleaved if the plasmid did not encode M•NgoMV (Figure 1, lane 2). The molecular sizes of the pBR322::M•NgoMV fragments obtained after digestion with HaeII indicated the loss of fragments of 21 bp, 54 bp, 60 bp, 181 bp, 227 bp, 430 bp and 439 bp and the appearance of fragments of 262 bp, 281 bp and 869 bp. The appearance of these bands corresponds to the lack of cleavage by HaeII at the sites located at the position of 413, 434, 548 and 1205 on the conventional pBR322 DNA sequence (2). Since HaeII recognizes PuGCGCPy and all of the sites that are not cleaved have a common sequence GGCGCC, recognized by both HaeII and M•NgoMV, these results demonstrate that cleavage by HaeII is inhibited not only when the first 5′ cytosine is methylated in its recognition sequence (3, 4) but also when the second 5′ cytosine in the recognition sequence is methylated.

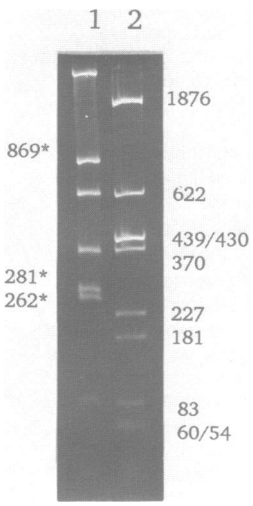

Figure 1. Lane 1 represents pBR322::M•NgoMV digested with HaeII. Lane 2 represents pBR322 digested with HaeII. The numbers represent the number of bases present in each band. The star indicates bands that have arisen as the result of the inability of HaeII to cleave the DNA.

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