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Cleavage by the restriction endonuclease *Asp*718, an isoschizomer of *Kpn*I, is sensitive to *Escherichia coli* Dcm methylation

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While attempting to use Asp718[1], a KpnI isoschizomer, to subclone KpnI fragments from pAn602[2], a plasmid known to have two KpnI sites, I found that one of these sites was cut very poorly by this enzyme (Fig. 1B, lane 1). DNA sequence analysis, Figure 1A, showed that the poorly cut site overlapped the sequence CCAGG, an Escherichia coli Dcm methylation site[3]. When this plasmid DNA was propagated in E. coli GM31 (dcm-6)[4], a host which does not methylate this site, and digested with either Asp718 or KpnI both sites were cut to completion, Figure 1B, lanes 3,4.

A. Site #1:

tagtgtGGTACCaggtaa

Site #2:

cacaccGGTACCgtagta

KpnI/Asp718 site = GGTACC
Dcm methylase site[3] = CCAGG

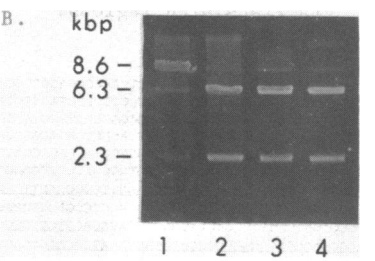

Figure 1. A. DNA sequence across the two KpnI sites in pAn602. The Dcm methylase site is underlined. B. Restriction enzyme analysis of pAn602 DNA prepared from either E. coli ED8654 (dcm+)[5] lanes 1 & 2, or GM31 (dcm-6) lanes 3 & 4, using Asp718 lanes 1 & 3 or KpnI lanes 2 & 4. Both enzymes were purchased from Boehringer Mannheim and used according to manufacture's specifications.

The Dcm methylase methylates the internal C of the recognition sequence[3] which corresponds to the last, 3', base of the restriction enzymes site. Though Asp718 and KpnI are isoschizomers, they cleave opposite one another with Asp718 generating a 5'- and KpnI leaving a 3'-protruding end. One wonders if this difference in specificity correlates with their difference in sensitivity to Dcm methylation.

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