DNA cleavage by restriction endonuclease *Pfl*MI is inhibited in recognition sites modified by *dcm* methylation

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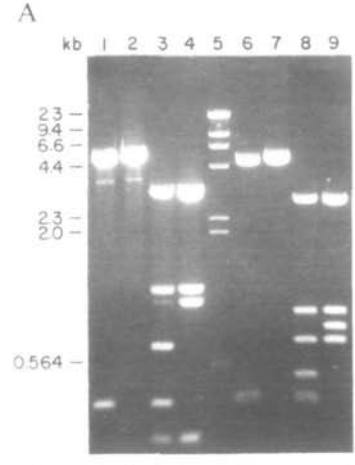
Plasmids pBS*oct*-1$^+$ (1) and pALTE1a (described below*) both contain two canonical *Pfl*MI recognition sites [CCANNNNNTGG (2)]. These plasmids, when purified from *Escherichia coli* *dam*$^-$/*dcm*$^-$ strain RB404 (3), release a 362- or 337-bp fragment, respectively, after digestion with *Pfl*MI (Fig.1A, lanes 1 and 6). However, when purified from *dam*$^+$/*dcm*$^+$ *E. coli* strains, these plasmids cleaved only once after digestion with *Pfl*MI (Fig. 1A, lanes 2 and 7). Digestion of unmethylated (lane 3) and methylated (lane 4) pBS*oct*-1$^+$ DNA with *Pfl*MI and *Eco*RI identified the *oct*-1 *Pfl*MI site at nucleotide position 1680 (1) (see Fig. 1B) as the recognition site that is resistant to cleavage. Digestion of unmethylated (lane 8) and methylated (lane 9) pALTE1a DNA with *Pfl*MI and *Puv*II identified the adenovirus *Pfl*MI site at nucleotide position 1461 (4) (see Fig 1C) as the resistant site. Both cleavage-resistant *Pfl*MI sites contain the *dcm* methylation sequence, CmCWGG (5). These results suggest that a subset of *Pfl*MI recognition sequences that contain a *dcm* methylation site are resistant to *Pfl*MI cleavage when methylated.



B

cag*CCAGGTGATGGtga

ccaCCAAGCAGTGGtgg

C

ctg*CCAGGATGTGGaat

gttCCATAGGTTAAaat

Figure 1: A. Restriction enzyme analysis of pBS*oct*-1$^+$ and pALTE1a. Plasmid DNAs were prepared from *dam*$^-$/*dcm*$^-$ *E. coli* RB404 (lanes 1, 3, 6 and 8) or *dam*$^+$/*dcm*$^+$ *E. coli* strains (lanes 2, 4, 7 and 9). Lane 5, Lambda *Hin*dIII-digested DNA markers; lanes 1, 2, 6 and 7, *Pfl*MI alone; lanes 3 and 4, *Pfl*MI/*Eco*RI; lanes 8 and 9, *Pfl*MI and *Puv*II. The fragments expected from complete digestion of pBS*oct*-1$^+$ with *Pfl*MI and *Eco*RI are 3188, 1190, 687, 362, and 220 bp (lane 3) and from non-digestion at *Pfl*MI site 1680 are 3188, 1190, 1049, and 220 bp (lane 4). The fragments expected from digestion of pALTE1a with *Pfl*MI and *Puv*II are 2840, 952, 707, 470, and 337 bp (lane 8); and from non-digestion at adenovirus 1461 *Pfl*MI site are 2840, 952, 807, and 707 bp (lane 9). **B.** DNA sequence around the *oct*-1 *Pfl*MI recognition sites (1) at nucleotide positions 1680 (top) and 1318 (bottom). **C.** DNA sequence around the pALTE1a *Pfl*MI recognition sites at adenovirus nucleotide coordinate 1461 (top) and SV40 nucleotide coordinate 4558 (bottom). The *Pfl*MI site is highlighted in capital letters, and the CCAGG *dcm* methylation recognition sequence contained within this sequence is underlined with the methylated cytosine indicated by an asterisk.

* Plasmid pALTE1a was derived from p12S.wt (6) by the replacement of the 465-bp *Puv*II (nucleotide position 451) to *Cla*I (nucleotide position 916) adenovirus fragment with 1075-bp of SV40 sequence that begins at the *Nae*I site (nucleotide position 345) and ends at nucleotide 4513, where a *Cla*I site was introduced by oligonucleotide mutagenesis.

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