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DNA cleavage by *AatI* and *StuI* is sensitive to *Escherichia coli* dcm methylation

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While attempting to subclone the StuI - EcoNI fragment of pRIF 309+ (1) we found that the AGGCCT site was not cleaved by StuI or its isoschizomer AatI. Upon examination of the DNA sequence it became evident that this site overlapped an E.coli dcm methylation site CCT/AGG,ie. AGGCCTGG, which when methylated could be resistant to cleavage . To test this proposition we prepared pRIF309+ in the dcm^- E.coli strain GM 2929 kindly supplied by Dr. B. Bachmann. As can be seen in Fig.1 pRIF309+ prepared from a dcm^- host is cleaved by StuI. An identical result was obtained with AatI (not shown).

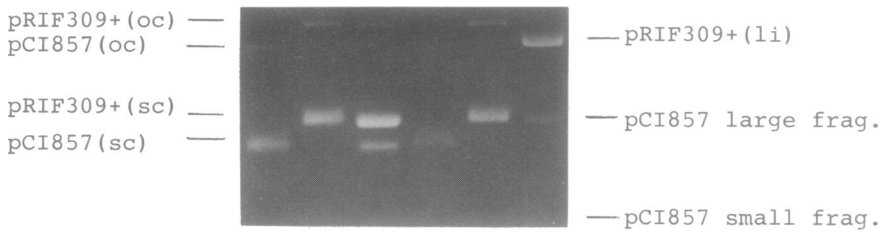

Fig.1: pRIF309+ which carries the gene for the EcoRI restrictionendonuclease under the control of the p_L promoter was isolated from E.coli LK111 (λ lysogen) or from E.coli GM 2929 together with pCI857 which codes for a thermosensitive CI repressor. The DNA was incubated in the absence or presence of StuI, then subjected to electrophoresis. Lane 1: pCI857(dcm^+); lane 2: pRIF309+(dcm^+); lane 3: pCI857 and pRIF309+(dcm^-); lane 4: pCI857(dcm^+)/StuI; lane 5: pRIF309+(dcm^+)/StuI; lane 6: pCI857 and pRIF309+ (dcm^-)/StuI.
This indicates that hemimethylation of AGGCCT arising from the modification by the dcm-methylase interferes with cleavage by AatI or StuI similarly as reported recently for ApaI and Asp718 (2,3)

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