

M.EcoP15 methylates the second adenine in its recognition sequence

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The type III restriction/modification system *EcoP15* recognizes the non-palindromic sequence 5'-CAGCAG-3'. The restriction enzyme cleaves the DNA 25–27 bp to the right of the sequence as written. The modification methylase methylates one of the two adenine residues in the recognition sequence (1). Attempts to determine which of the adenines is methylated by the enzyme were foiled by the internal repeated symmetry of the recognition sequence (1). In a new attempt to determine the methylated adenine, we searched the EMBL DNA sequence data base for *EcoP15* sites that overlapped sites for type II restriction enzymes in the hope of finding sites that might be rendered resistant to cleavage by *EcoP15* methylation. One promising region was found in the Ad2 viral genome. We cloned a 954 bp *SpeI/KpnI* fragment of this DNA (EMBL accession no. J01917; position 32,645–33,598 bp; ref.2) into the *KpnI* and *XbaI* sites of the pUC19 polylinker to generate the plasmid pUCAd192. The inserted Ad2 fragment contains 5 *EcoP15* sites, where one site overlaps an *AluI* site and a second site overlaps a *PstI* site (Figure 1). Since a methylated adenine residue in a *PstI* site protects against *PstI* restriction (3), methylation of the first adenine in the *EcoP15* sequence by *M.EcoP15* should change the *PstI* restriction pattern. If the second adenine is methylated, a change of the *AluI* cleavage pattern should result, since *AluI* is blocked by a methylated adenine in its recognition sequence (3).

For methylation, 6 µg of pUCAd192 DNA were incubated with *M.EcoP15* for 12 hours at 37°C in *EcoP15* buffer (100 mM HEPES, pH 8.0; 0.25 mM EDTA; 6.4 mM MgCl₂; 12 mM 2-mercaptoethanol) and 3 µM AdoMet. The reaction mixture was extracted with phenol/chloroform and the aqueous phase was ethanol-precipitated. The redissolved DNA was divided into 3 aliquots. As a control for the efficiency of methylation, one aliquot was digested with *EcoP15* in *EcoP15* buffer with 1 mM ATP. The second aliquot was treated with 10 U of *AluI* in NEB1 buffer and the third with 10 U of *PstI* in NEB3 buffer (New England Biolabs). The same procedure was done with non-methylated control DNA incubated in *EcoP15* buffer with 3 µM AdoMet but without *M.EcoP15*.

Figure 2 shows that *EcoP15* methylation blocks *AluI* cleavage but does not influence the *PstI* pattern. We thus conclude that *EcoP15* methylation generates 5'CAGC^m6AG. The ability to selectively block certain *AluI* sites by *EcoP15* methylation may be useful in some circumstances.

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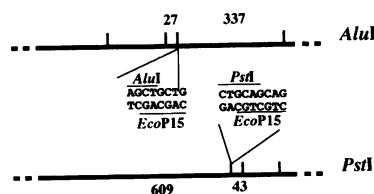


Figure 1. Localization of the *EcoP15* sites overlapping *AluI* and *PstI* sites in the cloned fragment of pUCAd192. Fragment lengths (in bp) are indicated.

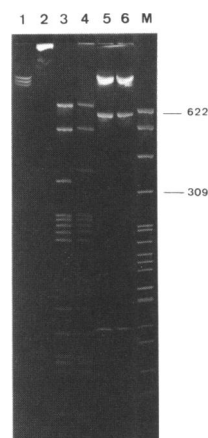


Figure 2. Comparison of the restriction pattern of *EcoP15*-modified and non-modified pUCAd192 DNA. 2 µg of the DNA cleaved with the enzymes indicated (5 hours at 37°C) were electrophoresed through a 5% polyacrylamide gel. Non-modified (1, 3, 5) or *EcoP15*-methylated DNA (2, 4, 6) was treated with *EcoP15* (1, 2), *AluI* (3, 4) or *PstI* (5, 6); marker DNA: pBR322 + *MspI* (M).

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