

*Mfo*AI, a novel isoschizomer of *Hae*III from *Mycobacterium fortuitum* recognizing 5'-GG/CC-3'

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We have isolated a novel class II restriction endonuclease, *Mfo*AI, from *Mycobacterium fortuitum* TMC 1529 which recognizes the sequence 5'-GG/CC-3' generating blunt ended fragments. *Mfo*AI was found to have a very high affinity for phosphocellulose and eluted late at a concentration of 1.8 M KCl. This permitted very rapid purification of the enzyme completely free from non-specific nucleases. In contrast to the situation in *Haemophilus aegypticus* which has *Hae*III and *Hae*II, *Mfo*AI was found to be the only activity associated with *M.fortuitum* TMC 1529, suggesting this organism to be a better source for this enzyme. In addition, the activity of *Mfo*AI was independent of salt concentration and purified *Mfo*AI was equally active in 10–200 mM concentrations of KCl, $MgCl_2$ and NaCl and in the wide pH range of 7.4–11.0.

A comparison of cleavage patterns experimentally obtained with *Mfo*AI on standard DNAs of known nucleotide sequence; pUC8 M13mp8, phiX174, pGEM3Z and pT7-7 with computer derived mapping data predicted the sequence 5'-GGCC-3' (Figure 1).

The cut positions within the *Mfo*AI recognition site were determined according to the enzymatic sequencing approach (1). A pUC8 recombinant with an insert containing an *Mfo*AI site was used for enzymatic sequencing reactions starting with a primer approximately 100 base pairs from the recognition site. In a parallel reaction the same primer end labelled with [gamma-^{32}P] ATP was annealed to the template and the labelled primer was extended through the *Mfo*AI site by treatment with Sequenase version 2.0 (2, 3) in the presence of all four dNTPs. The double stranded DNA was used as substrate for *Mfo*AI to produce 5' end labelled DNA fragments comparable to the sequencing ladder. Samples were analysed without and with (–/+) further incubation with T4 DNA polymerase and all four dNTPs by electrophoresis and subsequent autoradiography (Figure 2).

From the mapping and sequencing data the specificity of *Mfo*AI is concluded as:

5'-GG/CC-3'
3'-CC/GG-5'

REFERENCES

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2. Tabor,S. and Richardson,C.C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4767–4771.
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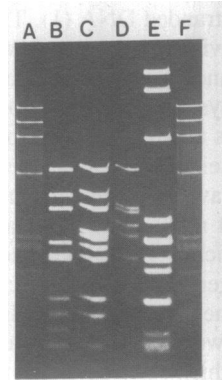

Figure 1. *Mfo*AI digests on [A] phiX174, [B] pUC8, [C] pGEM3Z, [D] pT7-7 and [E] M13mp8 DNA. [F] MW markers: phiX174-*Hae*III fragments.

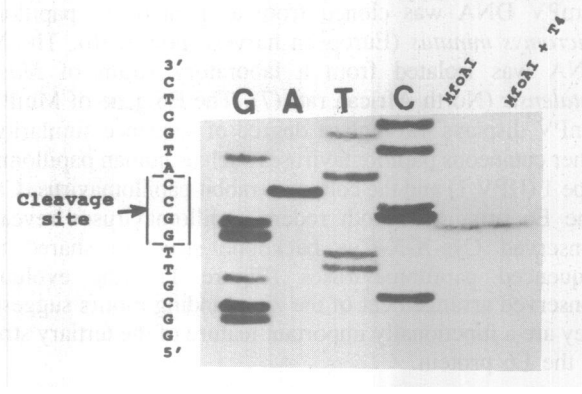

Figure 2. Determination of *Mfo*AI cleavage position.

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