

Isolation and identification of restriction endonuclease MspCI

M.Rina¹, M.Tzanodaskalaki¹, A.Karagouni³, M.Pagomenou¹ and V.Bouriotis^{1,2*}

¹Institute of Molecular Biology and Biotechnology, Enzyme Technology Division, PO Box 1515, Heraklion 71110, Crete, ²University of Crete, Department of Biology, Division of Applied Biology and Biotechnology, PO Box 1470, Heraklion 71110, Crete and ³Institute of General Botany, University of Athens, Athens 15784, Greece

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MspCI, an isoschizomer of AflII (1), has been purified from *Micrococcus* species. MspCI recognises the sequence 5'...C-TTAA G ... 3' and cleaves between C and T. The enzyme was purified using the following chromatographic steps: 1. Blue Sepharose F3GA; 2. Heparin-Sepharose; 3. DEAE Sepharose.

The enzyme was free of contaminating nuclease activity. After 100 fold overdigestion on Lambda DNA greater than 95% of the DNA fragments can be ligated and recut by the enzyme. Optimal conditions for enzyme activity are 150 mM NaCl, 10 mM Tris-HCl (pH 7.9), 10 mM MgCl₂, 1 mM DTT, at 37°C. The fragments produced by digestion of lambda DNA, Adeno-2 pBR322, (ΦX174, and SV40 match those predicted by cleavage at the sequence CTTAAG (Figure 1, lanes 4-8).

In order to determine the cleavage site within the recognition sequence the vector SV40 which contained a recognition site for the enzyme was digested by the enzyme then annealed with sequencing primers and extended with Klenow enzyme in the presence of ³²P-dATP. Dideoxy sequencing reactions were performed at this region with the same primers and run in parallel with the extended products (2). Results in Figure 2 show that the extended products of the forward (lane F) and the reverse (lane R) sequencing primers comigrate with the band corresponding to the 3' A in the sequence 5'...CTTAA G ... 3'. Therefore MspCI recognises and cleaves the following sequence 5'...CTTAA G ... 3'.



REFERENCES

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

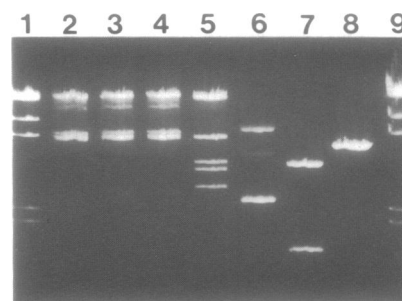


Figure 1. MspCI digests: lane 2: lambda DNA digested by AflII, 3: lambda DNA digested by AflII and MspCI, 4: lambda DNA, 5: Adeno-2, 6: pBR322, 7: ΦX174, 8: SV40, lanes 1,9: lambda-HindIII size standard.

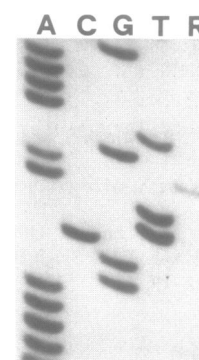


Figure 2.