Isolation and identification of restriction endonuclease MspCI

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MspCI, an isoschizomer of AflII (1), has been purified from Micrococcus species. MspCI recognises the sequence 5'...C-TTAAG ... 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 $MgCl_2$, 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 ^{32}P-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'...CTTAAG ... 3'. Therefore MspCI recognises and cleaves the following sequence 5'...CTTAAG ... 3'.

5' ...C$^{\downarrow}$TTAA G ... 3'
3' ...G AATT$_{\uparrow}$C ... 5'

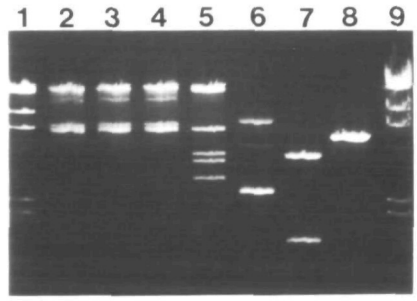

Figure 1. MspCI digests: lane 2: lambda DNA digested by AflII, 3: lambda DNA digested by AflII and MspCI, 4: lambda DNA, 5: Adeno-2, 6: pBR322, 7: ΦX174, 8: SV40, lanes 1,9: lambda-HindIII size standard.

Figure 2.

REFERENCES

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