

Isolation and identification of restriction endonuclease SgrBI

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SgrBI, an isoschizomer of SacII (1) has been purified from *Streptomyces griseus*. SgrBI recognises the palindromic sequence 5'...CCGCGG ... 3' generating 3'-protruding GC-dinucleotides. The enzyme was purified using the following chromatographic steps. 1. Phosphocellulose, 2. Heparin-Sepharose. The enzyme was free of contaminating nuclease activity. After 100 fold overdigestion on Adeno-2 DNA greater than 95% of the DNA fragments can be ligated and greater than 95% can be recut by SgrBI. Optimal conditions for enzyme activity are 10 mM Tris-HCl (pH 7.9), 10 mM $MgCl_2$, 1 mM DTT, 0.1% Triton X-100, at 37°C. The fragments produced by digestion of lambda-HindIII DNA, Adeno-2, SV40, Φx174, pBR322, and lambda DNA match those predicted by cleavage at the sequence CCGCGG (figure 1, lanes 4–9).

In order to determine the cleavage site within the recognition sequence the Bluescript KSII plasmid which contained a recognition site for the enzyme in the polylinker was digested by the enzyme, then annealed with forward or reverse sequencing primers and extended with Klenow enzyme in the presence of α^{32}P-dATP. Dideoxy sequencing reactions were performed at this region with the same primers and run in parallel with the extended products (2). The results show that the extended products of the forward primer (figure 2, lane F) and the reverse primer (data not shown), comigrate with the band corresponding to the second C in the sequence 5'...CCGCGG ... 3'.

From the mapping and sequencing data the specificity of SgrBI is concluded as:

5'...CCGC↓GG...3'
3'...GG↑CGCC...5'

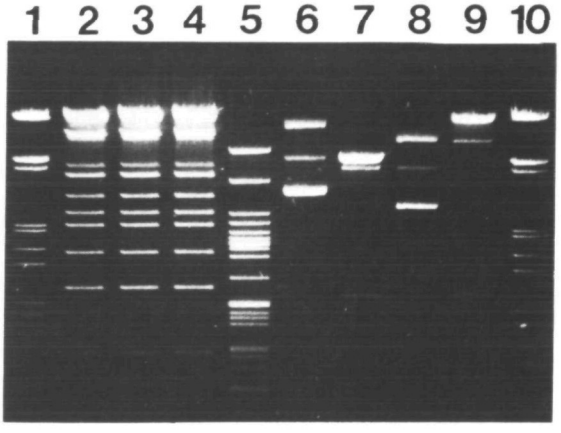

Figure 1. SgrBI digests: lane 2: lambda-HindIII DNA digested by SacII, 3: lambda-HindIII DNA digested by SacII and SgrBI, 4: lambda-HindIII DNA, 5: Adeno-2, 6: SV40, 7: Φx174, 8: pBR322, 9: lambda DNA, 1, 10: lambda-HindIII-EcoRI size standard.

Figure 2.

REFERENCES

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