Isolation and identification of restriction endonuclease SseBI

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SseBI, an isoschizomer of StuI (1) has been purified from Streptomyces species. SseBI recognises the sequence 5′ ... A-GGCCT ... 3′ and cleaves between G and C. The enzyme was purified using the following chromatographic steps: 1. Blue Sepharose F3GA, 2. Heparin−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 greater than 95% can be recut by SseBI. Optimal conditions for enzyme activity are 100 mM NaCl, 50 mM Tris−HCl (pH 7.9), 10 mM $MgCl_2$, 1 mM dithiothreitol at 37°C.

The fragments produced by digestion of lambda DNA, Adeno 2, pBR322, ΦX174 and SV-40 match those predicted by cleavage at the sequence AGGCCT (Figure 1, lanes 4−8). In order to determine the cleavage site within the recognition sequence, the vector ΦX174 which contained a recognition site for the enzyme 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).

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′G in the 5′ ... AGGCCT ... 3′ sequence. Therefore SseBI recognises and cleaves the following sequence:

5′ ... AGG↓CCT ... 3′
3′ ... TCC↑GGA ... 5′

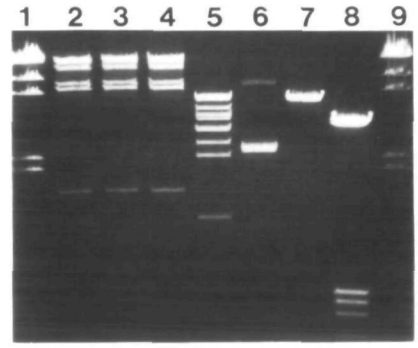

Figure 1. SseBI digests: lane 2: lambda DNA digested by StuI, 3: lambda DNA digested by StuI and SseBI, 4: lambda DNA, 5: Adeno-2, 6: pBR322, 7: ΦX174, 8: SV40, lanes 1,9: lambda-HindIII size standard.

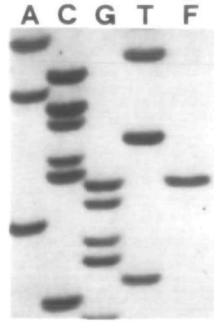

Figure 2.

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