Isolation and identification of restriction endonuclease SseAI

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SseAI, an isoschizomer of NarI (1), has been purified from *Streptomyces* species. SseAI recognises the palindromic sequence 5'...GGCGCC ... 3' and cleaves between second G and C. 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 recut by SseAI. Optimal conditions for enzyme activity are 6 mM Tris-HCl (pH 7.4), 6 mM $MgCl_2$, 6 mM β-mercaptoethanol at 37°C. The fragments produced by SseAI digestion of lambda-HindIII DNA, Adeno-2 DNA, SV40, Φx174, pBR322 and lambda DNA match those predicted by cleavage at the sequence GGCGCC (figure 1, lanes 4–9).

In order to determine the cleavage site within the recognition sequence a pUC19 vector which contained a recognition site for the enzyme was digested by the enzyme SseAI then annealed with 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 both primers (lanes F and R) comigrate with the band corresponding to the 3'G in the 5'...GGCGCC ... 3' sequence. Therefore SseAI recognises and cleaves the following sequence.

5'...GG↓CGCC...3'
3'...CCG↑CGG...5'

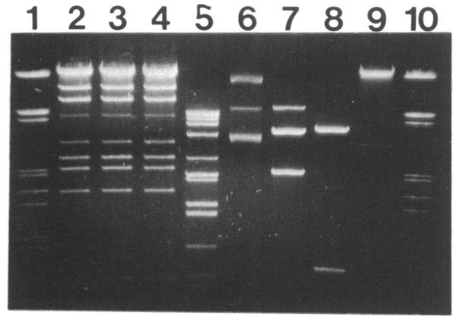

Figure 1. SseAI digests: lane 2: lambda-HindIII DNA digested by NarI, 3: lambda-HindIII DNA digested by NarI and SseAI, 4: lambda-HindIII DNA, 5: Adeno-2, 6: SV40, 7: Φx174, 8: pBR322, 9: lambda DNA, 1, 10: lambda HindIII-EcoRI size standard.

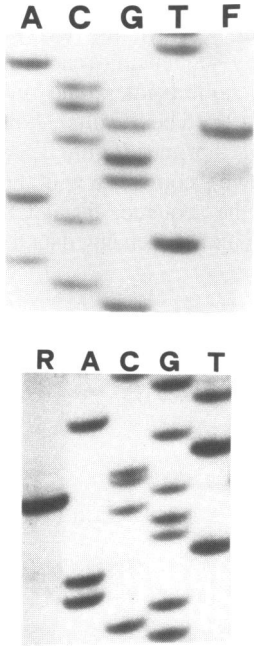

Figure 2.

REFERENCES

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