Isolation and identification of restriction endonuclease BseCI

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Submitted February 25, 1992

BseCI, an isoschizomer of ClaI (1) has been purified from Bacillus species. BseCI recognises the sequence 5'...ATCGAT ... 3' and cleaves between T and C. The enzyme was purified using the following chromatographic steps: 1. Phosphocellulose, 2. Heparin−Sepharose, 3. DEAE-cellulose. The enzyme was free of contaminating nuclease activity. After 100 fold overdigestion on lambda DNA greater than 95% of the fragments can be ligated and greater than 95% can be recut by BseCI. Optimal conditions for enzyme activity are 100 mM NaCl, 50 mM Tris−HCl (pH 7.9), 10 mM $MgCl_2$, 1 mM DTT, at 60°C. The fragments produced by digestion of lambda DNA, Adeno-2, pBR322, SV40, ΦX174, lambda dam$^-$ DNA match those predicted by cleavage at the sequence ATCGAT (Figure 1, lanes 4−9). In order to determine the cleavage site within the recognition sequence the pBR322 plasmid 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 a ^{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 both primers (Figure 2, lanes F and R) comigrate with the band corresponding to the G in the sequence 5' ... ATCGAT ... 3'.

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

5' ... AT↓CG AT ... 3'
3' ... TA GC↑TA ... 5'

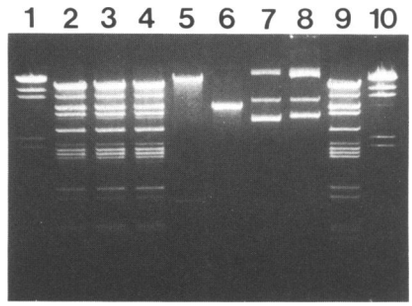

Figure 1. BseCI digests: lane 2: lambda DNA digested by ClaI, 3: lambda DNA digested by ClaI and BseCI, 4: lambda DNA, 5: Adeno-2, 6: pBR322, 7: SV40, 8: ΦX174, 9: lambda DNA (dam$^-$), lanes 1,10: lambda-HindIII size standard.

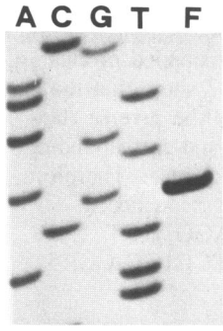

Figure 2.

REFERENCES

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