Isolation and identification of restriction endonuclease BseBI

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Submitted July 5, 1991

BseBI, an isoschizomer of BstNI (1) has been purified from *Bacillus stearothermopilus* species. BseBI recognises the sequence 5'...CCWGG...3' (W=A or T) and cleaves between C and W. 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 5-fold overdigestion on lambda DNA none of the DNA fragments can be ligated. Ligation can be achieved by 'filling in' the extensions with Klenow fragment. Optimal conditions for enzyme activity are 50 mM NaCl, 10 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 1 mM DTT at 60°C. The fragments produced by BseBI digestion of lambda DNA, Adeno 2, pUC19, Φx174, SV40 and pBR322 match those predicted by cleavage at the sequence CCWGG (Figure 1, lanes 3−8). In order to determine the cleavage site within the recognition sequence a pUC19-derivative with an insert containing a BseBI cleavage site, 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). Results in Figure 2 show that the extended product of the forward primer (lane F) comigrates with the band corresponding to the A in the sequence 5'...CC-AGG ... 3' while the extended product of the reverse primer (lane R) comigrates with the band corresponding to the T in the sequence 5'...CCTGG ... 3'. From the mapping and sequencing data the specificity of BseBI is concluded as:

5'...CC↓WGG...3'
3'...GGW↑CC...5'

REFERENCES

1. Schildkraut,I. and Greenough,L. unpublished observations.
2. Tabor,S. and Richardson,C.C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4767−4771.

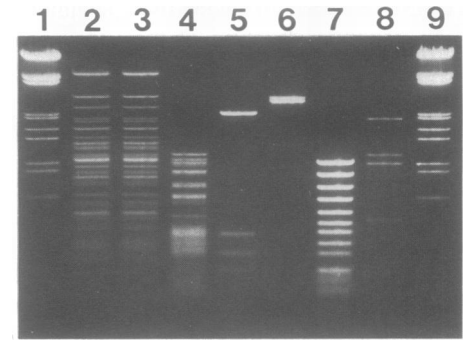

Figure 1. BseBI digests; lane 2: Lambda DNA digested by BstNI, lane 3: lambda DNA, 4: Adeno-2, 5: pUC19, 6: Φx174, 7: SV40, 8: pBR322, lanes 1 and 9 lambda−HindIII−EcoRI size standard.

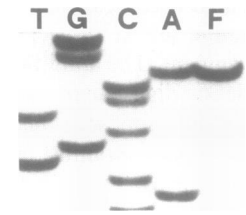

Figure 2.

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