Isolation and identification of restriction endonuclease BshGI

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BshG I, an isoschizomer of BstN I (1) has been purified from *Bacillus sphaericus* species. BshG I recognises the sequence 5'...CCA/TGG...3' and cleaves between C

Figure 1: BshG I digests; lane 2: lambda DNA, 3: Adeno 2, 4: pBR322, 5: ΦX 174, 6: SV40, lanes 1 and 7 lambda - Hind III - EcoR I size standard

and A/T. The enzyme was purified using the following Chromatographic steps 1. Blue F3GA-Sepharose, 2. DEAE-Sepharose, 3. Phosphocellulose. 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 BshG I. Optimal conditions for BshG I activity are 50mM NaCl, 10mM Tris (pH 7.6), 10mM MgCl₂, 5mM 2-mercaptoethanol at 37°C. The fragments produced by BshG I digestion of lambda DNA, Adeno 2, pBR322, ΦX 174 and SV40 match those predicted by cleavage at the sequence CCA/TGG (figure 1, lanes 2-6). A pUC 19 recombinant clone 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 α³²P-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 1) comigrates with the band corresponding to the A in the sequence 5'...CCAGG...3' while the extended product of the reverse primer (lane 2) comigrates with the band corresponding to the T in the sequence 5'...CCTGG...3'. Taken together the above results indicate that BshG I recognises and cleaves the following sequence

Figure 2

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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 Schildkraut, I. and Greenough, L., unpublished observations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