Isolation and identification of restriction endonuclease BshKI

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BshK I, an isoschizomer of Sau96 I (1) has been purified from *Bacillus sphaericus* species. BshK I recognises the sequence 5'...GGNCC...3' and cleaves between G and G. The enzyme was purified using the following Chromatographic steps. 1. Blue

F3GA-Sepharose, 2. DEAE-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 BshK I. Optimal conditions for enzyme activity are 50mM NaCl, 10mM Tris (pH 7.6), 10mM MgCl₂, 5mM 2-mercaptoethanol at 37°C. The fragments produced by BshK I digestion of: lambda DNA, Adeno 2, pBR322, ΦX 174, and SV40 match those predicted by cleavage at the sequence GGNCC (figure 1, lanes 2-6). A pUC 19 recombinant clone which contained a recognition site for the enzyme was digested by the enzyme BshK I 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 5'C in the sequence 5'...GGTCC...3' while the extended product of the reverse primer (lane 2) comigrates with the band corresponding to the 5'C in the sequence 5'...GGACC...3'. Taken together the above results indicate that BshK I recognises and cleaves the following sequence.

Figure 1. BshK 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.

Figure 2

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