Characterisation of *Bci*BII, an isoschizomer of *Bst*NI from a strain of *Bacillus circulans* B

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*Bci*BII, an isoschizomer of *Bst*NI (figure 1) has been isolated from *Bacillus circulans* B. The enzyme was purified by precipitation with polyethylenimine, ammonium sulfate and subsequent column chromatography on DEAE-sepharose, Blue sepharose and phosphocellulose. Restriction endonuclease *Bci*BII showed maximal activity at 37°C, pH between 7.4–7.8, MgCl₂ concentration in the range of 8–12 mM and at high ionic strength.

The *Bci*BII cleavage site was sequenced according to Brown and Smith (1) using the M13mp18 ss DNA template, the 'hybridisation primer' and T7 sequenase (2) instead of Klenow enzyme. The product of the primed synthesis reaction was digested with *Bci*BII after heat inactivation of T7 DNA polymerase, one half of it was incubated with Klenow fragment and 4 dNTPs and both mixtures were run in parallel with four standard sequencing reactions on the 5% sequencing gel. The obtained pattern (figure 2) clearly shows that *Bci*BII cleaves the sequence 5'-CC/A_TGG-3' as indicated by the arrow and thus is a true isoschizomer of *Bst*NI.

REFERENCES

1. Brown, N.L. and Smith, M. (1980) *Methods Enzymol.* **65**, 391–404.
2. Tabor, S. and Richardson, C.C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4767–4771.

Figure 1. Lambda DNA digests; lane 1, *Hind*III; lane 2, *Bci*BII; lane 3, *Bst*NI + *Bci*BII; lane 4, *Bst*NI.

Figure 2. *Bci*BII cleavage site. After heat inactivation of the T7 polymerase the extended nascent dsDNA template was cleaved with *Bci*BII (–), an aliquote of the digested sample was incubated with Klenow Fragment and all 4 dNTPs (+) and both samples were run in parallel with standard sequencing reactions (A,G,C,T).