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Isolation and identification of restriction endonuclease BstF I

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BstFI, an isoschizomer of HindIII, has been purified from Bacillus stearothermophilus FH58 isolated from the soil of the campus of Fudan University. Sequencing data show that the cleavage site of BstFI is A/AGCTT, the same as HindIII. 10,000 units BstFI can be obtained from each gram wet wt. of cells. BstFI is active over a temperature range from 37°C to 65°C. The optimal temperature for its action is 55°C. The optimal pH and ionic concentration of the assay buffer for the optimum activity of BstFI is 7.0 - 7.5 and 50-100 mM Nacl, respectively. BstFI is very stable during incubation at 45°C for as long as 10 hrs., but loses its activity easily at 50°C.

Fig. 1

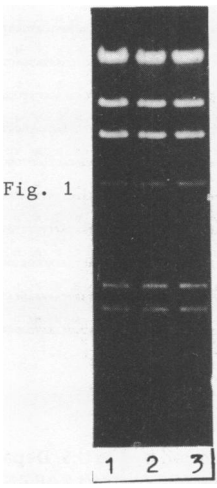

Fig. 2

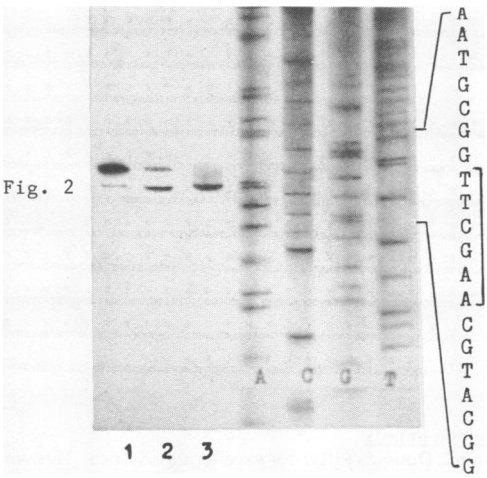

Figure 1: The cleavage patterns of BstFI (lane 1), BstFI + HindIII (lane 2), HindIII (lane 3). Lambda DNA is substrate.

Figure 2: Determination of the recognition sequence and the cleavage site of BstFI on M13mp19 using the dideoxynucleotide chain termination method. Lane 1: Digestion with BstFI. Lane 2,3: Incubation at 65°C, 20 min.for partially inactivating the Klenow Fragment, followed by digestion with HindIII (lane 2) or BstFI (lane 3).