***BfaI*, a new *MaeI* isoschizomer from *Bacteroides fragilis*, recognizes the sequence 5' C↓TAG 3'**

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A new type II restriction endonuclease, *BfaI*, has been isolated from *Bacteroides fragilis* (NEB # 668). *BfaI* recognizes the four base sequence 5' CTAG 3' and cleaves between the C and the T residues to produce a 2 base 5' extension; C/TAG.

BfaI was found to cut pBR322 DNA into fragments of approximately 1500 bp, 1270 bp, 1050 bp, 360 bp and 240 bp, and PhiX174 DNA into 3500 bp, 1150 bp, and 790 bp fragments. A computer search (1) was used to match these fragments to a CTAG recognition site. The fragments generated by *BfaI* on Lambda c1857, T7, Adeno2, pUC18, and M13mp18 (Figure 1) matched exactly those predicted by cleavage at a CTAG. In addition, digestion of Lambda c1857 with *MaeI* and *BfaI* together and separately generated identical patterns (data not shown). These data allow us to conclude that *BfaI* recognizes the sequence CTAG.

In order to determine the cleavage site within the recognition sequence, five similar reactions were run (2). In each, a single stranded M13mp18 template was treated with a primer (NEB 1211) specific for a site 40 base pairs 3' of a CTAG tetramer. Four of the reactions were used in standard dideoxy sequencing using DNA polymerase I large fragment (Klenow). In the fifth, the primer was extended through the CTAG site without any dideoxy analogs of the dNTP's. The extension reaction was heat inactivated, followed by digestion with *BfaI*. Subsequence to digestion by *BfaI*, the reaction was halved. One half of the mixture was treated with stop dye, while the other was incubated with Klenow and dNTP's. In Figure 2 the four sequencing reactions have been run alongside the reaction stopped after digestion with *BfaI* (-) and the reaction incubated with polymerase subsequence to *BfaI* digestion (+). The fragment generated by *BfaI* digestion (-) reaction comigrated with the C in the sequence CTAG, while the fragment produced by Klenow treatment of the *BfaI* digestion product in the (+) lane comigrated with the A of CTAG. These results demonstrate that the CTAG site is cleaved by *BfaI* to give a symmetric 2 base 5' staggered cut.

5' C↓TAG 3'
3' GAT↑C 5'

REFERENCES

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Figure 1. *BfaI* digests; lane 2: Lambda, 3: T7, 4: Adeno2, 5: pUC18, 6: pBR322, 7: PhiX174, 8: M13mp18, lanes 1 and 9: HindIII-Lambda and HaeIII-PhiX174 size standard.

Figure 2.

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