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*Bfi*I, a restriction endonuclease from *Bacillus firmus* S8120, which recognizes the novel non-palindromic sequence 5′-ACTGGG(N)$_{5/4}$-3′

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ABSTRACT

A new type IIS restriction endonuclease *Bfi*I has been partially purified from *Bacillus firmus* S8120. *Bfi*I recognizes the non-palindromic hexanucleotide sequence 5′-ACTGGG(N)$_{5/4}$-3′ and makes a staggered cut at the fifth base pair downstream of the recognition sequence on the upper strand, producing a single base 3′ protruding end.

More than 200 type II restriction endonucleases with different specifities have been identified (1). A subclass of the type II enzymes, so-called type IIS ENases (2), recognize assymetrical nucleotide sequences and cleave DNA at specified distances. Type IIS ENases are much less abundant than enzymes which operate within short symmetrical nucleotide sequences. Their ability to cut DNA without destroying their recognition sites has led to the development of many unique applications (2–6). Here we describe a new type IIS restriction endonuclease *Bfi*I which recognizes the assymetric hexanucleotide sequences 5′-ACTGGG-3′ and cleaves 5 and 4 nt away, producing a single base 3′ protruding end.

*Bfi*I was isolated from *Bacillus firmus* S8120. Cells of a log phase culture were disrupted by sonication and cell debris was removed by centrifugation. The supernatant was subjected to phosphocellulose chromatography. The *Bfi*I pool was passed over DEAE–cellulose. Fractions containing *Bfi*I activity were pooled and fractionated by heparin–Sepharose chromatography. The final preparation, free of contaminating nucleases, was obtained by passage over a hydroxyapatite column. The eluted *Bfi*I activity was pooled into storage buffer (10 mM Tris–HCl, pH 7.5, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.2 mg/ml BSA and 50% glycerol) and stored at –20°C.

To determine the substrate specifity of *Bfi*I, DNA substrates (λ phage, pBR322, pUC18 and M13mp18) were incubated at 37°C in 50 µl Tango-Y+ buffer (MBI Fermentas) containing 1 µg DNA (Fig. 1). Digestion of pBR322 DNA yielded five fragments. Four of five *Bfi*I cleavage sites on pBR322 DNA (approximate positions 300, 680, 2200 and 3400) were mapped by double digestion with *Hin*dIII, *Bsp*68I, *Eco*88I, *Bst*1107I, *Pst*I and *Ssp*I (data not shown). After digestion of λ DNA with *Bfi*I in combination with *Bsp*120I, *Eco*105I, *Pfl*23II, *Xba*I, *Xho*I, *Nhe*I and *Eco*81I (data not shown) four *Bfi*I cleavage sites at approximate positions 7000, 11 600, 25 500 and 30 300 were localized. A computer-aided search of homologous nucleotide sequences at the mapped *Bfi*I sites and areas surrounding them revealed only one common sequence 5′-ACTGGG-3′ (or its complement 5′-CCCAGT-3′) for all the positions mapped. The number and sizes of the fragments generated by *Bfi*I digestion of pUC18 (two fragments of 1380 and 1300 bp), M13mp18 (one cut site) and SV40 (two fragments; data not shown) DNAs were consistent with this sequence being the *Bfi*I recognition sequence.

pBR322 DNA was used as a template to characterize the cleavage site of *Bfi*I. A 20mer oligodeoxyribonucleotide complementary to pBR322 between positions 500 and 520 (cw strand) was used in forward sequencing through the *Bfi*I site located at position 609. Four dideoxy sequencing reactions (lanes G, A, T and C respectively) using [α-^{33}P]dATP were carried out (7). The same primer and template were used in a fifth non-terminating reaction, which also included T7 DNA polymerase and [α-^{33}P]dATP. The labelled DNA was cleaved with *Bfi*I, inactivated by heating and divided into two. One sample was treated with T4 DNA polymerase. Both samples were diluted with sequencing dye and loaded on a standard sequencing gel together with the dideoxy sequencing reactions.

The autoradiograph of the primed synthesis reaction used to characterize the cleavage site of *Bfi*I is shown in Figure 2. The single band produced by *Bfi*I cleavage co-migrates with the fifth nucleotide 3′ of the end of the recognition sequence (Fig. 2, lane 1). Lane 2 shows the result obtained when the *Bfi*I restriction product was further treated with T4 DNA polymerase. This sample produced a band that co-migrates with the fourth base from the recognition sequence, indicating that DNA cleavage by *Bfi*I generates a single base 3′ protruding end which is resected by the exonuclease activity of T4 DNA polymerase. From these results it can be inferred that *Bfi*I cleaves the template strand 4 nt away from the recognition sequence.

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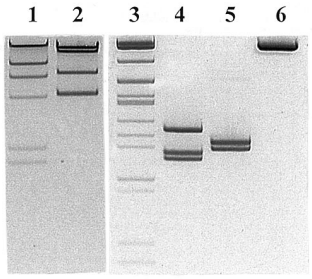

Figure 1. Cleavage of various DNAs with *Bfi*I. Lane 1, λ DNA *Hin*dIII digest as size markers; lane 2, λ DNA + *Bfi*I; lane 3, λ DNA *Eco*47I digest as size markers; lane 4, pBR322 DNA + *Bfi*I; lane 5, pUC18 DNA + *Bfi*I; lane 6, M13mp18 DNA + *Bfi*I.

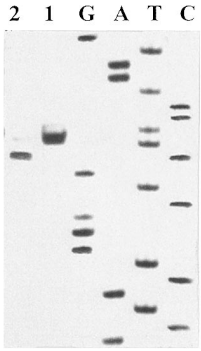

Figure 2. Determination of the *Bfi*I cleavage site. Lanes G, A, T and C, sequence ladder through the *Bfi*I recognition site on pBR322. Lane 1, product of the primed synthesis reaction cleaved with *Bfi*I. Lane 2, as lane 1 but treated with T4 DNA polymerase.

*Bfi*I is a new prototype of the type IIS restriction endonucleases whose recognition sequence and cleavage specificity is 5′-ACTGGG $(N)_{5/4}$-3′. Discovery of *Bfi*I and its novel specificity expands the possible applications of type IIS restriction enzymes.

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