Isolation and identification of restriction endonuclease Asp35HI from Acidiphilium species 35H

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Asp35HI, a type II restriction endonuclease, has been isolated from Acidiphilium species 35H. Asp35HI, an isoschizomer of BsmI (1), recognizes the six base non-palindromic sequence 5’G-AATGC3’, and cleaves one nucleotide outside of the recognition sequence on one strand, and within the recognition sequence on the opposite strand, to generate a two base 3’ overhang.

The enzyme was purified using the following chromatographic steps: 1) phosphocellulose, 2) DEAE-cellulose, 3) Heparin-Sepharose. The enzyme was free of contaminating nuclease activity. The crude extract contained approximately 40,000 units Asp35HI per gram of cells. Optimal conditions for Asp35HI activity are 10 mM Tris (pH 7.5), 7 mM MgCl₂, 7 mM 2-mercaptoethanol at 37°C. The fragments produced by Asp35HI digestion of Lambda DNA, Adeno 2, SV40, pBR322, and φX174 match those predicted by cleavage at the sequence GAATGC (Figure 1, lanes 1–5).

The cleavage site of Asp35HI was determined by cleavage of a primed synthesis reaction (2). An M13mp18-derivative with an insert containing an Asp35HI cleavage site was used for enzymatic sequencing reactions starting with a 5’-phosphorylated universal M13 sequencing primer. The four standard dideoxy DNA sequencing reactions were performed and a fifth reaction containing no dideoxy terminations was extended through the Asp35HI site. The fifth reaction was terminated by phenol treatment. The double-stranded DNA was used as substrate for Asp35HI. The cleaved product resulted in a single band (Figure 2, lane −) which comigrates with 5’ G in the sequence 5’GC-ATTTC3’. After the addition of Klenow a single band is produced which comigrates with the second 5’ nucleotide outside of the recognition site (Figure 2, lane +). These results indicate that Asp35HI cleaves one nucleotide 3’ outside of the recognition sequence on the 5’GAATGC3’ strand, and within the recognition sequence, between the G and C, on the opposite strand, 5’GC-ATTTC3’, generating a two base 3’ overhang.

5’ G A A T G C N/3’
3’ C T T A C/G N 5’

REFERENCES


Figure 1. Asp35HI digests of DNA: lane 1, Lambda; 2, Adeno 2; 3, SV40; 4, pBR322; 5, φX174. Marker: lane 6, HindIII-Lambda.

Figure 2. Determination of Asp35HI cleavage positions.