AspI, a novel isoschizomer of *Tth111I* from *Achromobacter* species 699 recognizing 5'-GACN/NNGTC-3'

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We have isolated *AspI*, a novel class-II (1) restriction endonuclease from *Achromobacter* species 699 recognizing the interrupted palindromic sequence 5'-GACN/NNGTC-3' generating 5'-protruding ambiguous mononucleotides. With respect to its isoschizomer *Tth111I* the novel enzyme can be isolated in higher purity because of its occurrence as a single restriction enzyme in the *Achromobacter* strain and absence of relaxed sequence specificity (1, 2).

A comparison of cleavage patterns experimentally obtained with *AspI* on standard lambda, Ad-2, SV40, ϕ X174, M13mp7, pBR322 and pBR328 DNAs of known nucleotide sequence (Fig. 1, lanes 2–8) with computer-derived mapping data (3) predicts the sequence 5'-GACNNNGTC-3'. The recognition sequence was confirmed by parallel digestion of lambda DNA with its isoschizomer *Tth111I* (2) (Fig. 1, lane 9) resulting in both cases in fragments of approximately 36000 bp and 12500 bp, which correlate with the computer-derived fragment lengths of 36123 bp and 12379 bp for the sequence 5'-GACNNNGTC-3'.

The cut positions within the *AspI* recognition site were determined according to the enzymatic sequencing approach described in (4). An M13mp18-derivative with an insert containing an *AspI* cleavage site was used for enzymatic sequencing reactions starting with a 5'-phosphorylated universal M13 sequencing primer. In a parallel reaction, the same primer, [32 P]-end-labeled with T4 PNK and [γ - 32 P]ATP, was annealed to the template, and the labeled primer was extended by treatment with Klenow enzyme and all four dNTPs through the *AspI* site. The double-stranded DNA was used as substrate for *AspI* to produce 5'-end-labeled DNA fragments comparable to the sequencing ladder. Samples were analyzed without or with (–/+) further incubation with T4 DNAP and all four dNTPs by electrophoresis and subsequent autoradiography (Fig. 2). In the *AspI* reaction the observed single band comigrated with A(4); after T4 DNAP treatment, the observed band shift refers to G(5) of the recognition sequence 5'-GACAGTGTC-3'.

From the mapping and sequencing data the specificity of *AspI* is concluded as:

5'-GACN/N-NGTC-3'
3'-CTGN-N/NCAG-5'

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Figure 1. *AspI* digests on lambda (2), Ad-2 (3), SV40 (4), ϕ X174 (5), M13mp7 (6), pBR322 (7), pBR328 (8). (9): Lambda[*Tth111I*]-fragments. (1, 10): MW marker.

Figure 2. Determination of *AspI* cleavage positions.