Ssp5230I, a novel isoschizomer of *AatII* from *Streptomyces* recognizing 5'-GACGT/C- 3'

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We have isolated *Ssp5230I*, a novel class-II restriction endonuclease from *Streptomyces* species recognizing the palindromic sequence 5'-GACGT/C-3' generating 3'-protruding ACGT-tetranucleotides. With respect to its isoschizomer *AatII* it can be isolated in higher purity and stability.

A comparison of cleavage patterns obtained with *Ssp5230I* using lambda, Ad-2, phiX174, pBR328, pBR322 and pUC18 DNAs of known nucleotide sequence (Figure 1, lanes 3–8) with computer-derived mapping data (1) predicts the sequence 5'-G-ACGTC-3'. The recognition sequence was confirmed by parallel digestion of lambda DNA with its isoschizomer *AatII* (2) (Figure 1, lane 2) resulting in both cases in fragments of approximately 14000, 12000, 5100, 4300, 3700, 3300, 2900, 1800, 1100 and 300 bp which correlate with the computer-derived length of 14062, 11770, 5109, 4289, 3731, 3316, 2906, 1849, 1134, 307 and 29 bp for the sequence 5'-GACGTC-3'.

The exact positions of the cuts within the *Ssp5230I*-recognition site were determined according to the enzymatic sequencing approach described in (3) An M13mp/8 derivative with an insert containing a *Ssp5230I* 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]-endlabelled with T4 PNK and [γ-^{32}P]ATP, was annealed to the template and the labelled primer was extended by treatment with Klenow enzyme and all four dNTPs through the *Ssp5230I* site. The double stranded DNA was used as substrate for *Ssp5230I* to produce an 5'-endlabelled DNA fragment comparable to the sequencing ladder. Samples were analyzed without or with (−/+) further incubation with T4 DNA polymerase and all four dNTPs by electrophoresis and subsequent autoradiography (Figure 2). In the *Ssp5230I* reaction the observed single band comigrated with T(5); after T4 DNAP treatment the observed band shift refers to G(1) of the recognition sequence 5'-GACGTC-3'.

From the mapping and sequencing data the specificity of *Ssp5230I* is concluded as:

5'-GACGT/C-3'
3'-C/TGCAG-5'

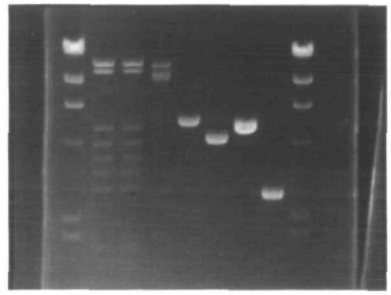

Figure 1. *Ssp5230I* digests on lambda DNA (3), Ad-2 (4), phiX174 (5), pBR322 (6), pBR328 (7) and pUC18 (8). (2): Lambda[*AatII*]-fragments. (1,9): MW marker.

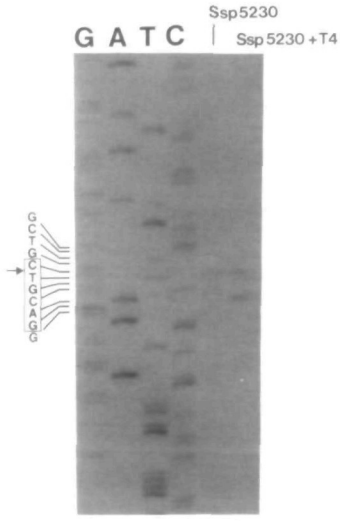

Figure 2. Determination of *Ssp5230I* cleavage positions.

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