SfuI, a novel *AsuII* isoschizomer from *Streptomyces fulvissimus* recognizing 5'-TT/CGAA-3'

S.Veitinger, G.G.Schmitz¹, K.Kaluza¹, M.Jarsch¹, V.Braun and C.Kessler¹

Universität Tübingen, Mikrobiologie II, Auf der Morgenstelle 28, D-7400 Tübingen and ¹Boehringer Mannheim GmbH, Biochemical Research Center, Department of Molecular Biology, Nonnenwald 2, D-8122 Penzberg, FRG

Submitted April 20, 1990

We have isolated *SfuI*, a novel class-II (1) restriction endonuclease from *Streptomyces fulvissimus* recognizing the palindromic sequence 5'-TT/CGAA-3' generating 5'-protruding CG-dinucleotides. With respect to its isoschizomers *AsuII*, *BstBI* and *NspV*, the novel enzyme can be isolated in high purity because of its occurrence as a single restriction enzyme in the *Streptomyces* strain and the presence of high specific activity even in the crude extract.

A comparison of cleavage patterns experimentally obtained with *SfuI* 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 (2) predicts the sequence 5'-TTCGAA-3'. The recognition sequence was confirmed by parallel digestion of lambda DNA with its isoschizomer *AsuII* (3) (Fig. 1, lane 10) resulting in both cases in 8 fragments of between approximately 18000 and 1150 bp, which correlate with the computer-derived fragment lengths between 18049 and 1170 bp for the sequence 5'-TT/CGAA-3'.

The cut positions within the *SfuI* recognition site were determined according to the enzymatic sequencing approach described in (4). An M13mp18-derivative with an insert containing an *SfuI* cleavage site was used for enzymatic sequencing reactions starting with a 5'-phosphorylated M13 universal sequencing primer. In a parallel reaction, the same primer, [³²P]-end-labeled with T4 PNK and [γ -³²P]ATP, was annealed to the template, and the labeled primer was extended by treatment with Klenow enzyme and all four dNTPs through the *SfuI* site. The double-stranded DNA was used as substrate for *SfuI* 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 *SfuI* reaction the observed single band comigrated with T(2); after T4 DNAP treatment, the observed band shift refers to G(4) of the recognition sequence 5'-TTCGAA-3'.

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

5'-TT/CG-AA-3'
3'-AA-GC/TT-5'

REFERENCES

1. Kessler, C. and Manta, V. (1990) *Gene* in press.
2. Devereux, J., Haeblerli, P. and Smithies, O. (1984) *Nucl. Acids Res.* **12**, 387–395.
3. De Waard, A. and Duyvesteyn, M. (1980) *Arch. Microbiol.* **128**, 242–247.
4. Brown, N.L. and Smith, M. (1980) *Meth. Enzymol.* **65**, 391–404.

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

Figure 2. Determination of *SfuI* cleavage positions.