***BpuAI*, a novel *BbsI* and *BbvII* isoschizomer from *Bacillus pumilus* recognizing 5'-GAAGAC-3'**

R.Pogge von Strandmann, P.Städtler, T.Walter^{1*}, B.Frey¹, K.Kaluza¹, W.Hengstenberg and G. Schmitz¹

Ruhr-Universität Bochum, AG Physiologie der Mikroorganismen and ¹Boehringer Mannheim GmbH, Biochemical Research Center, Department of Molecular Biology, Nonnenwald 2, D-8122 Penzberg, Germany

Submitted June 10, 1992

We have isolated *BpuAI*, a novel class-IIs (1) restriction endonuclease from *Bacillus pumilus* recognizing the sequence 5'-GAAGAC-3', cutting at N₂-3' and N₆-5'. High amounts of activity can be purified due to a fast protocol and presence of high amounts of specific activity in the crude extract.

A comparison of cleavage patterns experimentally obtained with *BpuAI* on standard Lambda, Ad-2, pBR322, pBR328, phiX174 DNAs of known nucleotide sequence (Figure 1, lanes 2-6) with the computer-derived mapping data (2) predicts the recognition sequence 5'-GAAGAC-3'.

The cut positions downstream of the recognition sequence were determined in two independent experiments according to the enzymatic sequencing approach described in (3). An M13mp18-derivative with an insert containing an *BpuAI* cleavage

site was used for the enzymatic sequencing reaction starting with a 5'-phosphorylated M13 universal sequencing primer. In a parallel reaction, the same primer, [³²P]-end-labeled with T4 PNK and [gamma-³²P]ATP, was annealed to the template, and the labeled primer was extended by treatment with Klenow enzyme and all four dNTPs through the *BpuAI* site. The double-stranded DNA was used as a substrate for *BpuAI* to produce 5'-end-labeled DNA fragments comparable to the sequencing ladder. Samples were analyzed with or without (+/-) further incubation with T4 DNAP and all four dNTPs by electrophoresis and subsequent autoradiography (Figure 2).

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

5'-GAAGACNN/NNNNNN-3'
3'-CTTCTGNNNNNN/NN-5'

REFERENCES

1. Kessler, C. and Manta, V. (1992) *Gene* **92**, 1-248.
2. Devereux, J., Haeblerli, P. and Smithies, O. (1984) *Nucleic Acids Res.* **12**, 387-395.
3. Brown, N.L. and Smith, M. (1980) *Method Enzymol.* **65**, 391-404.

Figure 1. *BpuAI* digest on lambda (2), Ad-2 (3), pBR322 (4), pBR328 (5), Φ X174 (6). (1, 7) MW marker.

Figure 2. Determination of *BpuAI* cleavage positions.

* To whom correspondence should be addressed