Bsp423I, a novel isoschizomer of *BbvI* from *Bacillus* recognizing 5′-GCAGC-3′

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We have isolated *Bsp423I*, a novel class-II restriction endonuclease from *Bacillus species* recognizing the palindromic sequence 5′-GCAGC-3′ generating 5′-protruding tetranucleotides within the sequence complementary to 5′-GCAGC$(N)_8$-3′. With respect to its isoschizomer *BbvI* it can be isolated in higher purity and stability.

A comparison of cleavage patterns obtained with *Bsp423I* using lambda, Ad-2, SV-40, phiX174, M13mp19, pBR322, pBR328 and pUC18 DNAs of known nucleotide sequence (Figure 1, lanes 3−10) with computer-derived mapping data (1) predicts the sequence 5′-GCAGC-3′. The recognition sequence was confirmed by parallel digestion of lambda DNA with its isoschizomer *Bbv*I (2) (Figure 1, lane 2) resulting in both cases in fragments of approximately 1750, 1500, 1200, 1000, 970, 900, and numerous smaller bands which correlate with the computer-derived length of 1741, 1538, 1523, 1249, 1246, 1242, 1027, 1020, 976, 974, 897, 894. 891 bp for the sequence 5′-GCAGC-3′.

The exact positions of the cut 3′ of the *Bsp423I*-recognition site were determined according to the enzymatic sequencing approach described in (3). An pBluescript Sk derivative with an insert containing a *Bsp423I* cleavage site was used for enzymatic sequencing reactions starting with a 5′-phosphorylated reverse M13 sequencing primer (CAGGAAACAGCTATGACC). 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 *Bsp423I* site. The double stranded DNA was used as substrate for *Bsp423I* 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 (Fig. 2). In the *Bsp423I* reaction the observed single band comigrated with the 12th nucleotide 3′ to the recognition sequence; after T4 DNAP treatment the observed band shift refers to the 8th nucleotide 3′ to the recognition sequence 5′-GCAGC-3′.

From the mapping and sequencing data the specificity of *Bsp423I* is concluded as:

5′-GCAGC$(N)_8$/-3′
3′-CGTCG$(N)_{12}$/-5′

REFERENCES

1. Devereux,J., Haeberli,P. and Smithies,O. (1984) *Nucleic Acids Res.* **12**, 387−3915.
2. Roberts,R.J. (1989) *Nucleic Acids Res.* **17**, r347−r387.
3. Brown,N.L. and Smith,M. (1980) *Methods Enzymol.* **65**, 391−404.

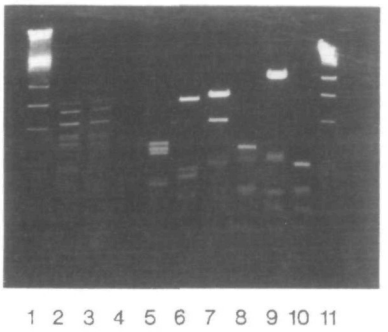

Figure 1. *Bsp423I* digests on lambda DNA (3), Ad-2 (4), SV40 (5), phiX174 (6), M13mp19 (7), pBR322 (8), pBR328 (9), pUC18 (10). (2) Lambda[*BbvI*]-fragments. (1, 11): MW marker.

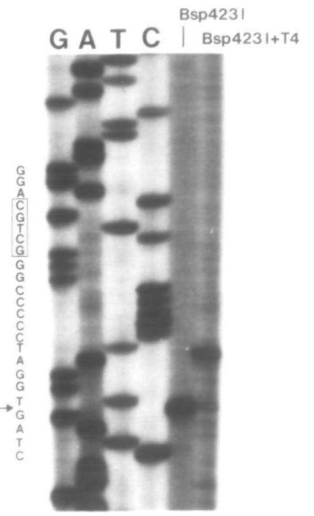

Figure 2. Determination of *Bsp423I* cleavage positions.

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