***KspI*, a novel isoschizomer of *SacII* from *Kluyvera* species recognizing 5'-CCGC/GG-3'**

B.J.Bolton, G.G.Schmitz, M.Jarsch and C.Kessler

Boehringer Mannheim GmbH, Biochemical Research Center, Department of Molecular Biology, Nonnenwald 2, D-8122 Penzberg, FRG

Submitted October 17, 1989

We have isolated *KspI*, a novel class-II restriction endonuclease from *Kluyvera* species recognizing the palindromic sequence 5'-CCGC/GG-3' generating 3'-protruding GC-dinucleotides. With respect to its isoschizomer *SacII* the novel enzyme can be isolated in higher purity because of its occurrence as a single restriction enzyme in the *Kluyvera* strain.

Fig. 1. *KspI* digests on lambda (2), Ad-2 (3), SV40 (4), phiX174 (5), M13mp7 (6), pBR322 (7), pBR328 (8). (9): Lambda[*SacII*]-fragments. (1, 10): MW marker.

Fig. 2. Determination of *KspI* cleavage positions.

ment of Klenow enzyme and all four dNTPs through the *KspI* site. The double-stranded DNA was used as substrate for *KspI* to produce an 5'-end-labeled DNA fragment 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 *KspI* reaction the observed single band comigrated with C(4); after T4 DNAP treatment the observed band shift refer to C(2) of the recognition sequence 5'-CCGCGG-3'.

From the mapping and sequencing data the specificity of *KspI* is concluded as: 5'-CC-GC/GG-3' 3'-GG/CG-CC-5'.

REFERENCES

- [1] Devereux, J., Haeberli, P. and Smithies, O. (1984) Nucl. Acids Res. **12**, 387-395
- [2] Arrand, J.R., Myers, P.A. and Roberts, R.J. as cited in Roberts, R.J. (1989) Nucl. Acids Res. **17**, r347-r387
- [3] Brown, N.L. and Smith, M. (1980) Meth. Enzymol. **65**, 391-404