# The cleavage site for the restriction endonucleases *Ban*I and *Hgi*C I is 5' ...G↓GPyPuCC ...3'

Ira Schildkraut, James Lynch and Richard Morgan

New England Biolabs, Inc., 32 Tozer Road, Beverly, MA 01915, USA
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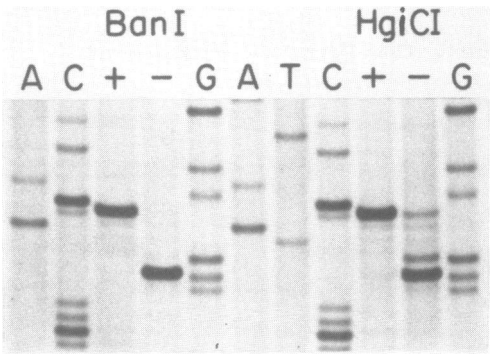
We have determined the cleavage site for the restriction endonucleases *Ban* I (1) and *Hgi*C I to be identical. Both cleaving the sequence 5'... G↓GPyPuCC ...3' leaving a 4 base 5' extension. This is in contrast to Kroger et al (2), who reported the cleavage for *Hgi*C I as 5'...↓GGPyPuCC...3' leaving a 6 base 5' extension.

To determine the cleavage site of *Ban* I and *Hgi*C I, the following experiment was performed: single-stranded M13 mp18 DNA , which contains the *BanI*/*Hgi*C I site in the the polylinker region ( the *Kpn* I site), was used as a template for a primer extension reaction. This template and a M13 sequencing primer were incubated with the Klenow fragment of *E. coli* DNA polymerase I, dGTP, dCTP, dTTP, and [α-$^{35}$S] dATP, such that the primer was extended through and beyond the Ban I site. Following elongation, the polymerase was inactivated by heat treatment and the extended chains were cleaved with *Ban* I or *Hgi*C I. To one half of the sample , an additional amount of DNA polymerase plus all four deoxynucleotide triphosphates were added (lane +). To the other half no additions were made (lane -). After further incubation , these samples were subject to electrophoresis on a DNA sequencing gel alongside the product of a set of standard dideoxysequencing reactions which were produced with the same primer. Both *Ban* I and *Hgi*C I treatment of the product of the initial DNA polymerase reaction (lane -) generates a fragment which co-migrates with the band in the G channel corresponding to the 5' G in the sequence GGTACC. Subsequent treatment of the *Ban* I or *Hgi*C I cleaved product with DNA polymerase removed the band co-migrating with the G residue and generated a band four nucleotides longer co-migrating with the 5' C residue in the sequence GGTACC (lane + ), which corresponds to cleavage between the two G residues on the unlabelled template strand. These results indicate that *Hgi*C I and *Ban* I cleave the sequence GGPyPuCC symmetrically between the two G residues on each strand to yield a four base 5' extension. The cleavage for both *Ban* I and *Hgi*C I is

5'... G↓G Py Pu C C ...3'

3'... C C Pu Py G↑G ...5'.




REFERENCES

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2. Kroger, M., Hobom, G., Schutte, H., and Mayer, H. (1984) Nucleic Acids Res. 12, 3127-3141.