*Aci*I, a unique restriction endonuclease from *Arthrobacter citreus* which recognizes 5′ CCGC 3′

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*Aci*I, a novel type II restriction endonuclease, has been isolated from *Arthrobacter citreus* (NEB #577). *Aci*I recognizes the 4 base non-palindromic sequence 5′ CCGC 3′, and cleaves between the two cytosines on the 5′ CCGC 3′ strand, and between the 5′guanine and cytosine on the 5′ GCGG 3′ strand to generate a 2 base 5′ extension. This extension is compatible with a large number of extensions produced by other restriction endonucleases: *Acc*I, *Aha*II, *Hin*PI, *Hpa*II, MspI, *Nar*I, and *Taq*I.

M13mp18 DNA was completely digested with *Aci*I. The size of the resulting fragments was determined by agarose gel electrophoresis (figure 1, lane 5). The sizes of the experimentally observed fragments were 1100, 965, 610 and 520 base pairs. Smaller sized fragments were also present, but the 1.0% agarose gel used did not allow for accurate sizing of these. The computer calculated (1) number and sizes of the fragments that would be generated by cleavage at the sequence 5′ CCGC 3′: 1105, 953, 605 and 528 base pairs, correlate with the observed fragments. To further test the sequence, 8 DNA molecules (1486 sites) were digested with *Aci*I and electrophoresed using a 1.6% agarose gel. The experimentally observed fragments (those fragments greater than 250 base pairs) also accurately matched the computer predicted fragments that would be produced by cleavage at 5′

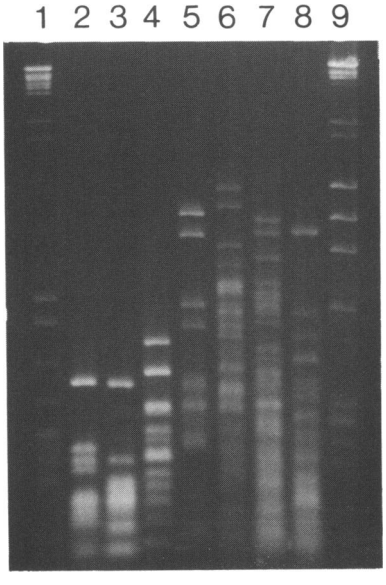

Figure 1. Agarose gel (1.6%) electrophoresis of *Aci*I digested DNAs. Lane 2, pUC19; lane 3, pBR322; lane 4, phiX174; lane 5, M13mp18; lane 6, T7; lane 7, Lambda; lane 8, Adeno2. Markers: Lane 1, *Bst*EII-Lambda and *Msp*I-pBR322; lane 9, *Hin*dIII-Lambda and *Hae*III-phiX174.

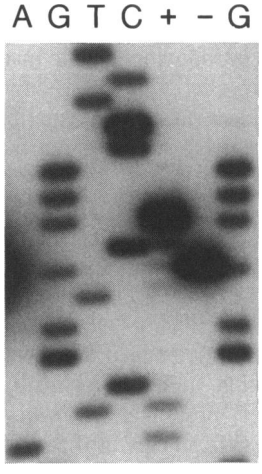

Figure 2.

CCGC 3′. *Aci*I has the following number of recognition sites on these commonly used DNAs: pUC19 (34), pBR322 (67), phiX174 (36), M13mp18 (42), SV40 (10), T7 (199), Lambda (516) and Adeno2 (582), (figure 1, lanes 2−8). The crude extract contains approximately 2000 units per gram of cells.

The cleavage site of *Aci*I was determined by cleavage of a primed synthesis reaction (2). Using M13mp18 DNA as template with an appropriate primer, the four standard dideoxy DNA sequencing reactions were performed and a fifth reaction containing no dideoxy terminals was extended through the *Aci*I site. The fifth reaction was terminated by heat treatment to inactivate the Klenow. *Aci*I was added to the fifth reaction. The cleaved product resulted in a single band (figure II; lane −) which comigrates with the 5′ G on the 3′ GGCG 5′ strand. The addition of Klenow subsequent to *Aci*I digestion (figure II, lane +) results in a band which is 2 nucleotides longer, comigrating with the internal G residue on the 3′ GGCG 5′ strand. This cleavage site results in a 2 base 5′ CG extension, as indicated below.

5′ C↓ C G C 3′
3′ G G C↑ G 5′

REFERENCES

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