AcpI, a novel isoschizomer of *AsuII* from *Acidiphilium cryptum* 25H, recognizes the sequence 5'TT↓CGAA3'

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AcpI, a type II restriction endonuclease, has been isolated from *Acidiphilium cryptum* 25H. *AcpI*, an isoschizomer of *AsuII* (1), recognizes the six base sequence 5'TTCGAA3', and cleaves between the T and the C residues to produce a two base 5' extension.

The enzyme was purified using the following chromatographic steps: 1) phosphocellulose, 2) Heparin–Sephadex, 3) FPLC(Mono Q). The purified enzyme is homogeneous as judged by polyacrylamide gel electrophoresis. The enzyme has a molecular weight of about 62,000 and consists of two identical subunits of M_r of 31,000. Optimal conditions for *AcpI* activity are 10 mM Tris (pH 8.0), 7 mM $MgCl_2$, 7 mM 2-mercaptoethanol at 50°C. The fragments produced by *AcpI* digestion of lambda DNA, Adeno 2, SV40, pBR322, and (ΦX174 match those predicted by cleavage at the sequence TTCGAA (Figure 1, lanes 1–5).

The cleavage site of *AcpI* was determined by cleavage of a primed synthesis reaction (2). An M13mp18-derivative with an insert containing an *AcpI* cleavage site was used for enzymatic sequencing reactions starting with a 5'-phosphorylated universal M13 sequencing primer. Four of the reactions were performed with the standard dideoxy sequencing method using DNA polymerase I large fragment. In the fifth, the primer was extended through the *AcpI* site without any dideoxy analogs of the dNTP's. The extension reaction was terminated by heat treatment, followed by digestion with *AcpI*. Subsequence to digestion by *AcpI*, the reaction was halved. One half of the mixture was treated with stop dye, while the other was incubated with Klenow enzyme and dNTP's. In Figure 2 the four sequencing reactions have been run alongside the reaction stopped after digestion with *AcpI* (–) and the reaction incubated with polymerase subsequent to *AcpI* digestion (+). The fragment generated by *AcpI* digestion-reaction comigrated with the second T in the sequence TTCGAA, while the fragment produced by Klenow treatment of the *AcpI* digestion product in the (+) lane comigrated with the G in the sequence TTCGAA.

These results indicate that *AcpI* recognizes and cleaves the following sequence:

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Figure 1. *AcpI* digests of DNA: lane 1, Lambda; 2, Adeno 2; 3, SV40; 4, pBR322; 5, ΦX174. Marker: lane 6, *HindIII*-Lambda.

Figure 2. Determination of *AcpI* cleavage positions.