*Tsp*45I, a new thermostable site-specific endonuclease that cleaves the recognition sequence 5′-↓GTSAC-3′

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A site-specific nuclease from *Thermus* strain 45 was purified by ammonium sulphate fractionation and then by chromatography on Phospho-Ultrogel, DEAE-Fractogel 650 M, and Sephadex G200 columns. The M_R by gel filtration was 80 kD, and a single band on SDS−PAGE was estimated as 38 kD (1). On the basis of the fragment patterns of digests of pBR322 and φX174RF DNA, and by reference to reported fragment sizes for these substrates (2), it was concluded that the recognition site was GTSAC. This was confirmed by the identity of *Tsp*45I patterns with those predicted by computer simulations of substrate hydrolysis at this site (kindly provided by R.Roberts), and by the agreement between observed and expected fragment sizes for digests of λ DNA, M13mp18RF DNA, pHC624 and pUC18. The optimum activity was observed at 65°C in 10 mM tris chloride buffer, pH 7.3 at 25°C containing no NaCl, 1 mM $MgCl_2$, 2 mM dithiothreitol and 100 mg/l albumin. The enzyme has a low requirement for inorganic ions, indeed activity was detected even when magnesium ions were omitted from the assay buffer (1).

The cleavage points were determined by the primed synthesis method from the M13 −40 sequencing primer (3) on a recognition site provided by two complementary synthetic oligonucleotides 5′ CCGTCGACGTGACGGATCCCC and 5′ GGGGATCCGTCACGTCGACGG that were annealed together and digested with SalI and BamHI. The central fragment that contained the recognition site was ligated into M13mp8 digested with the same enzymes and DNA was extracted and purified (4). The hydrolysis sites of *Tsp*45I (Figure 1) lie outside the recognition site, 5′ to the G residues in each strand:

5′ ↓GTGAC-3′

3′-CACTG↓ 5′

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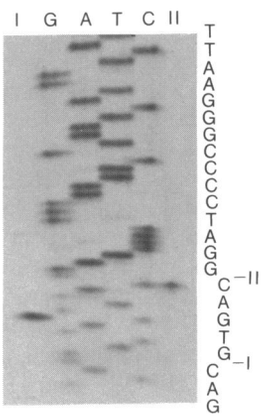

Figure 1. Determination of the *Tsp*45I cleavage site. I: Cleavage with *Tsp*45I, G,A,T,C: dideoxy sequencing reactions. II: As I but filled in to a blunt end with T4 DNA polymerase.

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