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# *Sts*I, a new *Fok*I isoschizomer from *Streptococcus sanguis* 54, cleaves 5′ GGATG(N)$_{10/14}$ 3′

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*Sts*I, an isoschizomer of *Fok*I (1) has been isolated from *Streptococcus sanguis* 54. *Sts*I recognizes the non-palindromic sequence 5′-GGATG-3′. Unlike its isoschizomer, *Sts*I cleaves DNA 10 nucleotides to the right from the noted recognition sequence and 14 nucleotides to the right on the opposite strand.

*Sts*I was purified from the cell extracts by combined chromatographies on phosphocellulose, DEAE-cellulose, heparin−Sepharose, hydroxylapatite, and Affi-Gel Blue agarose. The purified enzyme was homogeneous on SDS−polyacrylamide gel electrophoresis. The molecular mass of the enzyme was estimated at 70 kDa. The *Sts*I activity was eluted at 70 kDa from a gel filtration column. These results indicated that the active form of *Sts*I was monomer.

The recognition sequence of *Sts*I was identified using digestion on pUC19, pUC119, pBR322, ColE1, and ΦX174 DNA. The cleavage patterns obtained were compared with computer-derived data. The data predicts the sequences, 5′-GGATG-3′ and 5′-CATCC-3′, which were the *Fok*I recognition sites. Identical patterns were obtained when λ-DNA were cleaved with *Sts*I and *Fok*I.

The cleavage site was identified by cleavage of a primed-synthesis reaction on M13mp18 derivatives (2). M13mp18 template DNA was synthesized as follows. Oligonucleotides, 5′(GGCCATCCGG)3′ and 5′(CCGGATGGCC)3′, were annealed and ligated with *Sma*I-cleaved M13mp18RFI DNA. Single-stranded DNA containing the oligonucleotide in reverse orientation was selected and used for the template. M13 universal primer was end-labeled with $^{32}$P and then annealed with template DNA. Double-stranded DNA was synthesized in the presence of dNTP and Klenow fragment DNA polymerase I. The extension reaction was stopped by heat inactivation, followed by digestion with *Sts*I and *Fok*I. Cleavage products of *Sts*I and *Fok*I were analyzed alongside of a dideoxynucleotide sequence ladder produced using the same template and primer. At the site where the recognition sequence 5′-GGATG-3′ was located on the synthesized strand, *Sts*I cut the DNA 10 nucleotides 3′ to the end of the recognition sequence (Fig. 1, lane 2). At the site where the recognition sequence 5′-CATCC-3′ was located on the synthesized strand, the cutting site was 14 nucleotides 5′ to the end of the recognition sequence (Fig. 1, lane 4). *Fok*I cleaved 5′-GGATG(N)$_{9/13}$-3′ (Fig. 1, lanes 1 and 3) as described by Sugisaki *et al.* (1). These results showed that *Sts*I recognized the same sequence as *Fok*I, but cleaved DNA at different positions, 5′-GGATG(N)$_{10/14}$-3′.

## REFERENCES

1. Sugisaki,H. and Kanazawa,S. (1981) *Gene* **16**, 73−78.
2. Brown,N.L. and Smith,M. (1980) *Methods Enzymol.* **65**, 391−404.

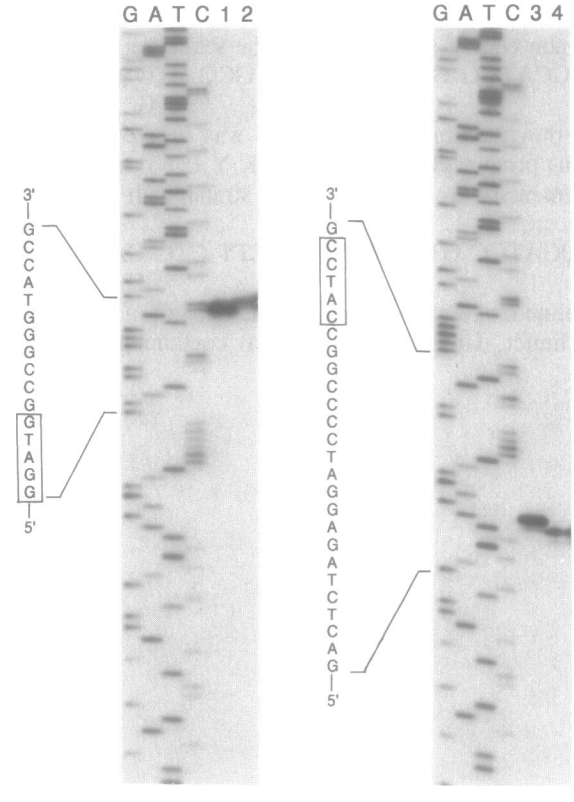


**Figure 1.** Identification of *Sts*I cleavage positions.