*Sgr*AI, a novel class-II restriction endonuclease from *Streptomyces griseus* recognizing the octanucleotide sequence 5′-CR/CCGGYA-3′

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We have isolated *Sgr*AI, a novel class-II restriction endonuclease from *Streptomyces griseus* (Soil Microbiology Associates, Inc.) recognizing the new octanucleotide palindromic sequence 5′-CR/CCGGYG-3′ generating 5′-protruding CCGG-tetranucleotides (1). *Sgr*AI complements *Not*I and *Sfi*I (2) both recognizing octanucleotide sequences; the novel enzyme may be a useful tool for rare cutting approaches (3).

A comparison of cleavage patterns experimentally obtained with *Sgr*AI on standard lambda, Ad-2, SV40, ϕX174, M13mp7, pBR322 and pBR328 DNAs of known nucleotide sequence (Fig. 1, lanes 2−8) with computer-derived mapping data (4) predicts the sequence 5′-CRCCGGYG-3′. Digestion of lambda DNA yielded in 7 fragments of approximately 17000, 15000, 7100, 4200, 2800, 1600 and 1300 bp, which correlate with the computer-derived lengths of 16678, 14850, 7064, 4190, 2775, 1616 and 1321 bp. Ad-2 yields in 6 fragments; pBR322 and pBR328 are linearized. The other DNAs are not cut by *Sgr*AI.

The cut positions within the *Sgr*AI recognition site were determined in two independent experiments according to the enzymatic sequencing approach described in (5). An M13mp18-derivative with an insert containing an *Sgr*AI cleavage site was used for enzymatic sequencing reactions starting with a 5′-phosphorylated M13 universal sequencing primer. In a parallel reaction, the same primer, [^{32}P]-endlabeled with T4 PNK and [γ-^{32}P]ATP, was annealed to the template, and the labeled primer was extended by treatment with Klenow enzyme and all four dNTPs through the *Sgr*AI site. The double-stranded DNA was used as substrate for *Sgr*AI to produce 5′-endlabeled DNA fragments comparable to the sequencing ladder. Samples were analyzed without or with (−/+) further incubation with T4 DNAP and all four dNTPs by electrophoresis and subsequent autoradiography (Fig. 2). In a first experiment with the *Sgr*AI reaction the observed single band comigrated with A(2); after T4 DNAP treatment, the observed band shift refers to G(6) of the recognition sequence 5′-CA/CCGGTG-3′. In a second experiment the *Sgr*AI-specific band comigrated with A(2); the observed band shift refers to G(6) of the recognition sequence 5′-CA/CCGGCG-3′.

From the mapping and sequencing data the specificity of *Sgr*AI is concluded as:

5′-CR/CCGG-YG-3′
3′-GY-GGCC/RC-5′.

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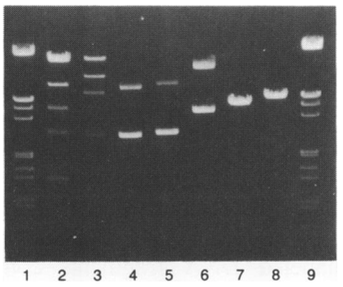

Figure 1. *Sgr*AI digests on lambda (2), Ad-2 (3), SV40 (4), ϕX174 (5), M13mp7 (6), pBR322 (7), pBR328(8). (1, 9): MW marker.

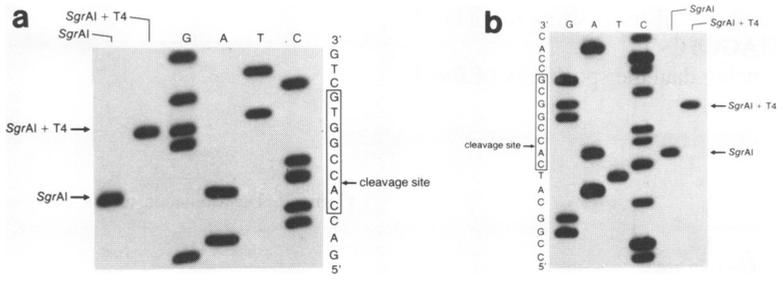

Figure 2a,b. Determination of *Sgr*AI cleavage positions in two independent sequencing reactions.