Cleavage of yeast and bacteriophage T7 genomes at a single site using the rare cutter endonuclease I-Sce I

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It is obvious that the availability of rare cutter restriction endonucleases would facilitate large scale genome mapping, cloning and sequencing projects. Restriction endonucleases of bacterial origin have recognition sites of 8 bp long, at most, and even when used in combination with methylases they can produce cleavage specificities of up to 12 bp only (1). Artificial endonucleases have been made by chemical modifications of either DNA binding proteins (2) or synthetic oligodeoxynucleotides (3). Although this last strategy can, in principle, be generalized to many sequences, cleavage occurs at low efficiency. Cleavage of yeast and *E. coli* genomic DNAs at a single predetermined site using a frequent cutter bacterial endonuclease has recently been achieved by the Achille's heel method, an elegant three step procedure combining the action of the lac repressor on artificially inserted sites with that of a methylase (4). We present here the first evidence for cleavage of total yeast genomic DNA at a single predetermined site in a one step procedure using a new endonuclease, I-Sce I, encoded by a mobile group I intron of yeast mitochondria. We further show that, although I-Sce I is extremely specific, cleavable sites can also be found in natural DNA.

The endonuclease I-Sce I recognizes an 18 bp long sequence 5' TAGGGATAACAGGGTAAT 3' (5, 6). Although single base substitutions are tolerated within this sequence, they all reduce cleavage efficiency by various degrees. Because such a partial sequence degeneracy does not allow prediction of the degree of specificity of the enzyme, we have decided to assay I-Sce I on the entire yeast genome (1.4×10^7 bp). To determine the optimal conditions of digestion in agarose plugs, a transgenic yeast strain (FY23/C6) has been constructed by inserting the wild-type I-Sce I recognition site in place of the non-essential gene YCR521 in chromosome III (7). The non-transformed isogenic strain (FY1679) was used as control. Results of optimal cleavage conditions are shown in Figure 1. It can be seen that I-Sce I specifically cleaves chromosome III to completion at its recognition site, producing two fragments of the expected sizes (ca. 220 and 105 kb). In the same conditions, no other cleavage site is revealed in the entire yeast genome lane (6). Thus, I-Sce I can be used as a very rare cutter restriction endonuclease for cloning and mapping artificially inserted sequences in pro- and eucaryotic genomes. Notice that reducing the Mg^{++} concentration to 0.002 M results in the appearance of a very weak band (lanes 1 and 4), corresponding to a secondary site not cleaved under optimal conditions.

On a statistical basis, the number of sites naturally present in more complex genomes should also remain very low. To determine whether I-Sce I could be used on naturally occurring sites, we have analyzed a number of genomes under optimal conditions. No sites were found for the bacteriophages λ , Φ X174, SSP 1, T4 or T5, for the Adenovirus (Ad 2) or for the bacteria *Bacillus anthracis*, *Borrelia burgdorferi*, *Leptospira biflexa* and *L. interrogans*. In contrast, one site is revealed for the DNA of phage T7 (Figure 2). Cleavage at this site, however, does not go to completion in the conditions used, consistent with the fact that no sequence corresponding to the 18 bp wild-type recognition site occurs in the entire T7 DNA sequence.

Figure 1. Agarose plugs (InCert™, FMC) containing 5×10^7 yeast cells in 50 μ l were prepared as described before (8). Prior to using, plugs were first equilibrated overnight @ 4°C in a buffer containing 0.1 M of diethanolamine-Cl and 0.001 M of dithiothreitol at pH 9.5 and then incubated twice for two hours @ 4°C in 200 μ l of the same buffer containing 200 μ g/ml of bovine serum albumin. Because I-Sce I is known to be unstable in the presence of Mg^{++} (9), diffusion of the enzyme in the plugs is needed before starting the reaction. We found that placing the plug into 50 μ l of the same buffer containing the enzyme for 2 hours @ 4°C gives the best results (note that increasing diffusion time gives better results than increasing reaction time or enzyme concentration, compare lanes 1,2,3 with lanes 4,5,6, respectively). The reaction is then started by addition of $MgCl_2$ and transfer of the tube at 37°C. Incubation is carried out for 1 h and agarose plugs are directly analyzed by pulse field gel electrophoresis in a 1% agarose gel (Seakem® GTG, FMC) and 0.25 $\times$ TBE buffer at 13°C on a Rotaphor equipment for 66 hours at 115 Volts with switch pulses from 120 to 60 sec. and a fixed electrode angle of 120°. Lanes 1 to 6: 30 Units of I-Sce I (Boehringer Mannheim). Lane 7: control without enzyme. Arrows indicates chromosome III and its cleaved products.

Figure 2. DNA of bacteriophage T7 was purified from infected *E. coli* B cells (Institut Pasteur collection) and cut by bacterial restriction endonucleases as indicated (2 h @ 37°C). Samples were then separated in two aliquots, one of which being further incubated with 3 Units of I-Sce I (Boehringer Mannheim) for 30 min. @ 37°C in 0.1 M diethanolamine-Cl, 0.0025 M MgCl₂, 0.001 M dithiothreitol, 200 µg/ml of bovine serum albumin at pH 9.5. Electrophoresis was performed in a 0.8% agarose gel. Lane 1: *Bcl*I + I-SceI; Lane 2: *Mlu*I + I-SceI; Lane 3: *Bcl*I; Lane 4: *Mlu*I. Arrows indicate I-SceI cleaved products.

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