***EarI*, a restriction endonuclease from *Enterobacter aerogenes* which recognizes 5'CTCTTC3'**

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EarI, a type II restriction endonuclease, has been isolated from *Enterobacter aerogenes* (NEB # 450). *EarI*, an isoschizomer of *Ksp* 632 I (1), recognizes the six base non-palindromic sequence 5'CTCTTC3', and cleaves one nucleotide 3' of the 3' cytosine on one strand, and four nucleotides 5' of the 5' guanine on the opposite strand, to generate a three base 5' overhang.

The single cleavage site of *EarI* on SV40 DNA was mapped to approximately position 4450 by analysis against *Bgl*I, *Eco*R I and *Taq*I cleaved SV40 DNA (figure I, lanes B-E). The sequence 5'CTCTTC3' occurs at position 4437. The number and sizes of the fragments generated by digestion with *EarI* on eight DNA molecules (119 sites) match the computer predicted (2) number and sizes of the fragments that would be generated by cleavage at the sequence 5'CTCTTC3'. *EarI* has the following number of recognition sites on these commonly used DNAs: pUC19, 3; pBR322, 2; phiX174, 2; M13mp18, 2; SV40, 1; Adeno2, 29; T7, 46; and Lambda, 34, (figure I, lanes F-L). From these data we conclude that *EarI* recognizes the sequence 5'CTCTTC3'. The crude extract contained approximately 8,000 units *EarI* per gram of cells.

The cleavage site of *EarI* was determined by cleavage of a primed synthesis reaction (3). Using M13mp18 DNA as template with an appropriate primer, the four standard dideoxy DNA sequencing reactions were performed and a fifth reaction containing no dideoxy terminations was extended through the *EarI* site. The fifth reaction was terminated by heat treatment. *EarI* was added to the fifth reaction. The cleaved product resulted in a single band (figure II, lane -) which comigrates with the first 3' nucleotide outside of the recognition sequence. The addition of Klenow subsequent to *EarI* digestion resulted in a single band, three nucleotides longer, comigrating with the fourth 3' nucleotide outside of the recognition sequence (figure II, lane +). These results indicate that *EarI* cleaves one nucleotide 3' of the recognition sequence on the 5'CTCTTC3' strand, and four nucleotides 5' of the 5' G on the opposite strand sequence 5'GAAGAG3', generating a three base 5' overhang.

Figure I. *EarI* digests of DNA. Lane B, uncut SV40; lane C, SV40 + *EarI* + *Bgl*I; lane D, SV40 + *EarI* + *Eco*R I; lane E, SV40 + *EarI* + *Taq*I. Lanes F-L: pUC19, pBR322, phiX174, M13mp18, Adeno2, T7, and Lambda, respectively. Markers: lane A, *Bst*E II-Lambda and *Msp*I-pBR322; lane M, *Hind*III-Lambda and *Hae*III-phiX174.

Figure II.

References:

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