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The sensitivity of DNA cleavage by *Spe*I and *Apa*LI to methylation by M.*Eco*K

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During work on site-directed mutagenesis of the human interleukin-2 gene an *Eco*K site (1) was created which overlapped recognition sequences for *Spe*I (1) and *Apa*LI (1) (Fig. 1a). Isolation of the DNA from m_K^+ strain DH1 (2) and m_K^- strain HB101 (3) and subsequent incubation with the two restriction endonucleases revealed that *Eco*K methylation completely or almost completely protected both DNA strands from cleavage by *Spe*I, but did not prevent cleavage of either strand by *Apa*LI (Fig. 1b). Thus, methylation of only one of the 5'-terminal A's of the *Spe*I site is sufficient to protect it against *Spe*I, whereas methylation of one of the two A's of the *Apa*LI sequence does not interfere with its cleavage by *Apa*LI.

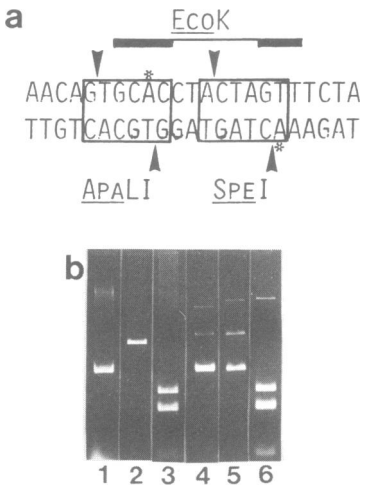

Figure 1. a: Overlap of the *Eco*K site with the *Apa*LI and *Spe*I sites as created by site-directed mutagenesis. The *Eco*K heptanucleotide, separated by a 6 nt spacer, is overlined by bars. The A's modified by M.*Eco*K are marked with asterisks. The restriction sites are boxed; potential cleavage positions are indicated by arrows. b: Analysis of cleavage of the sequence shown in fig. 1a by *Spe*I and *Apa*LI. Lanes 1-3: unmethylated DNA, lanes 4-6: methylated DNA. Lanes 1 and 4: intact vector, lanes 2 and 5: vector incubated with *Spe*I, lanes 3 and 6: vector incubated with *Apa*LI.

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