The restriction enzyme *EheI* (GGC/GCC) is sensitive to CpG methylation

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EheI cleaves the site GGC/GCC and is an isoschizomer of *NarI* (GG/CGCC) and *BbeI* (GGCGC/C) (1–4). Here I show that *EheI* is methyl sensitive and will not cleave its CpG methylated site (GG^mCGCC). *NarI* and *BbeI* are also CpG methyl sensitive (5–7). *EheI* methyl sensitivity is shown by digesting mixtures of methylated λ DNA and unmethylated plasmid DNA and vice-versa (Figure 1). I also digest eukaryotic DNAs with *EheI*. Figure 1 shows a digest of *Physarum* rDNA (which has published methylation patterns, ref. 6) and a digest of adenovirus DNA (which is unmethylated and is a standard substrate for *NarI*, ref. 8). *EheI* readily cleaves its unmethylated sites, including those in λ and pBR322 (1, 2 and this paper). *BbeI* is slow and *NarI* very slow to cleave some unmethylated sites including the one in λ DNA (5, 8 and my unpublished results). *EheI* readily cleaves its unmethylated sites and does not cleave GG^mCGCC sites. *EheI* should be very reliable for long range restriction mapping and methylation pattern mapping of eukaryotic genomes.

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Figure 1. This figure shows a λ /HindIII digest (lanes 1 and 7), an *EheI* digest of λ /HindIII (lane 2), pUB2 (a pBR322 based plasmid, ref. 9, lane 3), an *EheI* digest of pUB2 (lane 4), an *EheI* digest of a mixture of pUB2 and methylated λ /HindIII (lane 5), an *EheI* digest of a mixture of λ /HindIII and methylated pUB2 (lane 6), an *EheI* digest of *Physarum* rDNA (lane 8), an *EheI* digest of adenovirus DNA (lane 9) and BRL size standards (lane 10). To the right of lane 7 are sizes (in kbp) for lanes 1–7 and to the right of lane 10 are sizes (in kbp) for lanes 8–10. Each digest is 1 μ g of each DNA, 3 or 5 units of *EheI*, the buffer supplied with the enzyme and 100 μ g/ml BSA in 50 μ l at 37°C for 1 hour. Digests are separated by agarose gel electrophoresis and ethidium bromide stained. Methylation of plasmid and λ DNAs is with *HhaI* methylase (G^mCGC) as described (6). *EheI* is from Toyobo Ltd. (via U.S. Biochemical), *HhaI* methylase is from New England Biolabs and adenovirus DNA is from BRL.

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