

LpnI, from *Legionella pneumophila*, is a neoschizomer of *HaeII*Laurie Hamablet, Grace C.C.Chen¹, Arnold Brown¹ and Richard J.RobertsCold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724 and ¹Research Service, WJB Dorn Veterans' Hospital and Department of Medicine, Microbiology/Immunology and Biology, University of South Carolina School of Medicine, Columbia, SC 29201, USA

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LpnI is a Type II restriction endonuclease that was previously isolated from *Legionella pneumophila* strain 11 EJ and partially purified (1). Further purification by phosphocellulose and DNA-agarose chromatography, with an intermediate 50-75% ammonium sulphate concentration/fractionation step gave enzyme sufficiently pure for detailed characterization. *LpnI* cleaves pUC19 DNA at three sites. Double digests of pUC19 DNA with *LpnI* and either *AatII*, *EcoRI*, *PvuI* or *RsaI* mapped the *LpnI* cleavage sites to approximately 230, 690 and 1090 nucleotides. These sites lie close to those predicted for *HaeII*. A double digest between *HaeII* and *LpnI* on bacteriophage λ DNA confirmed that these enzymes are isoschizomers (Fig. 1a).

The precise site of cleavage by *LpnI* was determined by digestion of a primed synthesis reaction (2). Single stranded M13 template DNA containing the *HaeII* site at position 8734 on the Adenovirus 2 DNA genome was used. The cleaved product resulted in a single band (Fig 1b; lane 1) which comigrates with the 5' C in the sequence 5' PuGC↓GCPy3'. Treatment of this cleaved product with Klenow DNA polymerase following *LpnI* digestion showed no alteration in its migration (Fig. 1b, lane 2) indicating that *LpnI* produces blunt ended DNA fragments. This contrasts with *HaeII* which cleaves the same recognition site but produces a 4 base 3' extension (Fig. 2; lanes 3 and 4). *LpnI* thus resembles the *HaeII* isoschizomer, *BmeI42I* (3), and recognizes the sequence 5' PuGC↓GCPy3', cleaving as indicated by the arrow. We use the term neoschizomer to describe this situation, where isoschizomers recognize the same sequence but cleave at different positions within that sequence.

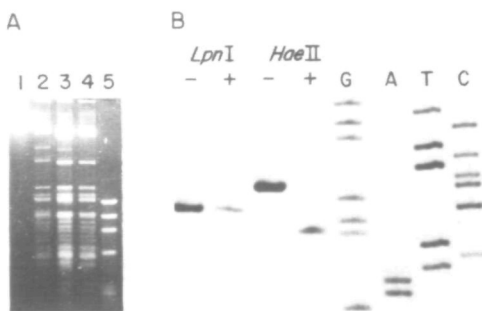


Figure 1.

- a. lane 1: uncut λ DNA
 lane 2: λ + *LpnI*
 lane 3: λ + *LpnI* + *HaeII*
 lane 4: λ + *HaeII*
 lane 5: ϕ X174/*HaeII*

b. *LpnI* and *HaeII* cleavage sites. The products of cleavage were either run directly (- lanes) or following treatment with Klenow polymerase (+ lanes). The four standard sequencing lanes through this region are shown.

References:

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