*Eco*29kI, a novel plasmid encoded restriction endonuclease from *Escherichia coli*

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*Eco*29kI is a type II restriction endonuclease from clinical strain *Escherichia coli* 29 isolated in Kiev. The genes for restriction modification *Eco*29kI system were located on one of its plasmids, namely pSACII1 about 4.0 kb in size as was determined by plasmid transformation of *E.coli* K802 (Figure 1) according to (1) except that the phage ϕ 80 *vir* was used for selection of clones.

Restriction endonuclease was separated and purified without contaminating nucleases by DEAE−cellulose (Whatman DE52), phosphocellulose (Whatman P11), heparin agarose (2), hydroxylapatite (Serva HA-Ultrogel) column chromatography with ammonium sulfate fractionation (30−45%, w/v) after P11 step. *Eco*29kI activity eluted at 200 mM NaCl from DE52, at 560−620 mM NaCl from P11, at 260−360 mM NaCl from heparin agarose and at 420−480 mM phosphate from HA-Ultragel. The enzyme was pure enough for 100 fold over-digestion on DNA.

The fragments produced from lambda DNA by *Eco*29kI are shown in Figure 1. The fragments profile of lambda DNA is identical to the one produced by *Sac*II, which recognizes the sequence 5′CCGC/GG3′. A double digest between *Eco*29kI and *Sac*II on bacteriophage lambda DNA confirmed that these enzymes are isoschizomers (Figure 1). In addition, fragment patterns of ϕ80 DNA produced by digestion with *Eco*29kI and *Sac*II are identical (data not shown) *Eco*29kI cleaves pUC128 DNA at unique sites in its polycloning linker, the localization of which corresponds to *Sac*II site and the position of phosphodiester bond cleavage within the recognition site was determined by examination of a primed synthesis reaction as described by Sanger *et al.* (3, Figure 2). It was found, that *Eco*29kI generated 3′ protruding GC-dinucleotide. So, *Eco*29kI and *Sac*II are true isoschisomers (4).

The optimal reaction conditions of *Eco*29kI are: 25−50 mM NaCl (75−100 mM KCl), 10 mM Tris−HCl (pH 7.5−8.5), 10 mM $MgCl_2$, 37°C.

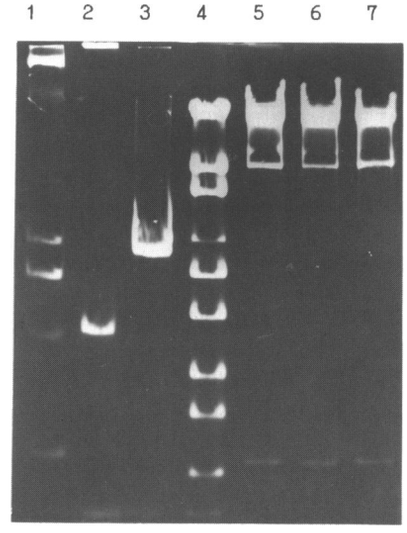

Figure 1. Plasmids and DNA fragments: lanes (1) *E.coli* 29k natural plasmids, (2) pSACII1, (3) linear form of pSACII1 after *Nru*I cleavage; digest of lambda DNA by (4) *Cfr*BI (*Sty*I), (5) *Eco*29kI, (6) *Eco*29kI + *Sac*II, (7) *Sac*II.

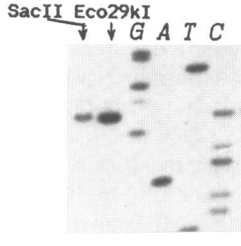

Figure 2. Determination of *Eco*29kI cleavage site.

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