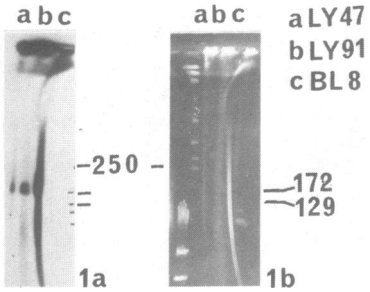
Volume 16 Number 9 1988 Nucleic Acids Research

Separation of episomal Epstein–Barr virus from Burkitt's lymphoma host cell DNA in pulse field gels

Ann Harris and David R.Bentley

Paediatric Research Unit, UMDS, Guy's Hospital, London, SE1 9RT, UK
Submitted February 12, 1988

Pulse field gel electrophoresis (PFGE) has been used to separate episomal EBV from host cell DNA in Burkitt's lymphoma (BL)-derived cell lines (1). Gel purification has only recently become applicable to very high molecular weight DNA and hence to direct isolation of large viral genomes. Previous methods of isolating episomal EBV were labour-intensive (2). We have isolated EBV episomes from 3 BL-derived cell lines: LY47, LY91 and BL8. Cells were embedded at concentrations of 3×10^7 cells/ml agarose and processed as described elsewhere (3). 1.2-1.5% agarose gels (BRL) were run in 0.5 x TAE (0.04M Tris-acetate, 0.001M EDTA) buffer. Alternating 40 sec, 250 mV cycles were used for 24h. DNA was transferred to nitrocellulose filters by Southern blotting and probed with ^{32}P-labelled B95-8 virion DNA. Fig 1a shows an autoradiograph of a Southern blot, probed with ^{32}P-labelled B95-8 DNA, of the BL cell lines LY47, LY91 and BL8 subjected to PFGE. A clear band is visible at 172 kb (the size of the EB viral episome) in the lanes of the gel containing these three BL cell lines. This size has been estimated from Lambda EMBL3 (43 kb) concatemers and intact chromosomes of S. cerevisiae (fig 1b). The circular structure of the EBV genome does not appear to alter its mobility relative to linear DNA markers, so the EBV episome is presumed to undergo reptation in a similar fashion to linear DNA (3). About one third of the episomal copies of EBV, as judged by relative intensity of hybridization, have been removed from the LY91 block and have migrated into the gel. A substantial degree of hybridization is also seen to the agarose blocks containing the BL cells. This is likely to reflect the presence of episomal EBV physically entrapped within, or closely associated with, the host chromosomal DNA as has been observed using other techniques (4). As there are about 120 copies of EBV per LY91 cell (5), this corresponds to a total EBV genome content of about 100 ng per PFGE block. If one third of this has migrated into the gel, about 35 ng can be isolated per lane by PFGE for molecular analyses, by electroelution and concentration dialysis (Biotrap, S & S).



References: 1. Carle, G.F. and Olson, M.V. (1985). P.N.A.S. 82, 3756-3760. 2. Griffin, B. et al. (1984). J. Virol. 40, 11-19. 3. Smith, C. (1986). Genet. Eng. 8, 45-70. 4. Harris, A. et al. (1985). J. Virol. 56, 328- 332. 5. Harris, A. et al. (1984). Mol. Biol. Med. 2, 135-150.

