Isolation of Epstein – Barr virus genomes using pulse-field gel electrophoresis

Sharon L.Silins, Victor P.Argaet and Thomas B.Sculley

Queensland Institute of Medical Research, Brisbane, 4029 Queensland, Australia

Submitted February 24, 1992

Epstein – Barr virus (EBV) DNA is normally difficult to isolate since the virus does not cause a productive lytic infection in EBV-transformed lymphocytes. Previous methods of isolating viral DNA required the production of virions from large quantities of partially permissive B-lymphoblastoid cell cultures which yielded extremely low amounts of pure EBV DNA (1 to 10 μ g DNA per 10^{10} cells). Here we report the isolation of approximately 400 ng of EBV DNA of the Ag876 and B95.8 strains from as little as 10^6 lymphoblastoid cells using pulse-field gel electrophoresis (PFGE).

Cells were treated for 7 days with 5 mM n-butyrate and 20 ng ml^{-1} 12-O-tetradecanoyl-phorbol-13-acetate (TPA) to stimulate virus production (1). DNA was prepared in agarose blocks by conventional methods (2). Electrophoresis was performed on the CHEF-DR II pulse-field gel electrophoresis system at 200 V (6 V/cm) at 14°C. Switch time was ramped from 50 to 90 seconds for 19 hours. In the ethidium bromide stained gel (Figure 1A), a discrete band was visible migrating at approximately 170 kb (the size of the EBV genome) (arrowed in lanes B95.8 and Ag876). This DNA was transferred to nylon membranes by Southern-blotting. The membrane was hybridized with a ^{32}P -radiolabelled probe prepared by PCR amplification from plasmid pMBg/IIC (EBV Bg/IIC in pH7C79) using the primer pair (5'-GCTGCCCCACCCTGAGGATTT-3') and (5'-TAGGTCTGCTTTAGGGCTGC-3') which amplify a 159-bp region of EBNA-2. The ethidium bromide stained band of interest was easily detected by hybridization with the EBV-specific probe (Figure 1B Lanes B95.8 and Ag876). Hybridization was virus-specific since DNA from the EBV-negative cell line BL-30 did not show any hybridization. The yield of purified EBV DNA was estimated densitometrically by comparison to a known amount of pMBg/IIC. EBV genomes can be excised from LMP agarose and the DNA isolated using β -agarase I (an agarose-digesting enzyme: New England BioLabs). The DNA obtained is suitable for production of EBV genomic libraries. Both *Eco*RI and *Hind*III restriction patterns of PFGE purified EBV DNA were also found to be consistent with published restriction maps (data not shown).

Pulse-field gel electrophoresis has proven to be a rapid and improved method of isolating intact EBV DNA directly from host cells.

IROI CA52250-01 from The National Cancer Institute, USA. S.L.S. is a recipient of an Australian Postgraduate Research Award.

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Figure 1. Pulse-field separation of EBV DNA from host cell DNA. Electrophoresis on a 1% LMP agarose gel for 19 hours at 50–90 second ramped pulse times, followed by ethidium bromide staining (A) and Southern-blotting (B). *Saccharomyces cerevisiae* chromosomes (Bio-Rad) and lambda concatemers (Bio-Rad) were used as size markers.

ACKNOWLEDGEMENTS

We thank Dr N.Hayward for use of the pulse-field equipment. This work was supported by grants from The National Health and Medical Research Council of Australia and grant number