

Short Communication – Technical Report

Molecular cytogenetic characterization of the EBV-producing cell line B95-8 (*Saguinus oedipus*, Platyrrhini) by chromosome sorting and painting

S. Müller¹, M. Neusser¹, P. C. M. O'Brien² & J. Wienberg^{1*}

¹*Institute of Anthropology and Human Genetics, University of Munich (LMU), Germany; Tel: 49 (0)89 21806728; Fax: 49 (0)89 21806719; E-mail: j.wienberg@lrz.uni-muenchen.de;* ²*Department of Pathology, Cambridge University, Cambridge, UK*

**Correspondence*

Received 30 June 2001; accepted for publication by Pat Heslop-Harrison 5 July 2001

Key words: Epstein–Barr virus, *Saguinus oedipus*

Abstract

The cell line B95-8 releases Epstein–Barr virus (EBV) with high titres of transforming activity and is widely used as a model in cancer research and virology. There are, however, controversial reports about the species of origin, cell line stability and karyotype. To address these questions, B95-8 chromosomes were analysed by chromosome sorting and painting by multicolour fluorescence *in-situ* hybridization. Reciprocal painting was performed between B95-8, ‘wildtype’ New World monkey and human chromosomes. *Saguinus oedipus* was revealed as the species of origin. A further five cell-line-specific marker chromosomes, resulting from translocations, deletions and an insertion were found. Although human chromosome 6 or 13 homologues were always involved in these rearrangements, co-hybridization of an EBV-specific DNA probe did not reveal site-specific hybridization to marker chromosomes or at translocation breakpoints. The multicolour probe set described here will be of special value for further evolutionary studies in New World monkeys.

Certain New World monkey cell lines release Epstein–Barr virus (EBV) with high titres of transforming activity. The lymphoblastoid cell line B95-8 is one of the most frequently used EBV resources. Despite widespread use of this cell line in basic and applied research, the literature and cell repository catalogues present conflicting, incomplete or misleading information about the species of origin, the karyotype, cell line heterogeneity and stability. Miller *et al.* (1972) named the Cotton-top marmoset (*Saguinus oedipus*) as the B95-8 species of origin. However, the Japanese

Collection of Research Bioresources online catalogue 2000 refers to B95-8 as *Callithrix jacchus*. Most other sources (Neitzel 1986, the European Collection of Cell Cultures (ECACC) and American Type Culture Collection online catalogues 2000) describe the species of origin as ‘marmoset’, defining a subgroup of the Callitrichidae family, comprised of three genera and over 10 species. All three cell-repository catalogues refer to Miller & Lipman (1973), in which neither species of origin nor the establishment of B95-8 is described. It is, however, well

known that cell lines from different species, sometimes even species subpopulations, are not equally susceptible to different culture conditions and external triggers. Thus, researchers who use B95-8 as an experimental model may be interested to know precisely which species they use and what genomic status their model cell line has, in particular whether it is karyotypically homogenous, stable or rearranged.

Over the past 25 years the karyotypes of most neotropical primates (Platyrrhini, New World monkeys) have been investigated with chromosome banding techniques. However, banding analysis is often not informative when rearrangements are complex or translocated chromosomal fragments involved are small. Today, sorting of chromosomes by flow cytometry provides chromosome-specific DNA probes for fluorescence *in-situ* hybridization (FISH). These probes allow karyotypes to be analysed with high resolution and accuracy. Here we used multicolour chromosome painting to identify the species origin of cell line B95-8 and further to characterize its karyotype.

The cell line B95-8 was purchased from ECACC (Ref. No. 85011419). The *S. oedipus* blood sample was provided by the New England Regional Primate Center, USA. Metaphase chromosomes were prepared according to standard protocols. The 'wild type' *S. oedipus* karyotype was found to be the same as described before (Nagamachi *et al.* 1997).

Human and B95-8 chromosome-specific painting probes were generated by bivariate chromosome flow sorting and subsequent degenerate oligonucleotide primed PCR amplification (DOP-PCR) as described in Telenius *et al.* (1992). With human chromosome painting probes, a multicolour approach was employed, which allowed us to assign 6 painting probes in a single experiment (Figure 1A–D) (Ried *et al.* 1992). DNA probes were combinatorially labelled with biotin-dUTP, digoxigenin-dUTP (Roche Diagnostics), and TAMRA-dUTP (Applied Biosystems/PE). Biotinylated DNA probes were detected by Avidin-Cy5, digoxigenin-labelled probes with sheep-antidigoxigenin-FITC antibody (Roche Diagnostics). A biotinylated EBV probe (Bioprobe) was purchased from Enzo Diagnostics. Reverse painting with B95-8 painting

probes to human and 'wildtype' *S. oedipus* chromosomes was performed in single or double hybridizations. *In-situ* hybridization and probe detection was as described (Müller *et al.* 1998, 1999). Metaphases were analysed with a cooled CCD camera (Photometrics NU200) coupled to an epifluorescence microscope. Image acquisition and processing was performed by SmartCapture software (DigitalScientific, Cambridge, UK).

The flow karyotype of B95-8 together with the chromosome assignment is illustrated in Figure 2A. Twenty-one individual B95-8 chromosome-specific paints could be generated by flow sorting, whereas three probes contained two chromosomes (3 + 12, 11 + 12 and 15 + 16). Three further B95-8 painting probes showed asymmetrical hybridization patterns on B95-8 metaphases, representing painting probes specific for derivative chromosomes (Figure 2A). Reciprocal painting between human and B95-8 revealed chromosomes homologous to human 1p, 1(q21-31), 1(q32-qter), 2(pter-q12)/16q, 2(q13-qter)/15(q25-qter), 3(p24-pter, p12-21, q12-13, q27-qter), 3(p21-24, q13-26), 3(p12)/21, 4, 5/7(p22, q11, q21), 6, 7(p11-21, q11-21, q22-qter) 8p/18, 8q, 10p, 10q/16p, 11, 12, 13(q14-qter)/9/22, 13(q11-13)/17/20, 14/15(q11-24), 19, X and Y. Figure 1 illustrates representative FISH experiments. To date, an association of human chromosome 2/15 homologues has only been observed in Callitrichidae and squirrel monkey. Further, *S. oedipus* is the only Callitrichidae species showing separate human 1p and 10p homologues (Neusser *et al.* submitted). Consequently, the cell line B95-8 could only have been derived from this species as described by Miller *et al.* (1972).

Reverse painting of B95-8 probes to 'wildtype' *S. oedipus* metaphases revealed B95-8 derivative chromosomes del(8), der(8)del(8)t(8;16) and der(1)t(1;2), as illustrated in Figure 2B. Reverse painting these probes to human metaphases mapped the two B95-8 chromosome 8 deleted regions to human homologous bands 6q14-24 and 6p22. The der(1)t(1;2) breakpoint mapped to human homologous band 13q14 (Figure 2C). Hybridization with human painting probes to B95-8 metaphases further delineated der(17)t(2;17) and ins(14;8). Table 1 summarizes the frequency of aberrant chromosomes observed.

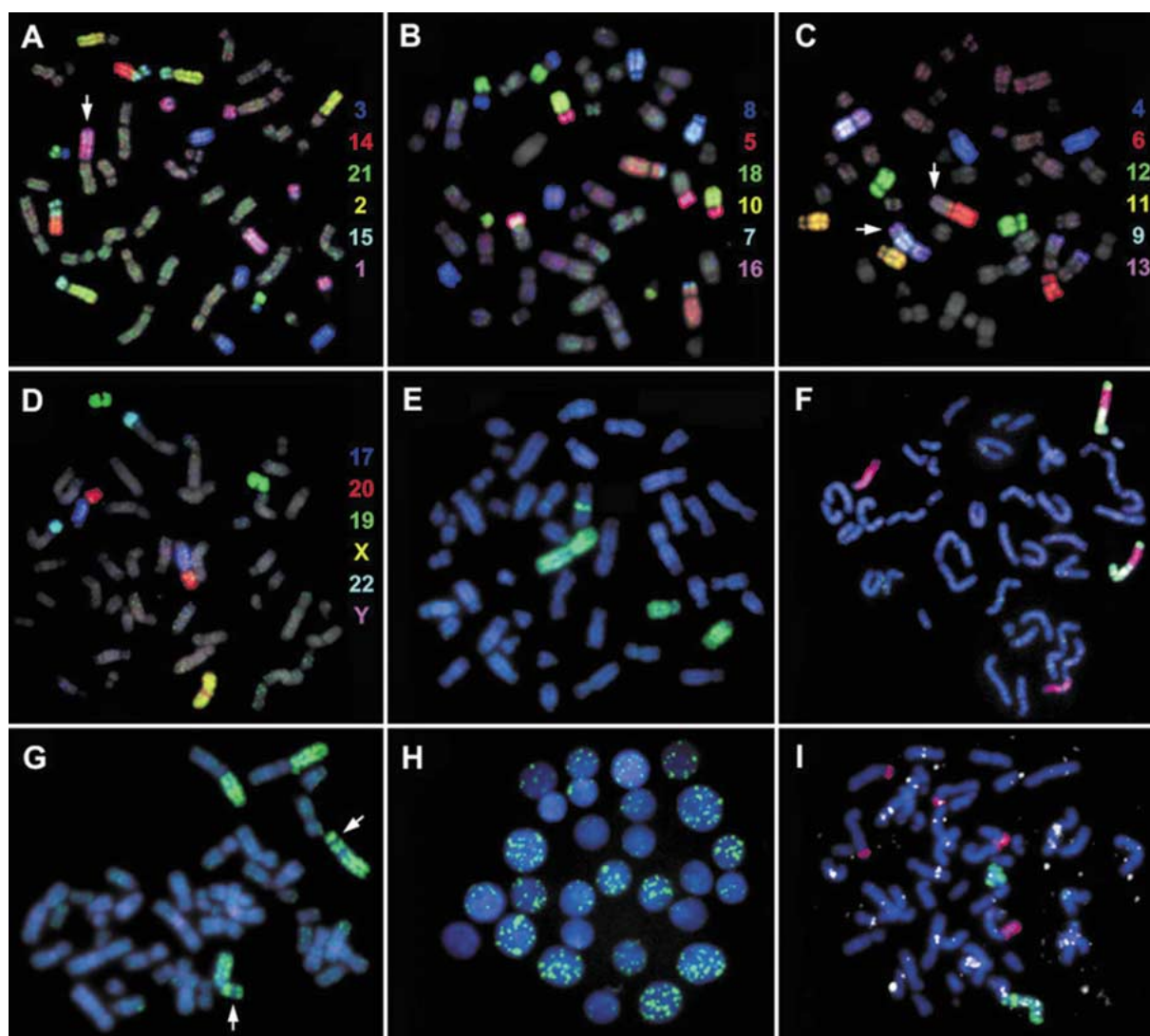


Figure 1. (A–D) Hybridization of human 6-colour multiplex probes to B95-8 metaphases. Arrows highlight B95-8-derivative chromosomes. (E) Hybridization of B95-8 chromosome der(8)del(8)t(8;16) to B95-8 metaphase, resulting in an asymmetrical hybridization pattern. (F) B95-8 der(8)del(8)t(8;16) (red) and del(8) (green) reverse painting to *S. oedipus* metaphases. (G) B95-8 der(8)del(8)t(8;16) reverse painting to human metaphases. Arrows indicate the deletion on human chromosome 6. (H) EBV Bioprobe (green), hybridized to B95-8 interphase nuclei and (I) cohybridization of human chromosome 6 (green), 13 (red) and EBV Bioprobe (white); no B95-8-specific rearrangements involving these chromosome regions were directly associated with hybridization sites of EBV probe in the B95-8 genome.

These experiments demonstrated that human chromosome 6 or 13 homologous regions were involved in all B95-8-specific chromosomal aberrations. In order to test whether the breakpoints of B95-8-specific marker chromosomes are associated with integration sites of EBV into the B95-8 genome, human chromosome

6- and 13-specific painting probes were cohybridized with a biotinylated EBV probe (Bioprobe). Significant variations in the amounts of EBV DNA per cell were observed in interphase nuclei (Figure 1H). In metaphases, the number of EBV-derived signals was also highly variable. Only some EBV hybridization signals were present

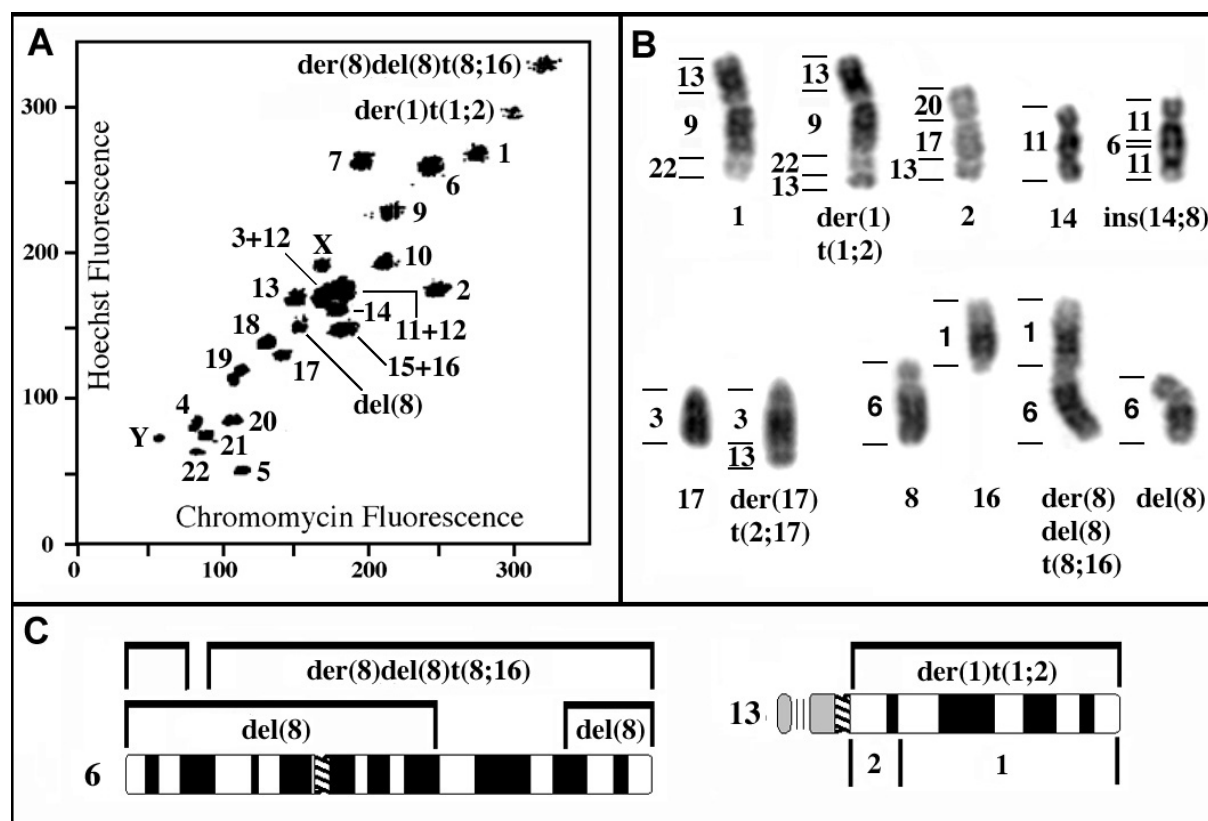


Figure 2. (A) Cell line B95-8 flow karyotype with chromosome assignment. **(B)** Comparison of banded 'wildtype' *S. oedipus* and B95-8-specific derivative chromosomes, together with the assignment of human homologous chromosome regions (left of each chromosome). **(C)** G-banded idiogram of human chromosome 6 and 13 with the assignment of the hybridization signals from the derivative B95-8-chromosome-specific probes, delineating deletion and translocation breakpoints.

on both chromatids at a specific chromosome region. The analysis of 50 cells showed no clustering of signals in regions homologous to human chromosome 6 or 13. Neither were EBV integration sites detected directly at the respective translocation or deletion breakpoints (Figure 1D).

Karyotype analysis has traditionally been an important tool to describe cell cultures. New molecular cytogenetic techniques can now define chromosome rearrangements at the molecular level, thus providing 'finger prints' for any cell line. Furthermore, we demonstrated that multicolour

Table 1. Frequency of derivative chromosomes in cell line B95-8. 50 cells.

Chromosome	<i>n</i> /total	% of total	Subclone A	Subclone B
del(8)	36/50	72	+	+
der(8)del(8)t(8;16)	50/50	100	+	+
ins(14;8)	22/50	44	+	
der(17)t(2;17)	24/50	48	+	
der(1)t(1;2)	20/50	40		+

Two subpopulations, A and B, were observed (each in approximately 40–50% of cells). Derivative chromosomes, der(8)del(8)t(8;16) and del(8), were present in both subpopulations. ins(14;8) and der(17)t(2;17) were subpopulation A specific. der(1)t(1;2) subclone B specific.

FISH is also applicable between distant related species. B95-8 chromosome painting probes will be very useful to delineate New World monkey taxonomy and karyotype evolution.

Acknowledgements

The authors would like to thank M.A. Ferguson-Smith, Cambridge University, UK, for enabling the flow sorting, R. Desrosiers, New England Regional Primate Center, USA, for providing *S. oedipus* blood samples, and R. Stanyon, National Cancer Institute, USA, for chromosome preparations. The work was funded by the Deutsche Forschungsgemeinschaft (DFG).

References

- Miller G, Lipman M (1973) Release of infectious Epstein-Barr virus by transformed marmoset leukocytes. *Proc Natl Acad Sci USA* **70**: 190–194.
- Miller G, Shope T, Lisco H, Stitt D, Lipman M (1972) Epstein-Barr virus: transformation, cytopathic changes, and viral antigens in squirrel monkey and marmoset leukocytes. *Proc Natl Acad Sci USA* **69**: 383–387.
- Müller S, O'Brien PC, Ferguson-Smith MA, Wienberg J (1998) Cross-species colour segmenting: a novel tool in human karyotype analysis. *Cytometry* **33**: 445–452.
- Müller S, Stanyon R, O'Brien PC, Ferguson-Smith MA, Plesker R, Wienberg J (1999) Defining the ancestral karyotype for all primates by multidirectional chromosome painting between tree shrews, lemurs and humans. *Chromosoma* **108**: 393–400.
- Nagamachi CY, Pieczarka JC, Schwarz M, Barros RM, Mattevi MS (1997) Comparative chromosomal study of five taxa of genus *Callithrix*, group *jacchus* (Platyrrhini, Primates). *Am J Primatol* **41**: 53–60.
- Neitzel H (1986) A routine method for the establishment of permanent growing lymphoblastoid cell lines. *Hum Genet* **73**: 320–326.
- Ried T, Baldini A, Rand TC, Ward DC (1992) Simultaneous visualization of seven different DNA probes by in situ hybridization using combinatorial fluorescence and digital imaging microscopy. *Proc Natl Acad Sci USA* **89**: 1388–1392.
- Telenius H, Pelmear AHP, Tunnacliffe A *et al.* (1992) Cytogenetic analysis by chromosome painting using DOP-PCR amplified flow-sorted chromosomes. *Genes Chromosomes Cancer* **4**: 257–263.