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CELL LINE INDIVIDUALIZATION BY STR MULTIPLEX SYSTEM IN THE CELL BANK FOUND CROSS-CONTAMINATION BETWEEN ECV304 AND EJ-1/T24

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Abstract: Short tandem repeat (STR) regions represent highly polymorphic microsatellite markers in the human genome that have tandemly repetitive sequence elements of 2 to 7 bp in length as a unit. The application of STR regions to population genetics and personal identification has been well studied. Recent technical advances have enabled us to analyze multilocus STR regions simultaneously by a method, called the STR Multiplex system, that uses a single PCR amplification in one tube. We established a new evaluation system for the identification of cell lines based on an STR Multiplex method that uses 9 different loci: D5S818, D13S317, D7S820, D16S539, vWA, TH01, Amelogenin, TPOX, and CSF1PO. The STR profiling data from 96 cell lines were examined and an efficiency of this approach for cell standardization was found. Using this method, we have analyzed the STR profiles of human cell lines, ECV304, EJ-1, and T24, recently reported by the DSMZ-German Collection of Microorganisms and Cell Cultures to have been cross-contaminated. Our results clearly detect the cross-contamination between ECV304 and EJ-1/T24. The cross-contamination was estimated to be derived from the T24 cells. Collectively, the STR Multiplex system provides a rapid, precise, and powerful method in cell line identification for quality control at the JCRB Cell Bank.

Key words: STR Multiplex system, Cell line identification, Cross-contamination, Cell Bank

Introduction

The importance of establishing methods in identifying the precise origin of cell-lines and in inhibiting cross-contamination has become clear since the reports of the widespread HeLa cell contamination[1]. To date, not only cytological analyses, including morphology, cytogenetics, and isozyme-typing, but also molecular approaches, such as DNA fingerprint/profiling methods[2] using VNTR regions, have been developed and used. Indeed, DNA profiling provides useful information for cell line identification, however, it has disadvantages of requiring large amounts of time and DNA. Therefore, simpler and more convenient alternatives have been investigated. Recently, an alternative method has been introduced in using polymorphic microsatellite markers, called short tandem repeat (STR) regions, which have tandemly repetitive sequence elements of 2 to 7 bp in length as an unit and are available for PCR-based analyses[3–5]. In particular, trimeric and tetrameric STR regions have been widely utilized in physical and genetic mapping of the human genome[3], disease diagnosis, and personal identification[6–8] in the medical and forensic science. In addition, STR regions have been used in human race analyses in the population genetics[9,10]. More recently, technical advances have enabled multilocus analysis of more than 8

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STR regions by one PCR amplification in a single tube, termed the STR Multiplex system[11,12]. Application of the STR Multiplex system has greatly benefited numerous fields of science, and its use in the verification of cell lines as unique have been attempted[13,14].

In this study, we establish a new evaluation system for cell line identification based on the STR Multiplex method using 9 loci: D5S818, D13S317, D7S820, D16S539, vWA, TH01, Amelogenin, TPOX, and CSF1PO. Further, we verify the identities of individual cultured cell lines deposited in the JCRB Cell Bank. This new system conveys digital code for each individual cell line as profiles and is available for the comparison of STR profiles of all other examined cell lines to check for cross-contamination.

Deutsche Sammlung von Mikroorganismen und Zellkurturen (DSMZ)–German Collection of Microorganisms and Cell Cultures has reported the cross-contamination[15] between the human cell lines, ECV304[16] and T24[17]. We investigated this cross-contamination using cytogenetic analysis and the STR profiles of ECV304, EJ-1[18], and T24. EJ-1 was previously reported to be cross-contaminated with T24 by HLA-typing analyses[19] and is now widely recognized as a delivertive of the T24. Our investigations use not only chromosome analysis, but also STR profiles of these three cell lines to evaluate how efficient this system is and to verify the cross-contamination.

Materials and Methods

Cell lines and morphology

Ninety-six human cell lines from the JCRB Cell Bank were examined in this study (Table 1). The cellular morphology of ECV304, EJ-1, and T24 was examined by light microscopy, using an Olympus CK40 microscope, at passage 73, 7*, and 5*, respectively (Asterisk indicates that the passage numbers are counted after the cells were deposited).

Cytogenetics and fluorescence in situ hybridization (FISH)

Chromosome spreads were obtained from cultured ECV304, EJ-1, and T24 cells according to the conventional methanol / acetic acid method with modifications for a hypotonic solution as 0.2% (W/V) sodium citrate / 0.06M potassium chloride. Chromosome numbers per cell from 50 metaphases were counted after Giemsa staining. QFH-banded metaphase images were captured by a cooled CCD camera (Photometrics PXL) and superimposed on the same metaphase image after fluorescence in situ hybridization (FISH) and the FISH signals indicating chromosomal localizations were detected. FISH, with human chromosome 5-specific painting probe (Vysis; SpectrumGreen-labeled whole chromosome painting probe WCP#5), was carried out according to the manufacture's instruction with slight modification[20]. Captured FISH images were colorized by a MultiFluor Electronic Photography system (Biological Detection Incorporation). A Zeiss Axiophot 2 microscope equipped with a 63× Apochromato objective lens was used for epifluorescence.

DNA extraction

Total genomic DNA was extracted from the cell lines by a DNA extraction kit (GenTle solution kit; Takara Co., Ltd., Kyoto) according the manufacturer's instructions.

STR Multiplex system

The *GenePrint* PowerPlex1.2 System (Promega, Madison, WI) was used for performing STR Multiplex analysis and all the procedures were obtained from the technical manual[12]. PCR amplification was performed using the Perkin-Elmer Gene Amp PCR System 9600 Thermal Cycler. Approximately 1 to 2 ng template DNA was included in each 25 ml reaction mixture containing 1× Gold ST★R buffer, 1× primer pair mixture, and 2.25 units of AmpliTaq Gold DNA polymerase. The cycling profile was as follows: preheating at 95°C for 11 min, 96°C for 1 min, then 10 cycles of 94°C for 30 sec, 60°C for 30 sec, 70°C for 45 sec; then 22 cycles of 90°C for 30 sec, 60°C for 30 sec, 70°C for 45 sec; then 60°C for 30 min. HeLa DNA template and nuclease-free water were used as positive and negative controls, respectively. This system contains two color fluorescent (fluorescein and carboxy-tetramethylrhodamine) primers that recognize the tetrameric

Table 1. STR data of the collected cell lines in the JCRB Cell Bank

The STR data is represented as 0 and 1, based on the graphical representation of the cell lines in the JCRB Cell Bank. The digital data was used to calculate the evaluation values to verify the probabilities of cross-contamination in specific comparison groups.

Cell Number	Cell Name	Lot No	Locus A (D5S818)	Locus B (D13S317)	Locus C (D7S820)	Locus D (D16S539)	Locus E (vWA)	Locus F (TH01)	G(A	Locus H (TPOX)	Locus I (CSF1PO)
JCRB0744	ECV304 (P73)	011499	0 0 0 1 0 0 0 0 0	0 0 0 0 0 1 0 0 0	0 0 0 0 1 1 0 0 0	0 0 0 0 1 0 0 0 0 0	0 0 0 0 0 0 1 0 0 0	0 1 0 0 0 0 0	1 0	0 0 1 0 0 1 0 0	0 0 0 0 0 0 1 0 0 0
NIHS0220	ECV304 (P10)	062599	0 0 0 1 0 0 0 0 0	0 0 0 0 0 1 0 0 0	0 0 0 0 1 1 0 0 0	0 0 0 0 1 0 0 0 0 0	0 0 0 0 0 0 1 0 0 0	0 1 0 0 0 0 0	1 0	0 0 1 0 0 1 0 0	0 0 0 0 0 0 1 0 0 0
JCRB0710	EJ-1	041597	0 0 0 1 0 1 0 0 0	0 0 0 0 0 1 0 0 0	0 0 0 0 1 1 0 0 0	0 0 0 0 1 0 0 0 0 0	0 0 0 0 0 0 1 0 0 0	0 1 0 0 0 0 0	1 0	0 0 1 0 0 1 0 0	0 0 0 0 0 1 0 1 0 0
JCRB0711	T24	101596	0 0 0 1 0 1 0 0 0	0 0 0 0 0 1 0 0 0	0 0 0 0 1 1 0 0 0	0 0 0 0 1 0 0 0 0 0	0 0 0 0 0 0 1 0 0 0	0 1 0 0 0 0 0	1 0	0 0 1 0 0 1 0 0	0 0 0 0 0 1 0 1 0 0
JCRB9068	293	012287	0 1 1 0 0 0 0 0 0	0 0 0 0 0 1 0 0 0	0 0 0 0 0 1 1 0 0	0 0 0 0 1 0 0 1 0 0	0 0 0 0 0 1 0 0 1 0 0	0 0 1 0 0 a 0	1 0	0 0 0 0 0 1 0 0 0	0 0 0 0 0 0 1 0 0 0
JCRB0826	8505C	060697	0 0 0 1 1 0 0 0 0	0 0 0 0 0 0 1 0 0	0 0 0 0 1 0 0 0 0	0 0 0 0 0 0 0 1 0 0	0 0 0 0 0 0 1 0 1 0 0	0 1 0 0 1 0 0	1 0	0 0 0 0 1 1 0 0 0	0 0 0 0 0 0 1 0 0 0
JCRB0228	A-172	030796	0 0 0 0 1 1 0 0 0	0 0 0 0 1 0 0 0 0	0 0 0 0 1 0 0 0 0	0 0 0 0 0 0 0 1 0 0	0 0 0 0 0 0 0 0 1 0	0 1 0 0 0 1 0	1 1	0 0 1 0 0 1 0 0	0 0 0 1 0 0 1 0 0 0
JCRB0101	A3/KAW	010788	0 0 0 1 1 0 0 0 0	0 0 0 1 0 1 0 0 0	0 0 0 0 0 1 0 0 0	0 0 0 0 0 1 0 0 0 0	0 0 0 0 0 0 1 0 0 0	0 0 1 0 1 0 0 1	0	0 0 1 0 0 0 0 0	0 0 0 0 0 1 0 0 0
JCRB0097	A4/Fuk	120186	0 0 0 0 0 1 0 0 0	0 0 1 0 0 1 0 0 0	0 0 1 0 0 1 0 0 0	0 0 0 0 1 0 0 1 0 0	0 0 0 0 0 0 0 1 1 0	0 0 1 0 1 0 0 1	0	0 0 0 0 0 1 0 0 0	0 0 0 0 0 1 0 1 0 0
JCRB0076	A-549	120498	0 0 0 0 1 0 0 0 0	0 0 0 0 1 0 0 0 0	0 0 1 0 0 1 0 0 0	0 0 0 0 0 0 1 0 0 0	0 0 0 1 0 0 0 0 0 0	0 0 0 1 0 a 0	1	0 0 1 0 0 1 0 0	0 0 0 0 1 0 1 0 0
JCRB0815	ABC-1	120996	0 0 0 0 0 1 0 0 0	0 1 0 0 0 0 0 0 0	0 0 0 0 1 0 1 0 0	0 0 0 0 1 0 0 1 0 0	0 0 0 0 0 1 0 0 0 0	0 0 0 0 0 1 0	1	0 0 1 0 0 1 0 0	0 0 0 0 0 0 1 0 0 0
JCRB0801	Caki-1	011097	0 0 0 0 1 1 0 0 0	0 0 0 0 1 1 0 0 0	0 0 1 0 0 0 1 0 0	0 0 0 0 0 0 0 1 0 0	0 0 0 0 1 0 1 0 0 0	0 1 0 1 0 0 0	1	0 0 1 0 0 1 0 0	0 0 0 0 1 0 0 0 0
JCRB0207	CaR-1	061298	0 0 0 1 0 0 0 0 0	0 0 0 1 0 1 0 0 0	0 0 1 0 0 0 1 0 0	0 0 0 0 1 0 0 0 0 0	0 0 0 1 0 0 0 1 0 0	0 1 0 0 0 0 0	1	0 0 1 0 0 0 0 0	0 0 0 0 1 0 0 0 0
JCRB9101	CCD-14Br	060487	0 0 0 0 1 0 1 0 0	0 0 0 1 0 1 0 0 0	0 0 1 0 0 0 1 0 0	0 0 0 0 1 0 0 0 0 0	0 0 0 1 0 0 0 1 0 0	0 1 0 0 0 1 0	1	0 0 1 0 0 0 0 0	0 0 0 0 1 0 0 0 0
JCRB0208	CCK-81	033198	0 0 0 0 1 1 1 0 0	0 0 1 0 0 1 1 0 0	0 0 1 b 0 a 1 0 0	0 0 0 0 1 0 0 0 0 0	0 0 0 1 0 0 0 1 0 0	0 1 0 0 0 0 0	1	0 0 1 0 0 0 0 0	0 0 0 0 1 0 0 0 0
JCRB9066	Chang Liver	011787	0 0 0 0 1 1 0 0 0	0 0 0 0 0 0 0 a 0	0 0 1 0 0 0 1 0 0	0 0 0 0 1 0 0 0 0 0	0 0 0 0 1 0 1 0 0	0 0 1 0 0 0 0	1	0 0 1 0 0 0 0 0	0 0 0 0 1 0 0 0 0
JCRB0257	CoCM-1	111798	0 0 0 0 1 1 0 0 0	0 0 0 1 0 0 0 0 0	0 0 0 1 0 1 0 0 0	0 0 0 0 0 0 1 1 0	0 0 0 0 1 0 0 0 0	0 0 1 0 1 0 0	1	0 0 1 0 0 0 0 0	0 0 0 0 1 0 0 0 0
JCRB0226	COLO201	071498	0 0 0 1 0 0 1 0 0	0 0 0 1 0 1 0 0 0	0 0 0 1 1 0 0 0 0	0 0 0 0 0 0 0 1 0	0 0 0 0 0 0 0 0 0	0 0 1 1 0 0	1	0 0 0 0 0 0 0	0 0 0 0 0 1 0 0 0
JCRB0225	COLO320 DM	112996	0 0 0 0 0 1 0 0 0	0 0 0 0 1 0 0 0 0	0 0 0 1 0 0 1 0 0	0 0 0 0 0 0 0 1 0	0 0 0 0 1 0 0 1 0	0 0 0 1 0 0 0	1	0 0 1 0 0 0 0	0 0 0 0 0 1 0 0 0
JCRB9094	DLD-1	051587	0 0 0 0 0 0 1 0 0	0 1 0 0 1 0 0 0 0	0 0 0 0 1 0 1 0 0	0 0 0 0 0 0 0 0 1 0	0 0 0 0 0 0 0 1 0 0	0 0 1 0 0 a 0	1	0 0 1 0 0 1 0 0	0 0 0 0 0 1 0 0 0
JCRB0820	EBC-1	031496	0 0 0 0 1 0 0 0 0	0 0 0 0 0 1 0 0 0	0 0 0 0 1 1 0 0 0	0 0 0 0 1 0 0 0 0 0	0 0 0 0 0 1 0 0 0 0	0 0 1 0 0 0 0	1	0 0 1 0 0 0 0 0	0 0 0 0 1 0 0 0 0
JCRB0067	FLOW2000	121485	0 0 0 0 1 0 0 0 0	0 0 0 0 0 1 0 0 0	0 0 0 1 0 0 1 0 0	0 0 0 0 0 0 0 1 0	0 0 0 0 1 0 0 1 0	0 0 1 0 0 a 0	1	0 0 1 0 0 0 0	0 0 0 0 1 0 0 0 0
JCRB0068	FLOW3000	122485	0 0 1 0 0 0 1 0 0	0 0 0 0 0 1 0 0 0	0 0 0 1 0 0 1 0 0	0 0 0 0 0 0 0 0 1 0	0 0 0 0 1 0 1 0 0	0 0 0 1 0 0 0	1	0 0 1 0 0 0 0	0 0 0 0 1 0 0 0 0
JCRB0612	GOTO	050698	0 0 0 0 1 1 0 0 0	0 0 0 0 0 0 0 0 1	0 0 1 0 0 0 1 0 0	0 0 0 0 0 0 1 0 1 0	0 0 0 0 0 0 0 1 0 0	0 0 0 1 0 0 0	1	0 0 1 0 0 0 0	0 0 0 0 1 0 0 0 0
JCRB9004	HeLa	071994	0 0 0 0 1 1 0 0 0	0 0 0 0 0 0 1 a 0	0 0 1 0 0 0 1 0 0	0 0 0 0 1 0 0 0 0 0	0 0 0 0 0 1 0 1 0 0	0 0 1 0 0 0 0	1	0 0 1 0 0 0 0	0 0 0 1 0 0 0 0 0
JCRB9086	HeLa 229	042887	0 0 0 0 1 1 0 0 0	0 0 0 0 0 0 1 a 0	0 0 1 0 0 0 1 0 0	0 0 0 0 1 0 0 0 0 0	0 0 0 0 0 1 0 1 0 0	0 0 1 0 0 0 0	1	0 0 1 0 0 0 0	0 0 0 1 0 0 0 0 0
JCRB0213	HeLa AG	011999	0 0 0 0 1 1 0 0 0	0 0 0 0 0 0 0 a 0	0 0 1 0 0 0 1 0 0	0 0 0 0 1 0 0 0 0 0	0 0 0 0 0 1 0 1 0 0	0 0 1 0 0 0 0	1	0 0 1 0 0 0 0	0 0 0 1 0 0 0 0 0
JCRB0649	HeLa.P3	120597	0 0 0 0 1 1 0 0 0	0 0 0 0 0 0 1 0 a	0 0 1 0 0 0 1 0 0	0 0 0 0 1 0 0 0 0 0	0 0 0 0 0 1 0 1 0 0	0 0 1 0 0 0 0	1	0 0 1 0 0 0 0	0 0 0 1 0 0 0 0 0
JCRB9010	HeLa S3	072485	0 0 0 0 1 1 0 0 0	0 0 0 0 0 0 0 a 0	0 0 1 0 0 0 1 0 0	0 0 0 0 1 0 0 0 0 0	0 0 0 0 0 1 0 1 0 0	0 0 1 0 0 0 0	1	0 0 1 0 0 0 0	0 0 0 1 0 0 0 0 0
JCRB0214	HeLa TG	062388	0 0 0 0 1 1 0 0 0	0 0 0 0 0 0 0 a 0	0 0 1 0 0 0 1 0 0	0 0 0 0 1 0 0 0 0 0	0 0 0 0 0 1 0 1 0 0	0 0 1 0 0 0 0	1	0 0 1 0 0 0 0	0 0 0 1 0 0 0 0 0
JCRB0215	HeLa TG Cap	070299	0 0 0 0 1 1 0 0 0	0 0 0 0 0 0 0 a 0	0 0 1 0 0 0 1 0 0	0 0 0 0 1 0 0 0 0 0	0 0 0 0 0 1 0 1 0 0	0 0 1 0 0 0 0	1	0 0 1 0 0 0 0	0 0 0 1 0 0 0 0 0
JCRB9095	HEPM	051887	0 0 0 0 1 1 0 0 0	0 1 0 0 1 0 0 0 0	0 0 0 0 1 0 1 0 0	0 0 0 0 0 1 0 1 0 0	0 0 0 0 0 0 1 0 0 0	0 0 1 0 0 0 0	1	0 0 1 0 0 1 0 0	0 0 0 0 0 1 1 0 0
JCRB0085	HL60	031395	0 0 0 0 0 1 0 0 0	0 1 0 0 1 0 0 0 0	0 0 0 0 0 1 1 0 0	0 0 0 0 0 0 1 0 0 0	0 0 0 0 0 1 0 0 0 0	0 0 1 0 0 0 0	1	0 0 1 0 0 1 0 0	0 0 0 0 0 0 0 1 1 0
JCRB0006	HL60RG	052092	0 0 0 0 0 1 0 0 0	0 1 0 0 1 0 0 0 0	0 0 0 0 0 1 1 0 0	0 0 0 0 0 0 1 0 0 0	0 0 0 0 0 1 0 0 0 0	0 0 1 0 0 0 0	1	0 0 1 0 0 1 0 0	0 0 0 0 0 0 0 1 1 0
JCRB0831	HO-1-N-1	110797	0 0 1 0 0 0 0 0 0	0 0 0 1 0 0 0 0 0	0 0 0 1 0 0 1 0 0	0 0 0 0 1 0 1 0 0 0	0 0 0 0 1 0 1 0 0 0	0 0 0 1 0 0 0	1	0 0 0 0 1 0 0	0 0 0 0 0 0 1 0 0 0
JCRB0828	HO-1-u-1	120997	0 0 0 1 0 0 0 0 0	0 0 1 0 0 0 0 0 0	0 0 0 1 0 0 0 0 0	0 0 0 0 0 0 0 1 0 0	0 0 0 0 0 0 1 0 0 0	0 0 0 1 0 0 0	1	0 0 0 0 1 0 0	0 0 0 0 0 1 0 0 0 0
JCRB0622	HSC-2	122198	0 0 0 1 0 1 0 0 0	0 0 0 0 0 1 0 0 0	0 0 0 1 0 0 1 0 0	0 0 0 0 0 0 0 1 0 0	0 0 0 0 0 1 0 1 0 0	0 1 0 0 0 0 0	1	0 0 1 0 0 0 0	0 0 0 0 0 1 0 0 0 0
JCRB0623	HSC-3	102198	0 0 0 0 1 0 1 0 0	0 0 0 0 0 1 0 0 0	0 0 0 0 0 0 1 0 0	0 0 0 0 1 0 0 0 0 0	0 0 0 1 0 0 1 0 0 0	0 1 0 0 0 a 0	1	0 0 1 0 0 0 0	0 0 0 0 1 0 0 0 0
JCRB0624	HSC-4	112796	0 1 0 0 1 0 0 0 0	0 1 0 0 0 0 1 0 0	0 0 1 0 0 0 0 0 0	0 0 0 0 0 1 0 0 0 0	0 0 0 0 0 0 1 0 0 0	0 1 0 0 0 0 0	1	0 0 1 0 0 0 0	0 0 0 0 1 0 0 0 0
JCRB0403	HuH-7	040396	0 0 0 0 0 1 0 0 0	0 0 0 1 1 0 0 0 0	0 0 0 0 1 0 0 0 0	0 0 0 0 0 0 1 0 0 0	0 0 0 0 1 0 1 0 0 0	0 0 1 0 0 0 0	1	0 0 1 0 0 0 0	0 0 0 0 1 0 0 0 0
JCRB0413	HuO-3N1	122895	0 0 0 1 0 1 0 0 0	0 0 1 1 0 0 0 0 0	0 0 0 1 1 0 0 0 0	0 0 0 0 0 0 0 1 0 0	0 0 1 0 1 0 0 0 0	0 0 1 0 0 0 0	1	0 0 1 0 0 0 0	0 0 0 0 1 0 0 0 0
JCRB0427	HuO-9	081996	0 0 0 0 0 0 1 0 0	0 0 0 0 1 0 0 0 0	0 0 0 0 1 0 0 0 0	0 0 0 0 0 0 1 0 0 0	0 0 0 1 0 0 0 0 0 0	0 0 0 0 1 0 0	1	0 0 1 0 0 0 0	0 0 0 0 1 0 0 0 0
JCRB0428	HuO-9N2	122596	0 0 0 0 0 0 1 0 0	0 0 0 0 1 0 0 0 0	0 0 0 0 1 0 0 0 0	0 0 0 0 0 0 1 0 0 0	0 0 0 1 0 0 0 0 0 0	0 0 0 0 1 0 0	1	0 0 1 0 0 0 0	0 0 0 0 1 0 0 0 0
JCRB9050	IMR-32	102594	0 0 0 0 1 1 0 0 0	0 0 1 0 0 0 0 0 0	0 0 0 1 0 0 0 0 0	0 0 0 1 0 0 0 0 0 0	0 0 0 0 1 0 0 0 0 0	0 0 1 0 0 1 0	1	0 0 0 0 1 0 0	0 0 0 0 0 1 0 0 0
JCRB9054	IMR-90	121186	0 0 0 0 0 1 1 0 0	0 0 0 0 1 0 1 0 0	0 0 0 1 0 0 1 0 0	0 0 0 0 1 0 0 1 0 0	0 0 0 0 1 0 0 1 0 0	0 0 0 1 0 0 0	1	0 0 1 0 0 0 0	0 0 0 0 0 1 0 0 0
JCRB0073	J-111	100698	0 0 0 0 1 1 0 0 0	0 0 0 0 0 1 0 a 0	0 0 1 0 0 0 1 0 0	0 0 0 0 1 0 0 0 0 0	0 0 0 0 0 1 0 1 0 0	0 0 1 0 0 0 0	1	0 0 1 0 0 0 0	0 0 0 1 0 0 0 0 0
JCRB0019	K-562	020190	0 0 0 0 1 1 0 0 0	0 1 0 0 0 0 0 0 0	0 0 0 1 0 1 0 0 0	0 0 0 0 0 0 1 0 1 0	0 0 0 0 0 0 1 0 0 0	0 0 0 0 1 0 0	1	0 0 1 0 0 0 0	0 0 0 1 0 0 0 0 0
JCRB9027	KB	120398	0 0 0 0 1 1 0 0 0	0 0 0 0 0 0 1 0 a	0 0 1 0 0 0 1 0 0	0 0 0 0 1 0 0 0 0 0	0 0 0 0 0 1 0 1 0 0	0 0 1 0 0 0 0	1	0 0 1 0 0 0 0	0 0 0 1 0 0 0 0 0
JCRB0432	KMS-6	042198	0 0 0 1 0 1 0 0 0	0 0 0 0 1 0 0 0 0	0 0 1 0 0 0 1 0 0	0 0 0 0 1 0 0 0 0 0	0 0 0 0 0 1 0 1 0 0	0 0 1 0 0 0 0	1	0 0 1 0 0 0 0	0 0 0 1 0 0 0 0 0
JCRB0430	KMS-12-PE	022897	0 0 0 0 0 0 0 1 0	0 1 0 0 0 0 0 0 0	0 0 0 0 1 1 0 0 0	0 0 0 0 1 0 0 0 0 0	0 0 0 0 0 1 0 1 0 0	0 0 1 0 0 0 0	1	0 0 1 0 0 0 0	0 0 0 1 0 0 0 0 0
JCRB0123	KO52	081497	0 0 0 0 1 0 0 0 0	0 1 0 0 0 1 0 0 0	0 0 0 1 1 0 0 0 0	0 0 0 0 1 0 0 1 0 0	0 0 0 0 0 1 0 0 0 0	0 1 0 0 0 0 0	1	0 0 1 0 0 0 0	0 0 0 0 1 0 0 0 0
JCRB0127	KOSC-3	042198	0 0 0 0 0 1 0 0 0	0 0 0 0 1 0 0 0 0	0 0 0 0 0 1 0 1 0	0 0 0 0 1 0 0 0 0 0	0 0 0 0 0 1 0 0 0 0	0 1 0 0 0 0 0	1	0 0 1 0 0 0 0	0 0 0 0 0 1 0 0 0

JCRB0098	KURAMOCHI	090298	
JCRB0627	KYM-1	103197	
JCRB0829	LK-2	040196	
JCRB9083	LoVo	032687	
JCRB0055	LU65B	100898	
JCRB0044	LU99A	071985	
JCRB0058	LU99C	091697	
JCRB0235	Lu-134-A-H	121898	
JCRB0240	MCAS	070798	
JCRB0810	ME-180	090498	
JCRB0066	MeWo	121797	
JCRB0070	MIA PaCa-2	110498	
FDSC0011	MKN28	-	
FDSC0011	MKN28.6TG	-	
FDSC0013	MKN74	-	
JCRB9008	MRC-5	042287	
JCRB9060	MRC-9	061395	
JCRB0832	NH-6	070798	
JCRB0821	NUGC-2	100496	
JCRB0822	NUGC-3	112596	
JCRB0834	NUGC-4	031097	
JCRB0614	NY	070197	
JCRB0077	PC-3	121986	
JCRB0604	PSV811	110796	
JCRB0256	RCM-1	110698	
JCRB0102	RERF-LC-FM	091686	
JCRB0812	RERF-LC-MA	091498	
JCRB0081	RERF-LC-MS	053097	
JCRB0811	RERF-LC-OK	032798	
JCRB9058	RPMI2650	121986	
JCRB0034	RPMI8226	030397	
JCRB0260	SAS	120798	
JCRB0251	SCH	060198	
JCRB0075	SF-TY	033198	
JCRB9069	SW-13	012287	
JCRB0618	TGW	032596	
JCRB0112.1	THP-1	082687	
JCRB0128	TK-1	041798	
NIHS0181	TK-1	030598	
JCRB0234.0	TYK-nu	102198	
JCRB0814	VMRC-LCD	082598	
JCRB0074	VMRC-MELG	101298	
JCRB0813	VMRC-RCW	050697	
JCRB0827	VMRC-RCZ	030497	
JCRB0224	WiDr	051598	
JCRB9063	WIL2-NS	103095	
JCRB0746	YKG-1	062496	
JCRB0823	YMB-1	120996	
JCRB0825	YMB-1-E	121297	

repeat sequences of either 5'-AGAT-3' or 5'-AATG-3', both of which can amplify 9 separate loci of D5S818 (5q21→q31), D13S317 (13q22→q31), D7S820 (7q11.21→q11.22), D16S539 (16q24→qter), vWA (12pter→p12), TH01 (11p15.5), Amelogenin (Xp22.31→p22.1 and Yp11.2), TPOX (2pter→p25.1), and CSF1PO (5q33.3→q34). Amplification products were examined by 2% agarose gel electrophresis. The 26 ml reaction mixture containing fluorescent allelic ladder (internal standard) 1μl, deionized formamide 24 μl, and PCR product 1μl was prepared and the Gene Scan was performed by an ABI PRISM 310 Genetic Analyzer. Fluorescent peaks were detected and displayed by ABI PRISM Gene-Scan Analysis software Ver2.1.1. Each allele was assigned a specific typing by Genotyper software (Perkin-Elmer Applied Biosystems) and represented with the peak positions as 1 and all other non-peak positions as 0. Digital code with a total of 76 figures was constructed as STR profiling data for each cell line. To determine similarity between STR profiles from two different cell lines, we defined an evaluation value (EV) as follows:

EV = (Number of coincident at figure "1") × 2 / (Total number of figure "1" in cell A + Total number of figure "1" in cell B)

Results

Cellular morphology and cytogenetics

The ECV304 cell line was derived from human umbilical cord endothelial cells derived by spontaneous transformation[16] and deposited into the JCRB Cell Bank in 1991 (JCRB0744). EJ-1 and T24 cell lines were derived from human bladder carcinomas[17,18], deposited in 1985, and distributed as JCRB0710: EJ-1 and JCRB0711: T24. As shown in Figure 1, the three cell lines possess slight morphological differences, although all are epithelial-like in shape. The three cell lines were found to be hypertriploid, with chromosome number distributions from 62 to 132 in ECV304 and EJ-1, and from 78 to 95 in T24, resulting in no remarkable differences was implied. The chromosome modal numbers of ECV304, EJ-1, and T24 were 73 (14%), 86 (10%), and 83 (16%), respectively. QFH-banding results demonstrate the absence of

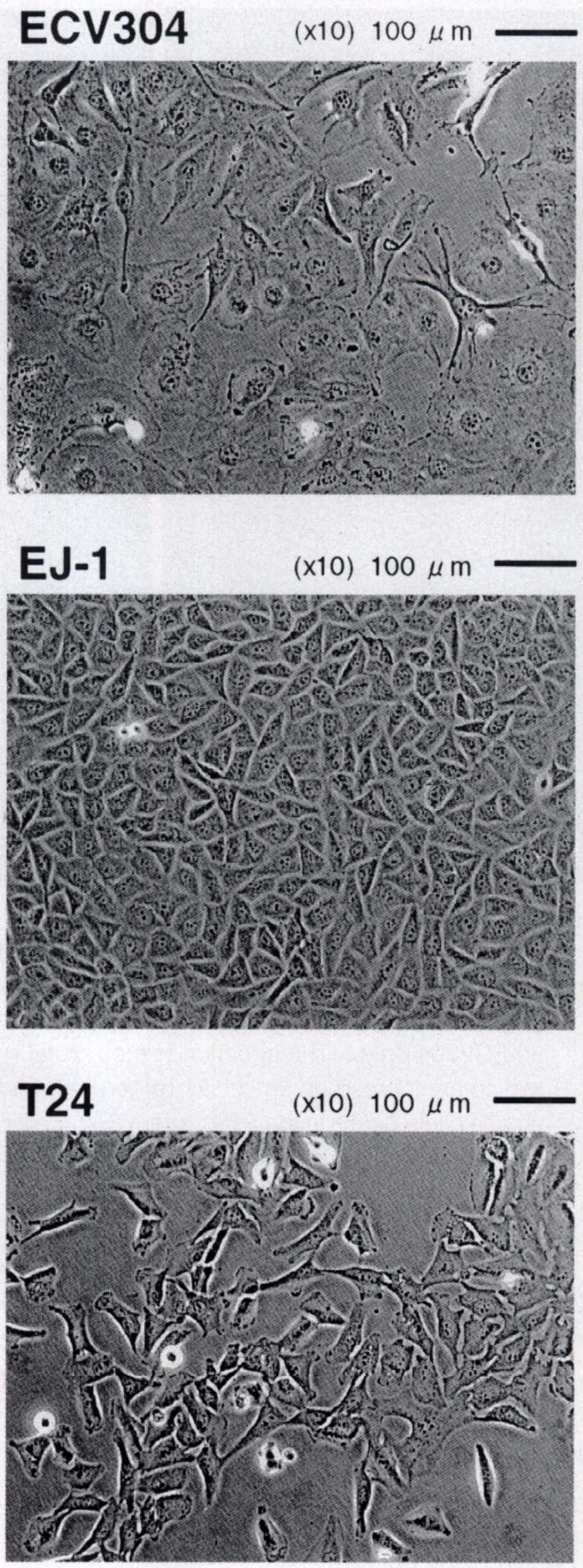

Figure 1. Cellular morphology Light micrographs of the cellular morphology of the three cell lines: ECV304, EJ-1, and T24.

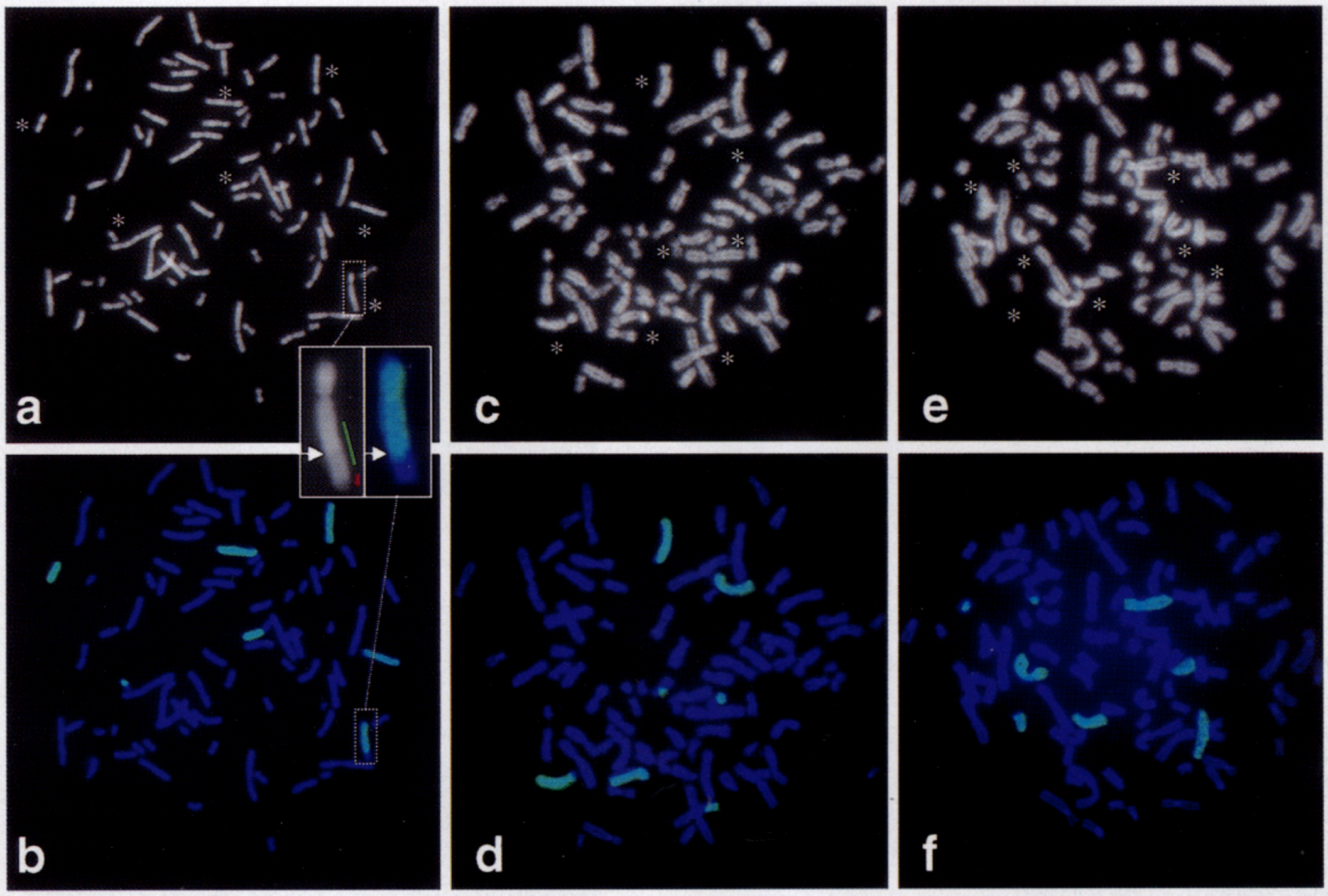

Figure 2. QFH-banding and FISH with WCP#5 QFH-banded metaphase chromosomes of three cell lines, (a) ECV304, (c) EJ-1, and (e) T24 are shown. FISH with WCP#5 was performed after QFH-banding, and the same metaphase images of (b) ECV304, (d) EJ-1, and (f) T24, respectively, are shown. Asterisks indicate the localization of painted regions. Insets show the translocated chromosome 5, with its breakpoint at 5q31 indicated by arrows, observed in ECV304 only. The specific deleted distal region of 5q31 includes two STR markers, D5S818 and CSF1PO, which are located on 5q21→31 (green bar) and 5q33.3→q34 (red bar), respectively. These two markers show single allele peaks in the STR pattern (Fig. 3) of ECV304 only, suggesting a relationship between the chromosomal deletion and the two STR loci.

a brightly stained quinacrine-positive Y chromosome in all three cell lines (Fig. 2a, c, e). FISH using the WCP#5 probe resulted in 7 to 8 signals per cell in all three cell lines. However, only ECV304 possessed a specific partial deletion of distal region of long arm at band 5q31(qter accompanying the translocation (Fig. 2b, d, f).

STR Multiplex system

Each allele of the 9 examined loci, in addition to 3 other loci examined in the three cell lines, represented fluorescent peaks and specific typing was assigned, by comparing the position of the allelic ladder of an internal standard, and graphically displayed (Fig. 3). The peak positions were represented as 1, and all other non-peak positions were represented as 0. A digital code with a total of 76 figures was produced that represented the STR profiling data for each cell line (Table 1). By examining the degree of matching of STR profile patterns between two cell lines, namely the evaluation value (EV), we found cross-contamination between the cell lines. Our results suggest that STR profile patterns among the three cell lines revealed identical typing patterns in all 12 loci, with the exception that one allele deletion was observed in ECV304, in both D5S818 (5q21→q31) and CSF1PO (5q33.3→q34) loci on chromosome 5 (Figs. 2, 3, Table 1). The EV was 0.917 between ECV304 and EJ-1/T24 and 1.00 between EJ-1 and

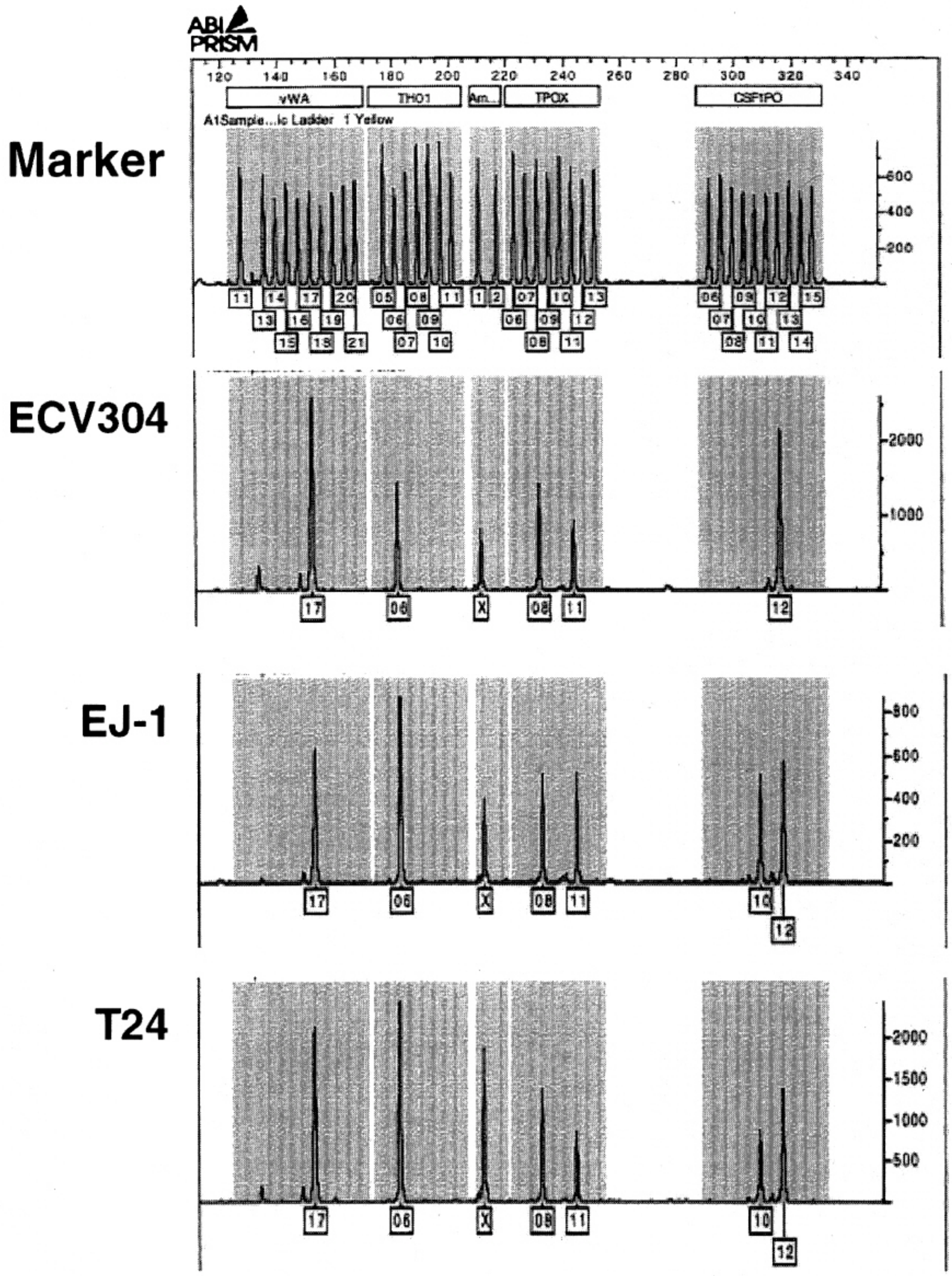

Figure 3. STR graphical representation An example of the graphical representation of the STR patterns of ECV304, EJ-1, and T24. The upper low represents the internal standard of the allelic ladder as posision markers. Note that all loci have identical peak positions among three cell lines except for the marker, CSF1PO in this figure, which indicate the loss of one allele in ECV304 as well as an additional loss appearing in the D5S818 marker(data not shown).

T24. The distribution of EVs in our comparison of ABC-1 and all other 95 cell lines was from 0.10 to 0.70. However, when we compared ECV304 and the same cell line group, the distribution was from 0.10 to 1.00 (Fig. 4). This result suggests that the range of 0.8 or more is critical to the case for cross-contamination, and if the EV distribution appears in that range in other comparison groups, we consider cross-contamination likely.

Discussion

While the power of exclusion (PE) value for

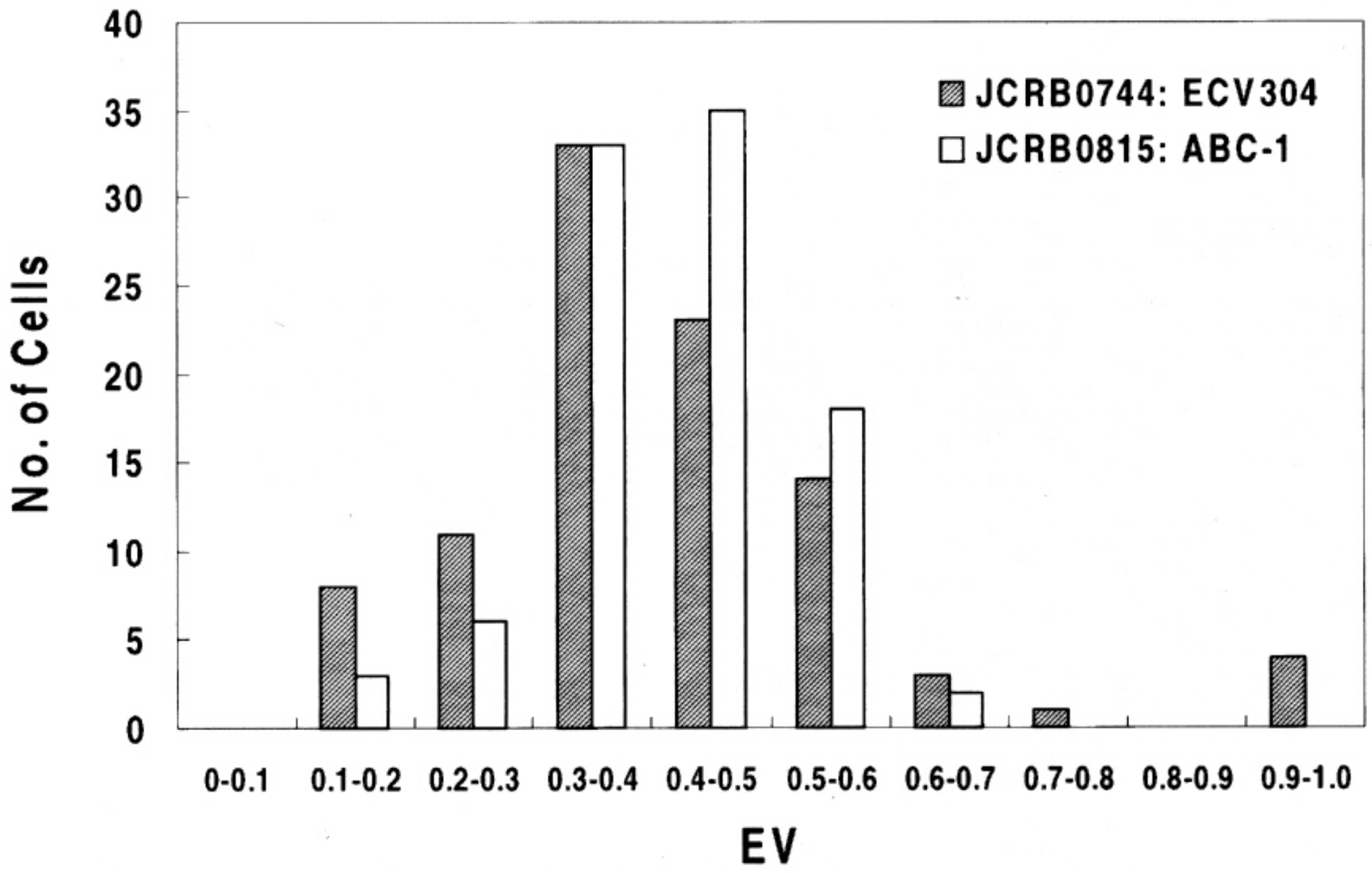

Figure 4. Distribution of evaluation values (EVs) in two comparison groups An example of the distribution of cell numbers in relation to evaluation values (EV) in two different comparison groups is demonstrated. EJ1, T24 and ECV304 appear at the EV range between 0.9 and 1.0 when the cells were compared with ECV304 (shaded bar). However, no cells appear between 0.7 and 1.0 when the cells were compared with ABC-1 (No cross-contamination expected).

each STR locus is small, the PE value can be increased greatly by combining several STR loci. For example, in Caucasian-Americans, the matching probability is $1/1.14\times10^{8}$ in 8 STR loci, and is $1/3.03\times10^{11}$ in 12 STR loci[12]. Among the ECV304, EJ-1, and T24 cell lines, STR profiles possessed identical typing patterns in all 12 loci, except for a single allele deletion that was observed in ECV304 only, at both D5S818 (5q21 →q31) and CSF1PO (5q33.3→q34) loci on the chromosome 5 (Figs. 2, 3, Table 1). In addition, the EV distribution of the above three cell lines indicated a high range of from 0.9 to 1.0 (Fig. 4). These results are consistent with reports from DSMZ and a confirmation of the cross-contamination of ECV304. According to the descriptions of establishment of these cell lines[16–18], the sexual origin of T24 is female, but that of both EJ-1 and ECV304 is male origine. These descriptions contradict our evidence that QFH-banding resulted in no brightly stained quinacrine-positive Y chromosome in three cell lines, and the only X allele peaks in all three cell lines were detected in the Amelogenin locus. This suggests that the three cell lines have no Y chromosome. However, since the Y chromosome is highly inclined to delete itself during culture passages (26 cases / 38 cases; 68.4% in Table 1), these results do not exclude the possibility of a male origin for both cell lines. Previous reports of EJ-1 cross-contamination with cells of a T24 origin are consistent with our STR profiling and EV distribution data. Together, these findings suggest that the cross-contamination of ECV304 found by DSMZ indicates a T24 origin and is the same as an EJ-1 origin. In addition, the date of contamination is considered to be at very early stages, since the STR profile pattern at passage 73 is identical with at passage 10 (Table 1).

From the results of FISH analysis with the WCP#5 probe, the deleted allele of both D5S818 and CSF1PO loci in ECV304 correlates well with the region of a partially deleted region of chromosome 5 (Fig. 2, insets). This suggests that the deleted allele that carries the two STR loci corresponds to a chromosomal deletion of the distal region at 5q31 (Figs. 2, 3). This deletion might be the specific event that occurred in ECV304 in early stages, and it is an interesting examples of the production of different patterns in the STR pro-

files from cells of the same origin. As in this case, chromosomal analyses provides important information.

The STR Multiplex system provides a rapid and reliable method of cell identification and reveals cross-contamination immediately by means of an evaluation test system developed in the present study. Combining chromosomal analyses with STR profiling data, the STR Multiplex system provides us with a more informative characterization of the cell lines. Further information of the current status of STR data will be available through the web site (http://cellbank.nihs.go.jp/) and we hope it will be of value for investigators.

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