QUALITY CONTROLS FOR CELL CULTURES: IDENTIFICATION OF INTERSPECIES CROSS-CONTAMINATION BY PCR-RFLP ANALYSIS OF THE CYTOCHROME B GENE

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INTRODUCTION

Cross-contaminations of a cell line with cells of different species represent a potential risk in laboratories handling human and animal cells. Therefore, it is necessary to control such contaminations.

Tests based on mitochondrial DNA (mtDNA) are used in forensic analysis, phylogenetic studies and in food authentication. However, the use of mtDNA in quality controls of cell cultures is recent. Mitochondrial sequence differences of closely related animal species are five- to tenfold higher than those of nuclear genes. On the contrary, intraspecies variation in mitochondrial sequences is low in most animal species. Moreover, each cell contains 100–10.000 mitochondrial genomes. The amount of mtDNA is greater than nuclear DNA, so that mtDNA can be analyzed also from small or partially degraded samples.

In the present study, a method based on a PCR-Restriction Fragment Length Polymorphism (RFLP) analysis of the mitochondrial cytochrome b gene was used (2). This gene has some stable sequences which are recognized from universal primers and some variable sequences used for animal species identification by PCR-RFLP method.

METHODS

Cells. In this study over 28 cell lines belonging to 13 different species were analyzed (Table 1). These cell lines included some human and animal cell lines among the most widely used for diagnostic and research purpose. Moreover mesenchymal stem cells of horse, dog and rat were tested.

An experimental contamination between cell lines of 2 different species (human and rat) was also performed.

Sample preparation and DNA extraction. Cell cultures stored in liquid nitrogen were thawed at +37°C, diluted with minimum essential medium containing 10% fetal calf serum, and centrifuged at 180xg; the pellets were resuspended in 200 µl of phosphate buffer saline (samples analyzed are listed in Table 1). DNA extraction was performed with DNeasy Blood & Tissue Kit (Qiagen) according to manufacturer's protocol.

PCR. Primers L14816 an H15173 (1) were used to amplificate a fragment of the cytochrome b gene (2).

RFLP analysis of PCR products. The amplification product was digested with 10U of six restriction enzymes: AluI, HinfI, HaeIII, TaqI, RsaI, MboI (3), and the derived pattern was resolved on 3% high-resolution agarose gel and visualized with a UV transilluminator.

Species	Cell line	Type	AluI	HinfI	HaeIII	TaqI	RsaI	MboI
Human	A549	ECL	358	198 160	231 106 21	217 141	358	192 115 51
	MRC5	ECL						
	CACO	ECL						
	MDA-MB-231	ECL						
	Hep-2	ECL						
Pig	PK-15	ECL	244 114	358	153 132 73	217 141	358	243 115
	MPK	ECL						
Mouse	RAW	ECL	358	313 45	358	217 141	282 76	243 115
	AML-12	ECL						
Rat	MSCs	PRC	358	358	358	358	267 60 31	245 79 34
Cow	MDBK	ECL	191 167	196 117 45	283 75	358	358	358
	REB	ECL						
	AUBEK	ECL						
Horse	MSCs	PRC	167 105 86	243 79 45	160 125 73	358	358	358
	E-DERM	ECL						
Cat	CRFK	ECL	190 114 54	117 79 45	253 75 19 11	358	215 143	358
Rabbit	RK13	ECL	358	236 122	153 128 45 32	358	358	243 115
Dog	MDCK	ECL	243 85 30	294 55 9	233 125	358	285 42 31	213 115 30
	A72	ECL						
	MSCs	PRC						
Rhesus Monkey	FRhK-4	ECL	358	198 160	159 123 76	358	204 154	358
	LLC-MK2	ECL						
African Green Monkey	VERO	ECL	358	198 160	159 123 76	358	327 31	295 63
	BGM	ECL						
	MA-104	ECL						
Chicken	Fibroblasts	PRC	358	188 160 10	159 123 76	358	204 154	358
Sheep	RFO	PRC	358	294 64	160 125 73	358	358	244 114
Hamster	BHK-21	ECL	190 114 54	358	264 74 20	217 141	327 31	326 32

Table 1. Cell lines tested for each species (MSCs: Mesenchymal Stem Cells, ECL: Estabilished Cell Line, PRC: Primary Cell line) and restriction profiles of different species.

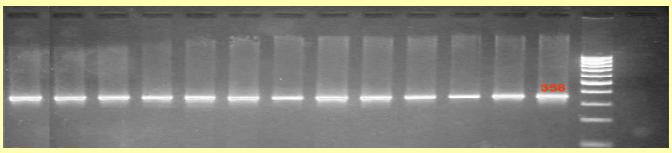

Figure 1. PCR amplification products of all species analyzed.

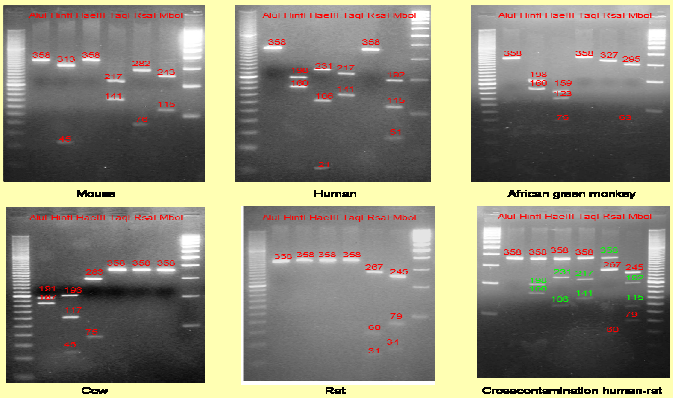

Figure 2. Restriction profiles of some species and of a crosscontamination uman-rat.

RESULTS

The couple of primers allowed the amplification of a 358 bp fragment of the cytochrome b gene in all species analyzed (Figure 1). For each species, RFLP produced a specific restriction pattern (Table 1) and the origin of these animal cells was confirmed by this analysis. Restriction profiles of some species are shown in Figure 2. The species of the two cell lines used for the cross-contamination were identified by the reading of the restriction profile obtained (Figure 2).

CONCLUSIONS

The obtained data showed that this method could be used in detecting the presence of cross-contamination and to identify the species of origin of cells. This allows to hypothesise the application of this method in the implementation of quality controls of cell cultures and mesenchymal stem cells.

BIBLIOGRAFIA

1) Bravi C. M., Lirón J. P., Mirol P. M., Ripoli M. V., Peral-Garcia P., Giovambattista G. (2004). A simple method for domestic animal identification in Argentina using PCR-RFLP analysis of cytochrome b gene. Leg. Med. 64: 246–251.

2) Losi C. G., Ferrari S., Sossi E., Villa R., Ferrari M. (2008). An alternative method to isoenzyme profile for cell line identification and interspecies cross-contaminations: cytochrome b PCR-RLFP analysis. In Vitro Cell. Dev. Biol. Anim. 44 (8-9): 321-9.

3) Meyer R., Höfelein C., Lüthy J., Candrian U. (1995). Polymerase chain reactionrestriction fragment length polymorphismanalysis: a simplemethod for species identification in food. J. AOAC Int. 786: 1542–1551.