The Amplified Fragment Length Polymorphism Study of Locus pMCT118 and Its Application in Forensic Biology

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INTRODUCTION

DNA restriction fragment length polymorphism (RFLP) analysis has been used more and more in forensic science on individual identification and paternity test. But the RFLP analysis needs relatively large quantity of undegraded DNA (>300ng for mutilocus probe) and the manupulation is complicated (Jeffreys 1985, 李伯龄 1991). In actual cases, the samples to be detected usually contain too little high molecular DNA to conduct RFLP analysis. The development of in vitro DNA amplification provides a new way to solve this problem. The sequences flanked the target VNTR locus are chosen as primers, and the use of polymerase chain reaction to amplify the VNTR can result in different length DNA fragment. The polymorphism is called amplified fragment length polymorphism (Amp—FLP) (Bugoele 1991).

According to the results of Kasai (1989), we chose pMCT118 locus to analysis the Amp—FLP. The question related to forensic sciences were discussed.

MATERIALS AND METHOD

DNA extraction: The DNA extraction from blood, semen, tissue and organ were discribed elsewhere (李伯龄 1991).

Amplification of pMCT118 locus: The PCR was carried out in a final volume of 100μl or 20μl. The content were 50mM KCl, 10mM Tris—HCl, pH8.3, 1.5mM $MgCl_2$ 0.01mg/ml gelatin, 200mM of each dNTP, 0.1μM of each primer, 1—100ng sample DNA. After denatured at 95°C for 5min, 2.5U or 1.25U of Taq DNA polymerase was added. Then overlay the mix with 50μl or 20μl of mineral oil. The PCR was carried out in Thermal Cycler (PE co. USA) with 95°C for 1 min. 65°C for 1 min. 70°C for 3 min. as one cycle. Ten second was added after each cycle. Thirty three cycles were conducted for each PCR.

Detection of the amplified sample: 5%T, 3%C, 0.75mm mini vertical plate polyacrylamide gel electrophoresis and silver stain was used to detect the Amp—FLP.

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RESULTS

Blood extracted DNA of 98 unrelated Chinese was analyxed by PCR of pMCT118 locus. The Amp—FLP results show 22 alleles, ranging from 340 to 780bp. The allele distribution frequencies range from 0.5—30% with a heterozygosity of 0.79.

Two families of three generation with 5 persons and four families of two generation with 3 persons were analysed of pMCT118 locus Amp—FLP. The Amp—FLP bands of the child were inherited from either mother or father. These were consistant with the Mendelian Inheritance Law.

The pMCT118 locus Amp—FLP of sperm DNA prepared from mixed stains were compared with that of the male and female blood DNA. The results showed that the sperm DNA prepared from the mixed stain was not contaminated by the female DNA and that a genotype was the same as that of the male blood DNA. Fifty samples were conducted and none of them showed female alleles.

The result of pMCT118 locus Amp—FLP of DNA extracted from 1μl saliva has been got.

Using the method discribed above, the PCR have been conducted with DNA prepared from blood, semen, and different organs of the same person. The results are the same.

DNA from a sample, even the quantity varied more than 100 times(1ng to 100ng)or have different degrade level, can all get the same results. The sample quantity as little as 1ng DNA, 1μl blood, 0.1μl semen or semen stain, single hair root and 1μl saliva can all get PCR results. In a study of 20 blood samples, 1μl blood was analysed and all get good results. If use 0.1μl blood, 16 of 20 samples were positive in a percentile of 80%.

DISCUSSION

The pMCT118 locus is a VNTR with a core sequence as GNNGTCCC. The analysis of unrelated 98 Chinese shows that pMCT118 locus has 22 alleles ranging from 340 to 780 bp compared to the results of Kasai (1989)with 21 alleles ranging from 387 to 723bp when studying of 67 Japanese. There is obvious difference between the two results. There are also differences on the alleles distribution frequency of the two studies. And some results infer the repeat unit of the alleles is an 8 base sequence(will be reported elsewhere). So the Amp—FLP result of this locus is different in different race. In 200 samples we have detected, two samples show three bands. The reason of that would be studied further.

About 60% of the 98 studied samples show one or two extra bands, some show three or four. These extra bands are stable. They do not change according to the change of DNA sample quantity. The Amp—FLP of different organs from one individual also show stability. They seemed to be individual specific. But they can not be inherited and do not interfere the analysis. In addition, samples of some individuals show a weak band on allele except ordinary bands. These may be caused by the sensitive detect method. When we use 3% agarose gel electrophoresis and ethidium bromide fluorescence to detect, the weak band can not be seen. In our analysis, we overlooked this band. If the primer concentrition was raised to 1μM, the extra bands are

likely to exist according to the sample DNA quantity and the bands were visible by agarose electrophoresis detection.

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