The Study of apoB Locus Apm—FLP and Its Application in Forensic Sciences

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INTRODUTION

The restriction fragment length polymorphism (RELP) analysis needs a relatively large quantity of high molecular DNA, which is hard to meet in actual cases. In 1985, Saiki et al reported DNA amplification in vitro, which provided a new method to solve the problem. The technique used polymerase chain reaction (PCR) to amplify specific template DNA sequence to more than 10^6 times (Saiki 1988). This method is sensitive and suitable to the sample with minute quantity or degraded DNA.

ApoB gene, which located on the chromosome 2 of human genome, is a marker to show the probability to suffer cardiovscular diseases. In 3' end of the gene has a VNTR locus which contains an A—T rich core sequence. Using special flanked primers, the locus can be amplified. Based on the method reported by Boerwinkle (1989), we have studied blood, blood stain, semen, semen stain, mixed stain, tissue, hair and degraded DNA with PCR. Reliable result has been got.

MATERIALS AND METHOD

PCR was carried out in 100μl system. Each cycle includes denaturing at 95°C for 1 min, annealling and extension at 58°C for 6 min. Thirty five cycles as a PCR.

The results were detected with mini vertical PEAG and silver stain.

The composition of the gel is as follows:

30% acrylamide	810μl
20% bis	375μl
10x TBE	500μl
DDW	3285μl

Mix and then add 3. 5μl TEMED and 35μl 10% $(NH_4)_2S_2O_8$, gel for 1 hour. Pre—electrophoresis for 10 min and then the sample was loaded. Electrophoresis was carried out under 200V for 45 min. Silver staining was carried out as follows: fix with 10% ethanol and 5% acetic acid for 15 min and stain with 12 mM $AgNO_3$, for 30 min, 0. 25M Na_2CO_3, 0. 04% HCHO stain for 10 min, stopped with 10% acetic acid. Gel was dried with drier.

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RESULTS

We have investigated the apoB locus Amp—FLP of 100 unrelated individuals in Beijing area. Eleven alleles have been determined. The fragments range from 600—1000bp. Gene frequences are 0. 5—52. 5% with a heterozygosity of 70%.

ApoB Amp — FLP of four families each contains 3 persons have been analysed. With family 3 as example:

The mother, father and the son each show two bands. The two bands of the son match either the mother or the father, which is consistant with Mendelian Law.

Compare the Amp—FLP get from semen stain with that from blood, the same results were got.

The apoB Amp—FLPs of the sperm DNA prepared from mixed stain were got and compared with that of the male and female blood DNA. The Amp—FLP of the sperm DNA prepared form mixed stain is the same as that of the male and different from that of the female.

The Amp—FLP of different organs show the same bands.

1μl blood, 0. 1μl semen, 1 ng DNA and single hair root can all be detected respectively with the method described above.

We have used this time—saving technique as a prThe unexcluded sample cann' t be used to conduct DNA fingerprinting. The identification rate has been raised.

DISCCUSION

Uniformity study of different nucleate cells DNA (include kinds of tissue, blood, blood stain, semen, semen stain, hair and so on) has been done. The results show that DNA from different nucleate cells of one individual gives the same form Amp — FLP pattern. Even to the DNA easily degraded organs such as liver, spleen, kidney, which usually get only partial fingerprints, can also get good amplified fragment same to other tissues. The sameness is the base for the application of this technique. We repeat amplification of some sample DNA which show sameness. In apoB locus Amp—FLP, some samples show extra bands. The reason need farther discussion.

Blood or blood stain separated from the substrate have been amplified directly and good results have been got. And the method was proved to be a simple and time—saving method . PCR, as a simple, time—saving preexperinment of DNA fingerprint, have rasied the identification rate of DNA fingerprint. A series of VNTR locus Amp—FLP should be an even more prowerful tool in forensic sciences.

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