DNA Fingerprinting in Paternity Testing in Lithuania

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The classical methods of personal identification which up till now have been used in forensic and criminologic practice, were mainly based on biochemical and immunobiological practice (i.e. on blood group ABO system, Rh, HNSs, or on serum tests, such as P, Gm, Lewis Hp, or enzymatic system of erythrocytes), as well as dactyloscopic analysis of papillary line comparison.

On the basis of the observations by Vassart, G. et al. (1987) and Dzhincharadze, A. et al. (1987), proving that wild-type M13 bacteriophage DNA could serve as a molecular probe for detection of hypervariable minisatellite regions in human genome, the DNA fingerprinting technique for disputable paternity testing in forensic medicine was applied. The method used included the DNA extraction from the mother, father in dispute and child, digestion with Hinf I (preferentially) or Bsu RI restriction endonuclease, electrophoresisthrough agarose gel, and Southern blot-hydridization to ^{32}P-labeled M13 probe. The latter is prepared from single-stranded M13 DNA by partial synthesizing of the second strand from the forward sequencing primer in the DNA polymerase I Klenow fragment - catalyzed reaction. Since 1990, the DNA fingerprinting method above was approved by Lithuanian authorities for the use in paternity testing. Since the fall of 1990, 28 cases of disputed paternity using M13 probe were studied. The following regularities were observed in the fingerprints obtained: in average, 20 well identifiable DNA fragments were scored for every individual, whereas 6 fragments were shared by all the individuals in the case. For nearly 20% of the cases studied, the number of co-migrating identifiable fragments made up to 10-13. The ambiguity obtained was eliminated by parallel use of restriction endonuclease Bsu RI. Two cases of paternity exclusion were obtained, whereas in one case the exclusion was obtained by DNA fingerprinting only, where no conventional biochemical or blood group marker system was effective

The DNA fingerprint was made using a biotin-labeled M13 DNA fragment. For such a purpose, two primers have been synthesized - TCCTATTGGGCTTGCTATCC and TTTCGGTCATAGCCCCCTTA - surrounding the hybridizing sequence in the protein III gene. The M13 DNA fragment was then labeled with biotin using the amplification reaction by Bellany, R. et al. (1990). For development of the membrane, streptavidin-alkaline phosphatase conjugate and BEIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/Nitro Blue Tetrazolium) was used.

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REFERENCES

Vassart G et al. (1987). A sequence in M13 phage detects hypervariable minisatellites in human and animal DNA. Science 235:683-684
Dzhincharadze A et al. t. 1987. Genomnaja "daktiloskopija". Dokl. AN SSSR 295:230-233
Bellamy R et al.(1990) Better fingerprinting with PCR. Trends in genetics 6:32