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Detection of short tandem repeat polymorphisms from human nail samples by direct polymerase chain reaction

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Abstract

Human nail is often used for DNA identification as evidence material in forensic medicine. Hard tissues such as hair, bone and tooth can be preserved for longer periods than soft tissues such as blood, and can be cleansed easily to remove contaminating substances. Due to these characteristics, they are one of the useful materials preferentially chosen for DNA analysis especially in large-scale disasters. In the present study, we succeeded to analyze multiple short tandem repeat (STR) polymorphisms by performing PCR directly on nail samples without prior extraction of DNA. Human nail sample were collected from 15 volunteers. For each subject, nails were cleansed in neutral detergent and 2-3 mg of nail was cut and collected. Each sample was subjected to PCR directly and after pretreatment. In the direct PCR method, the sample was placed in a tube and analyzed by PCR directly. In the pretreatment PCR method, the nail sample was pretreated with Direct PCR Lysis Reagent (DPLR; Viagen Biotech), proteinase K or alkaline solution (50 mM NaOH and 1M Tris-HCl) and then centrifuged, and 2 µl of supernatant was analyzed by PCR. STR polymorphism was detected using the SilverSTR® III Multiplex Kit. Three loci; D16S539, D7S820 and D13S317 were amplified and PCR products were analyzed by urea gel electrophoresis. In addition, the Investigator ESSplex Plus Kit (QIAGEN) was used for PCR amplification of 16 STR polymorphisms comprising D10S1248, D22S1045, D12S391, D8S1179, D2S1338, D2S441, D18S51, FGA, TH01, D3S1358, vWA, D2S11, D16S539, D1S1656, D19S433 and SE33, as well as amelogenin (X-Y gender discrimination marker). ABI PRISMTM 310 Genetic Analyzer (Applied Biosystems) was used to analyze the STR polymorphisms. As PCR enzyme, KOD FX Neo (Toyobo) which is effective for amplification from crude samples was used. Compared with the DNA of each subject obtained from blood sample, STR polymorphisms were correctly detected from the nail samples by both direct and DPLR- or alkali-pretreated PCR method. Using the SilverSTR® III Multiplex Kit, the DPLR- and alkali -pretreated methods allowed the detection of not only the main band but also extra bands, which may facilitate typing. Bands obtained from direct PCR of nail sample could be typed more easily. Moreover, when the nail samples remaining in the tube after PCR were collected, washed, and subjected to PCR again, DNA was successfully amplified. Therefore nail sample is considered to be ideal for DNA identification in forensic medicine. Using the Investigator ESSplex Plus Kit, 16 types of STR polymorphisms were correctly detected. For typing STR loci using the SilverSTR® III Multiplex Kit, no influence was observed with both direct and pretreated nail samples used for PCR amplification. The present method is potentially applicable to forensic medicine.

Biography

Jian Tie has completed his M.D. at the age of 26 years from China Medical University and postdoctoral studies from China Medical University and Nihon University School of Medicine. He is the Associated Professor in Nihon University School of Medicine. He has published more than 50 papers in reputed journals.