USAGE OF THE POWERPLEX® S5 MINI-STR SYSTEM IN LCN DNA TESTING

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INTRODUCTION: The primary value of DNA typing has been significantly increased in the last fifteen years due to introduction of short tandem repeat (STR) loci in routine paternity testing, as well as in forensic cases and mass disaster human identification. Nevertheless, ability to obtain DNA profiles from very small amounts of sample still presents certain types of challenges in forensic casework. As it is already known, low-copy number (LCN) DNA testing typically refers to examination of less than 100 pg of input DNA. In this analysis the number of PCR cycles is often increased to improve the amplification yield. Nevertheless, application of LCN results should be approached with caution due to the possibilities of allele dropout, allele drop-in, and increased risks of collection-based and laboratory based contamination. Therefore, sometimes, additional DNA analysis of these samples is required to be performed with an available miniSTR system.

CASE DESCRIPTION: Two single hairs, which were provided to our laboratory, were microscopically observed. Hair root, with extremely poor presence of epithelial cells, was detected on both of them. In addition, both samples were briefly washed in absolute ethanol and extracted by usage of the Qiagen Dnaeasy™ Tissue Kit. DNA concentration was determined using Quantifiler Human DNA Quantification Kit as described previously. The reaction was carried out in AB 7300 Real-Time PCR System according to the manufacturer's recommendations. Measured mean quantity was 1.40 × 10−2 ng/μL for the first and 3.20 × 10−2 ng/μL for the second sample. Initially, the PowerPlex®16 kit has been used to amplify 15 STR loci. The total volume of each reaction was 10μL. The PCR amplification was carried out in PE Gene Amp PCR System Thermal Cycler. The number of cycles was increased to 32 and elongation time extended to 90 seconds. The very same protocol we are already successfully using in analysis of degraded bone samples from WWII. But this time, we have obtained partial DNA profiles with few possible allele dropouts, allele drop-in situation. Therefore, amplification step using PowerPlex S5® system was additionally performed. The total volume of each reaction was 10μL. As the result of that, full and clean profiles (over all 5 loci) were obtained for both samples. That helped us in final distinction of real alleles on the one, and stochastic fluctuation on the other side over the analyzed loci.