B44 The Study of Using Next Generation Sequencing (NGS) Technologies to Analyze Mixed DNA Patterns

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Learning Overview: The goal of this presentation is to analyze the DNA mixture results obtained through capillary electrophoresis and NGS.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by informing attendees of the DNA mixture results using NGS.

In forensic science laboratories, capillary electrophoresis is currently employed to perform routine analysis of Short Tandem Repeat (STR) fragments and DNA sequences. However, the technique has limited discrimination for mixed forensic evidences. The DNA quantity of most forensic evidence is extremely small, and the mixing ratio is unknown. Therefore, it is difficult to identify trace components of DNA in mixed samples by capillary electrophoresis. NGS can overcome the problem of excessively large proportions of DNA in mixed samples by increasing the sequencing depth, and can analyze Single Nucleotide Polymorphism (SNP) or mitochondrial DNA sequence to assist in the study of DNA composition in mixed forensic evidence.

In this study, a total of 28 forensic cases were collected and analyzed through both capillary electrophoresis and NGS, including human STR, human mitochondrial HV1 and HV2 sequence, and animal mitochondrial 12S rRNA, 16S rRNA, and Cyt b sequences. A comparison of the results obtained through two methods was performed to validate the accuracy and reliability of NGS technologies. Moreover, NGS technologies can further aid to identify the sources of two-person mixed samples. Among the 15 DNA mixed samples analyzed were STR and Y-chromosomal Short Tandem Repeat (Y-STR) DNA patterns using the above two methods, and four mixed samples were examined of human mtDNA HV1 and HV2 sequence. In addition, nine animal cases were analyzed, which included mtDNA 12S rRNA, 16S rRNA, and Cyt b sequences.

The results of this research are respectively stated as follows. Human STR: the 15 cases were analyzed by the above two methods. The 15 cases can be correctly detected by capillary electrophoresis; however, 13 cases can be correctly detected by NGS. The detection rate of NGS was lower than traditional capillary electrophoresis due to the low DNA quantity of two cases. The detection rate of STR DNA of the remaining 13 cases of NGS was higher than traditional capillary electrophoresis. The study found that increasing the sequencing depth or supplementing with SNP sites can assist in the judgment of mixed samples. Human mtDNA: NGS analysis of forensic mixed evidences of mitochondrial DNA can break through the dilemma that capillary electrophoresis can only study the existence of two kinds of bases in mixed samples, and it is not easy to quantify the ratio. Animal species identification: Capillary electrophoresis only detected five cases, and the remaining four cases were not detected. The NGS technologies are more sensitive than capillary electrophoresis. Except for one case that was not detected due to severe decomposition, the other eight cases were all detected mitochondrial DNA sequences.

In conclusion, the NGS method still needs to invest a lot of manpower, material resources, and time, combined with molecular biology, forensic sciences, and statistical analysis to effectively use this huge amount of information to help identify forensic evidences. It is hoped that in the future we can continue to refine NGS technologies and achieve a forensic energy that could not be achieved in the past.

NGS, DNA Mixtures, DNA Analysis

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