ANALYZING DNA MIXTURES USING PCR/CE COMPARED TO NEXT GENERATION SEQUENCING

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NGS (Next Generation Sequencing) or MPS (Mass Parallel Sequencing) is a newer technology that can simultaneously sequence multiple genomic regions. This technology has been widely used in many scientific areas and recent endeavors have begun testing it with forensic samples. Current DNA analysis methods in forensic science use a combination of PCR (Polymerase Chain Reactions) and CE (Capillary Electrophoresis) to determine DNA identity. Variable genomic regions known as STRs (Short Tandem Repeats) are amplified using PCR and these variable PCR fragments sizes are determined using CE. The allelic variation in the PCR fragment sizes are used to identify unique individuals. One advantage of NGS technology compared to traditional methods is that it permits the sequencing of the STR region which enables the ability to distinguish between STR alleles that are the same size but have a different sequence. Furthermore, NGS technology has been shown to be more sensitive allowing for the ability to see alleles that traditional methods will have missed. These advantages of NGS technology compared to PCR/CE should allow for better interpretation of DNA mixtures. Complex DNA mixtures are some of the most difficult samples to get complete DNA profiles, due to shared alleles and potential drop out. Our study attempts to evaluate whether the potential advantages of NGS can enhance mixture interpretation.

DNA mixtures were prepared using purified DNA that was extracted from saliva of known individuals. The 2-, 3- and 4-person mixtures were prepared in the following rations; 1:1, 1:3, 1:10, 1:1:1, 1:1:3, 1:1:10, 1:1:1:1, 1:1:1:3 and 1:1:1:10. For the traditional DNA analysis the mixtures were amplified using Identifiler® Plus (Applied Biosystems) and GlobalFiler™ (Applied Biosystems) and then run on a 3130 XL Genetic Analyzer (Applied Biosystems) capillary electrophoresis and analyzed with the GeneMapper ID-X software. In parallel, the mixtures were prepared using the PowerSeq NGS library preparation kit (Promega), sequenced on a MiSeq (Illumina) and sequences analyzed using the ExactID software (Battelle).

The results show an increase in total alleles due to sequence variation of shared alleles and overall sensitivity of the NGS instruments compared to the traditional methods. Furthermore, the increase in allele number enhances the ability to determine the number of contributors at some loci. Overall NGS technology improves the ability to interpret mixtures.