# USE OF MASSIVELY PARALLEL SEQUENCING (MPS) TO ASSIST WITH DECONVOLUTION OF STR MIXTURE PROFILES

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Forensic DNA profiling by PCR of short tandem repeats (STRs) is considered a robust and reliable method of human identification. However, difficulties can arise in interpretation when the starting DNA template is extremely low or if the profile is mixed, particularly in cases where alleles are shared by multiple contributors.

Current methods of STR analysis only provide information regarding the STR fragment length. However, MPS provides further information to assist with deconvolution of mixture profiles. Repeats contained within each MPS read are counted, and resulting counts are used to calculate a mixture ratio, enabling parsing of alleles to each contributor. Shared alleles can also be deconvoluted. STR fragments may contain single nucleotide polymorphisms (SNPs), or different sequence configurations that would not be detected with traditional capillary electrophoresis (CE) genotyping analysis.

We propose a method to use MPS methods to sequence PCR product generated using a commercial STR kit designed for traditional CE analysis. While STR kits designed specifically for MPS are in development, the ability to sequence previously amplified samples could provide further insight into profiles that were difficult to interpret with STR length information alone.

Single source and two-person mixture samples of various mixture ratios were amplified with the GlobalFiler® PCR Amplification Kit. All samples were run on the 3500xL Genetic Analyzer to obtain reference data. The same PCR product was then prepared for MPS using the Nextera® XT kit followed by deep sequencing on the Illumina® MiSeq®. Data were then analyzed using STRait Razor software.

Preliminary results indicate that the Nextera® XT step successfully removes fluorescent tags incorporated into PCR products during amplification with the GlobalFiler® kit. This is essential for successful sequencing on the MiSeq®, since this method involves fluorescence detection. Furthermore, analysis of STR sequences in mixed DNA profiles has revealed some different sequence motifs for shared alleles that are kept in contributor-defined ratios. This has allowed some shared alleles that were initially masked in CE data to be assigned to the major or minor contributor.