

## USE OF MASSIVELY PARALLEL SEQUENCE ANALYSIS TO FURTHER CHARACTERIZE A UNIQUE SINGLE GENETIC INCONSISTENCY OBSERVED IN A PARENTAGE TEST AT THE vWA LOCUS

Gary M. Stuhlmiller<sup>1</sup>, Sarah Cavanaugh<sup>2</sup>, Robert Bever<sup>2</sup>, George C. Maha<sup>1</sup>

<sup>1</sup>Laboratory Corporation of America Holdings

<sup>2</sup>Bode Cellmark Forensics

A standard paternity trio of mother, child and alleged father was amplified with the PowerPlex<sup>®</sup> 16 kit and PowerPlex<sup>®</sup> CS7. The amplicons were separated using the ABI Genetic Analyzer 3730xl and interpreted using GeneMapper<sup>®</sup> v3.2.1. The results indicated that the alleged father shared obligate paternal alleles at all loci except one. A single genetic inconsistency was observed at vWA: the mother's genotype is 16, 18, the alleged father's genotype is 16, 16, and the child's genotype is 16, 16.2. The 16.2 created the single inconsistency. These results were confirmed and the total number of loci examined in the case extended to 27 loci using PowerPlex<sup>®</sup> ESI and PowerPlex<sup>®</sup> ESX, ABI Genetic Analyzer 3130xl and GeneMapper<sup>®</sup> v3.2.1. The DNA from the paternity trio was further analyzed by massively parallel sequence analysis using the Promega PowerSeq<sup>™</sup> Auto System STR amplification kit and the Illumina MiSeq<sup>®</sup> System genome analyzer. Sequence analysis determined that at vWA both parents have 16 alleles with the repeat motif of TCTA [TCTG]<sub>4</sub> [TCTA]<sub>11</sub> TCCA TCTA, and the mother's 18 allele repeat motif is TCTA [TCTG]<sub>4</sub> [TCTA]<sub>13</sub> TCCA TCTA. The child's genotype consists of one 16 allele with the same motif and a 16.2 microvariant allele with the sequence motif of TCTA [TCTG]<sub>4</sub> [TCTA]<sub>4</sub> TA [TCTA]<sub>7</sub> TCCA TCTA. This analysis indicates a meiotic mutation event within one parental allele: either a 2 base pair insertion mutation within one parent's 16 allele or a 6 base pair deletion within the maternal 18 allele. vWA has an observed paternal meiosis mutation rate of 0.17% and a maternal meiosis mutation rate of 0.03% (American Association of Blood Banks 2003 Annual Report); however, no further determination regarding parental phasing could be deduced from the sequence information. To date, this vWA variant has not been annotated in the NIST STRbase vWA fact sheet nor reported in current literature detailing STR variants identified through massively parallel sequencing. This case study provides an example of how massively parallel sequencing technology can be used to further characterize single genetic inconsistencies that are observed in parentage and kinship casework.