Next Generation Sequencing of Forensic DNA Loci Using 454 Life Sciences Technology

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Technology continues to propel the evolution of forensic DNA analysis and its applications. From simple PCR methods to high throughput instrumentation and robotics, the forensic community has embraced innovative molecular tools for more than two decades now. While not as far reaching as fragment length analysis, DNA sequencing of forensically-relevant loci has played a significant and successful role in solving criminal cases, and cases of human remains identification. The technology of choice began with Sanger dideoxy-terminator-based sequencing in the 1980's, and while the instruments used to separate the products of a sequencing reaction have improved over time, the chemistry has remained relatively unchanged. However, we are currently transitioning from the Sanger era of DNA sequencing, and have embarked on the journey of cyclic-array, hybridization-based, nanopore and single molecule sequencing, all commonly referred to as Next Generation Sequencing (NGS). Applications of NGS are having an immediate impact on medical genetics, biodiversity and basic molecular research. Given the imminent prospects of a $1000 human genome sequence, the potential impacts are far reaching and will shape personalized medicine in the near future. So, how will NGS be applied in forensics? The answer to this question is complex, and will require careful thought and planning. For example, if we assume that STR analysis will remain the core forensic approach in the foreseeable future, how does NGS fit into this model? The development of an amplicon sequencing method using the 454 Life Sciences NGS technology may be the answer. Experiments were run with the 454 Genome Sequencer FLX instrument to evaluate the system's ability to generate reliable sequence data from forensic STR and Y STR loci, as well as mtDNA control region sequences, all from the same sequencing reaction. The ability to identify individual loci using primer binding sequences, and identify "individuals" using embedded sequence-based barcodes, allows for the analysis of dozens of loci and hundreds of individuals in a single sequencing run. In addition, the size of the amplicons being sequenced is not limited by fragment length requirements, allowing for development of systems with enhanced sensitivity. Therefore, the advantages of an NGS approach include greatly increased discrimination potential and a low-cost solution to expanded profile development; well below the cost for conventional STR analysis, and far below current DNA sequencing approaches. This presentation will focus on our assessment of the 454 system to 1) generate reliable sequence data from a cocktail of STR, Y STR and mtDNA loci at different levels of sensitivity, 2) resolve mixtures of individuals with different barcodes, and 3) resolve conventional mixtures.