MEASUREMENTS OF SENSITIVITY REGARDING THE POWERPLEX™ 1.1, 2.1, AND COFILER™ KITS USING THE HITACHI FMBIO® AND ABI 310 FORMATS

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Performing sensitivity studies is a routine part of any validation for the use of new DNA typing formats and new loci. The standard protocol for measuring sensitivity involves the amplification of incrementally smaller quantities of input DNA and subsequently determining the minimum amount of DNA placed into the reaction mix from which the analyst can obtain a complete DNA profile. During our validation studies with the PowerPlex™ 2.1 kit, the COfiler™ kit and the ABI 310, questions arose as to just what was our post-PCR yield of the STR products and what was the limit of sensitivity of the two instruments used in our laboratory, the Hitachi FMBIO®-100 and the ABI 310. These are two independent questions that needed to be addressed separately. If there are differences between the sensitivity of the two instruments, it could influence our choices as to which format for analyzing STR products we should utilize when there is very little amplified product available.

Previous sensitivity studies have demonstrated that 3 out of 4 DNA samples typed for the PowerPlex™ 1.1 loci provided a complete (all eight loci clearly visible) profile at only 50 plcograms of input DNA when analyzed using the Hitachi FMBIO®-100. As part of our validation studies for the PowerPlex™ 2.1 kit, we performed a sensitivity study. We determined that we could obtain a complete profile (all nine loci clearly visible) at approximately 150 pg of input DNA, but not less than that. We also performed a sensitivity study using the COfiler™ kit and analyzed the PCR products using the ABI 310. Likewise, a complete profile was obtained from the COfiler™ kit (all six loci with allele peak heights above 150 rfus) at 150 pg of input DNA, but not less than that. Of the six STR loci amplified in the COfiler™ kit, five of them are included in the PowerPlex™ 1.1 kit (CSF1pO, TPOX, THO1, D7S820, and D16S539) and three of them are included in the PowerPlex™ 2.1 loci (THO1, TPOX, and vWA). Given the apparent differences in sensitivity of detection between the PowerPlex™ 1.1 loci and the COfiler™ loci (50 pg of input DNA vs. 150 pg of input DNA), we began a series of experiments to determine whether the differences were due to the yields of STR products or differences in the limits of detection by the instruments used.