VALIDATION OF DIRECT AMPLIFICATION OF STRs USING POWERPLEX® 18D AND IDENTIFILER® DIRECT SYSTEMS

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Direct amplification is a process that minimizes sample manipulation, also reducing potential handling errors, by eliminating the extraction and quantitation of DNA. Currently, there are two commercially available direct amplification kits for processing forensic type samples. Both kits offer the core CODIS loci plus amelogenin, and the loci D2S1338, D19S433. The PowerPlex® 18D kit offers two additional loci, Penta E and Penta D. To determine the robustness and reliability of the PowerPlex® 18D and Identifier® direct amplification systems, buccal cell samples from 400 anonymous donors were collected and deposited onto FTA paper using the Whatman EasiCollect™ device (Florham Park, NJ) and analyzed under analytical conditions defined to achieve a high first-pass success rate. Working interpretation guidelines were established to create an unbiased typing of STR results. These guidelines were used for effectively interpreting low level and saturated DNA profiles. First-pass success rates, defined as complete profile typing for PowerPlex® 18D with a 5 sec injection --- 96.25%, Identifiler® Direct with a 10 sec injection --- 96.25%, Identifiler® Direct with a 5 sec injection --- 95%). Profiles that could not be typed are not a result of the kit performance but are a result of the inherent variation in the amount of DNA obtained with the collection device. Weak samples can be reanalyzed by either re-injecting for a longer time or by re-amplification with an additional PCR cycle. Overloaded samples can be reanalyzed by re-injecting for a shorter time or by re-amplification with one less cycle. Peak height ratios (PHR) for all samples were determined across the spectrum of peak heights. All called typing results were consistent under the prescribed conditions, different injection times, and 26-28 PCR cycles for both chemistries. Both kits were well balanced with peaks >2000 RFUs while remaining well balanced in samples with one or more peaks with heights <100RFU. A sloping PowerPlex® 18D ILS was used as an indicator of a poor injection, whereas for Identifiler® Direct an ILS that leveled off was used as an indicator. Fragment Ratio Analysis (FRA) for PowerPlex® 18D ILS, determined by dividing the peak height of the smallest ILS fragment by the peak height of the largest ILS fragment, provided additional quality assessment data. For PowerPlex® 18D a FRA minimum threshold was set at 0.72 (3 standard deviations higher than the average). 39/400 (9.75%) samples demonstrated a sloping ILS, and 4 of these samples contained a compromised profile (samples with allele drop-out). Often reinjection effectively overcame the sloping ILS phenomenon. For Identifiler® Direct the FRA minimum threshold was set at 0.65, with (45/400) samples experiencing a leveling off of the ILS at 10 sec and (98/400 at 5 sec); however, of these samples, none affected the overall success rate. The results of this study demonstrate that PowerPlex® 18D and Identifiler® Direct are both robust kits for direct amplification. Reliable results were obtained for profiles displaying a wide range of peak heights and PHR were extremely well-balanced even for low levels of DNA. The interpretation guidelines used for this study can form a basis for internal validation studies by databasing laboratories.