IMPROVEMENTS TO A HIGH THROUGHPUT STRATEGY FOR STR TYPING OF DNA FROM FINGERPRINTS AND TRACE EVIDENCE

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STR DNA profiles from fingerprints and other trace evidence can be obtained in an automated, high throughput manner. Low copy number DNA profiling, however, creates many stochastic effects that are difficult to resolve. Recent improvements in procedures employed at the Office of the Chief Medical Examiner of the City of New York regarding sample collection, DNA purification and quantification, amplification of the loci, and interpretation of STR analysis will be presented.

Experiments with fingerprints from unwashed hands left on a variety of surfaces revealed that the type of swabbing solution and the swab material as well as the swab shape affect DNA recovery. To better understand these results, the absorbency of the swab materials was compared. By quantifying the amount of DNA recovered when a known amount of DNA was applied to these materials, the ability of the swab materials to release captured DNA was also measured. In addition, the efficacy of using one versus two swabs per sample was tested.

Samples were extracted with a simple detergent method, which minimized sample manipulation and subsequent sample loss, while maintaining prospects for automation. Specifically, samples were digested with 0.01% SDS with Proteinase K at 56°C for two hours, further incubated at 100°C for 8 minutes, and purified and concentrated with a microcon 100. The addition of 1 ng of poly A RNA to the sample solution applied to the microcon, significantly enhanced sample recovery. Samples were then quantitated with an ALU based PCR method with an endpoint determination using a fluorescent plate reader. Based on suggestions by Dr. Eric Buel and Dr. Jan Nicols, 1 pg of DNA could be detected, although the dynamic range of the assay was reduced.

Using 25 pg or less of purified DNA, the PowerPlex® 16 STR typing kit was optimized for sensitivity and specificity. Parameters for testing included adjusting annealing and extension times and cycle numbers, titrating the dNTP, $MgCl_2$, and enzyme concentrations, and the addition of PCR enhancers. Results indicate that amplification for 35 cycles with the recommended kit components produced the most accurate allelic calls with only on average one spurious allele, or drop-in. Since drop-ins often appeared in the stutter position, several commercially available Taq enzymes and buffers with supposedly increased processivity were compared. Thus far, none were found to produce on average less than one drop-in per sample with the same degree of sensitivity. The rate of stutter and the most common stutter positions for low copy number samples, amplified under the aforementioned conditions, will be discussed.

No significant dye artifacts were apparent with amplified samples from the PowerPlex® 16 kit with a 2 µL sample injection at 3 kV for 20 seconds and a threshold of 75 RFUs. Increased allelic calling, at all cycle numbers, was observed with a 4 µL sample injection at 7kV for 30 seconds. However, the increases included more drop-ins. Since the drop-ins, even at the default injection conditions, were often in the stutter positions, a macro, produced by Promega, was used that attempted to account for increased stutter. A general filter of 10% was also

applied.

Presently, we are resolving sample interpretation issues. These include the parameters for stutter editing, peak height ratios and thresholds, and the consequence of drop-ins. Based on our studies, an additional stutter filter for low copy analysis with PowerPlex® 16 will be created if necessary. To produce reliable profiles, samples will be amplified twice, and alleles will only be called if they occur in both amplifications. Moreover, preliminary testing with automated allelic calling for low copy number templates will be introduced.