A162 Improved Genotyping Performance of Complex Multiplexes on DNA Mixtures

Chien-Wei Chang, PhD, Life Technologies, 850 Lincoln Centre Drive, MS 404-1, Foster City, CA 94404; Robert Lagace, BS, Applied Biosystems, 850 Lincoln Centre Drive, Mail Stop # 404-3, Foster City, CA 94404; Dennis Wang, PhD, and Julio J. Mulero, PhD, Applied Biosystems, 850 Lincoln Centre Drive, MS 404-1, Foster City, CA 94404; Lisa M. Calandro, MPH, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404; and Lori Hennessy, PhD, Life Technologies, 850 Lincoln Centre Drive, MS404-1, Foster City, CA 94404*

After attending this presentation, attendees will understand the performance of complex STR multiplexes in DNA mixtures and the parameters influencing mixture interpretation.

This presentation will impact the forensic science community by providing information on factors that affect the performance of different STR multiplex systems in compromised samples and solutions to improve the recovery of information from complex multiplexes.

Evidence submitted for DNA analysis can be recovered from a variety of biological samples including blood, saliva, or semen stains on different substrates, body surface swabs, hair, bones, and finger nail scrapings. Forensic analysts seek technologies to maximize the quality of results obtained and increase the success rate of obtaining a DNA profile from these types of samples. Multiplex short tandem repeat (STR) assays simplify genotyping processes and preserve sample by eliminating the need for multiple amplifications and/or electrophoretic injections. Observations have previously been presented that there is varying performance with different STR multiplexes. Therefore, a study was undertaken to investigate the effect of multiplex complexity on genotyping performance in mixtures by comparing STR assays of different complexity (e.g., 10-16 markers/reaction) using simulated inhibited samples at various mixture ratios (1:0, 1:1, 1:5, and 1:7). Amplifications were performed with a total DNA input of 1.5 ng in either 1X TE, 15µM hematin, or 6ng/µl humic acid concentrations, chosen to provide a moderate level of inhibition and generate a pattern characteristic of inhibited profiles. Allelic drop-out of the minor alleles in prepared inhibited samples was observed in all of the assays at each mixture ratio; but when compared to the 10-plex assay, the 16-plex assay detected an equivalent or greater number of alleles from the minor contributors in all the mixture samples. Comparison of the intra-locus balance values of the major and minor contributors were similar for all assays. Some loci exhibited greater sensitivity to PCR inhibition in a larger multiplex so modifications were made to the PCR buffer and thermal cycling parameters in an effort to improve performance of these loci. The improvements in PCR buffer and cycling conditions enabled recovery of all alleles in the inhibited mixtures amplified using the 16- plex assay and eliminated the ski slope effect seen with the other kits tested. This study illustrates how large multiplexes can be optimized successfully for use on challenging casework samples.

Multiplex, STR, Mixture

 ** Presenting Author*