B2 Developmental Validation of Reduced-Size STR Miniplex Primer Sets

Kerry L. Opel, MA, BS, and Bruce R. McCord, PhD, Florida International University, Department of Chemistry and Biochemistry, 11200 SW 8th Street, Miami, FL 33199; Denise T. Chung, PhD, Center for Neurological Diseases, Brigham & Women's Hospital, Harvard Institutes of Medicine, 77 Avenue Louis Pasteur, Room 785, Boston, MA 02115-5817; Jiri Drabek, PhD, Palacky University, Department of Biochemistry, Slechtitelu 11 783 71 , Olomouc, CZ-783 71, Czech Republic; and John M. Butler, PhD, National Institute of Standards and Technology, 100 Bureau Drive, Mail Stop 8311, Gaithersburg, MD 20899-8311*

After attending this presentation the attendee will be familiar with the results of the developmental validation of the reduced size STR Miniplex Primer sets.

This presentation will impact the forensic community and/or humanity by providing information on the robustness and reliability of the reduced size STR Miniplex kits for forensic casework.

In heavily degraded DNA, poor amplification of the larger sized amplicons (300-500 base pairs) in the standard multiplex typing kits is common. Due to sample decomposition, the DNA template can become highly fragmented, and the yield of template fragments having a complete target sequence is reduced. Thus, a "decay curve" is seen, in which the larger loci have much lower intensity, and often fall below the detection threshold.

New primer sets, known as Miniplexes, have been designed to place the target sequence much closer to the repeat region. This new primer set produces smaller amplicons, and increases the probability of obtaining a usable profile from degraded DNA. These kits were designed for use with difficult (degraded and compromised) samples. These primers were com- bined to produce five kits of "Miniplexes" of 3-4 loci each (for use with mul- ticolor detection systems). Two of the kits, Miniplex 1 and Miniplex 3 were combined to create a six loci multiplex kit known as "Big Mini." The kits cover 12 of the 13 CODIS loci in the 4 dye detection system, plus three non- CODIS loci. These kits produce a reduction in amplicon size for the loci used in the range of 33-191 base pairs when compared to a commercial kit. The size of the alleles in the Miniplex kits range from 60-284 base pairs.

Developmental validation studies of the Miniplex primer sets had been completed in accordance with the Scientific Working Group on DNA Analysis Methods (SWGDAM) Guidelines, and the validation studies on Miniplex primer sets 2, 4, and Big Mini will be presented. Because the Miniplexes were designed for the analysis of degraded and compromised samples and since low quantities of DNA template are usually recovered in these situations, most of the studies conducted with the Miniplexes were performed with 100 pg of DNA template per 25 µL of reaction volume (4 pg/µL) and 33 amplification cycles.

A range of tests were performed: primer concentration, extraction technique, cycle number, annealing temperature, matrix, environmental, magnesium concentration, Taq polymerase concentration, reaction volume, mixtures, and non human DNA. Each study was conducted with a range of at least 3 conditions and 3 replicates for each condition.

Using the Miniplex kits, DNA was extracted and successfully amplified from a range of matrices, including denim, leather, wood, metal, and organic matter. DNA was also extracted and successfully amplified after exposure to a range of temperatures from -20 to 50 °C, and sunlight for up to 84 days of exposure. Standard sample tests demonstrated the utility of the kits to amplify samples from blood and saliva from stains and FTA cards. The optimum primer concentrations for the Miniplexes ranged from 0.16 µM to 0.56 µM for the various loci and the optimum cycle number was determined to be 33 cycles for degraded samples with an annealing temperature of 55 degrees. Magnesium concentrations ranged from of 1.5 to 2.5 µM with an optimum of 1.5 µM, and the best Taq con- centration was 2U/25 µL. Forensic samples analyzed included blood stains, hair, and bone. The Miniplex sets were tested and found to give satis- factory results in a range of reaction volumes from 10 to 25 µL. In mix- tures, the minor component is detected in ratio of 9:1. Non-human DNA was not amplified with the Miniplex primers, with the exception of mouse DNA which amplified outside the size range of human DNA and human DNA. In general the results demonstrate the Miniplex procedure to be a robust and sensitive method for the analysis of degraded DNA.

Degraded DNA, Developmental Validation, Miniplex Reduced-Size STRS

 ** Presenting Author*