B165 Some Interesting Point Mutations and Deletions Found Through STR Allele Sequencing

Margaret C. Kline, MS, Michael D. Coble, PhD, Jill E. Appleby, BS, Richard Schoske, PhD, and John M. Butler, PhD, National Institute of Standards and Technology, 100 Bureau Drive, MS 8311, Building 227, Room B250, Gaithersburg, MD 20899-8311*

Attendees will learn a methodology for sequencing variant STR alleles will be described along with some interesting findings from samples producing allele dropout upon PCR amplification with various primer sets.

This presentation will impact the forensic community and/or humanity by enhancing the knowledge of the forensic community in regards to methodologies used to define the differences found in variant alleles.

Polymorphisms exist in the flanking regions of short tandem repeat (STR) loci that can cause allele dropout when they fall underneath PCR primer binding sites. The resulting "null alleles" are typically detected when concordance studies are performed using sets of PCR primers with different annealing positions (1,2). Some interesting deletions have been discovered in the flanking regions of D13S317 and VWA through concordance studies between miniSTR assays and commercial kits (3). In addition, several forensic laboratories have supplied samples possessing some novel variants that have been characterized. These STR typing and sequencing results will be discussed in the context of the growing number of more than 230 variant alleles reported and cataloged as part of the National Institute of Standards and Technology STRBase website: http://www.cstl.nist.gov/biotech/strbase/.

Methods and Materials: DNA sequencing primers lying outside of PCR amplification assay primer binding sites have been designed and tested for all 13 core STR loci used in the Combined DNA Index System (CODIS) as well as the D2S1338, D19S433, Penta D, and Penta E loci that are contained in commercial STR kits such as PowerPlex® 16, SGM Plus, and Identifiler. A variety of polyacrylamide gel electrophoresis conditions have been developed to separate closely spaced heterozygous alleles so that these alleles can be individually sequenced. Gel cutouts (individual alleles) are re-amplified prior to sequencing each allele. Variations in the individual alleles are determined by aligning their sequence to a reference sequence from GenBank. These alignments are assisted by the use of the software program Sequencher 4.1 (GeneCodes, Ann Arbor, MI).

Summary of Results: The novel sequencing primers developed encompass the primer binding regions of all known published primer sequences for loci included in commercial STR kits and thus enable an examination of polymorphisms giving rise to allele dropout upon PCR amplification.

Conclusions: Methodologies for DNA sequencing of STR alleles can aid in understanding the molecular basis for allele dropout due to point mutations or insertion/deletions in template DNA that disrupt PCR primer annealing. An increasing number of rare variant alleles are being discovered and information is being uncovered through DNA sequencing that can be helpful in assessing natural human variation and developing improved detection assays in the future.

References:

1. Budowle, B., *et al.* (2001) STR primer concordance study. *Forensic Sci. Int* 124: 47-54.
2. Clayton T.M., *et al.* (2004) Primer binding site mutations affecting the typing of STR loci contained within the AMPF*l*STR SGM Plus kit. *Forensic Sci.Int.* 139: 255-259.
3. Drabek, J., *et al.* (2004) Concordance study between miniplex STR assays and a commercial STR typing kit, *J. Forensic Sci.* 49(4): 859-860.

Short Tandem Repeat DNA Typing, DNA Sequencing, Variant Alleles

 ** Presenting Author*