B158 Understanding Null Alleles and STR Allele Mobility Issues Through Variant Allele Sequencing

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After attending this presentation, attendees will develop a better understanding of "null" alleles and mobility shift issues after confirming sequence variation.

This presentation will impact the forensic science community 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 mobility shifts due to sequence variation have been elucidated using sequence analysis. Additionally there can be sequence variations found within the STR repeat that do not cause mobility shifts. Some of the STR loci of interest that will be discussed are: D16S539, D8S1179, D7S820, D18S51 and D19S433. These STR typing and sequencing results will be discussed in the context of the growing number of more than 364 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 four additional loci that are contained in commercial STR kits. A variety of polyacrylamide gel electrophoresis conditions have been developed to separate closely spaced heterozygous alleles so that these alleles can be individually sequenced without the need for cloning. 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.

Summary of Results: The novel sequencing primers developed in our laboratory 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. With the introduction of "mini" STRs additional variants have been seen.[3]

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 AMPFlSTR SGM Plus kit. *Forensic Sci.Int.* 139: 255-259.

3 Hill, C.R., Kline, M.C., Mulero, J.J., Lagace, R.E., Chang, C.-W., Hennessy, L.K., Butler, J.M. (2007) Concordance study between the AmpFlSTR MiniFiler PCR Amplification Kit and conventional STR typing kits. J. Forensic Sci. 52(4): 870-873.

Short Tandem Repeat, DNA Sequencing, Variant Alleles

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