A85 Identification and Secondary Structure Analysis of a Region Affecting Electrophoretic Mobility of the STR Locus SE33

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After attending this presentation, attendees will understand the difficulties in designing suitable primer binding sequences to amplify the SE33 locus due to a highly polymorphic region outside of the repeat region which, if amplified, affects the electrophoretic mobility of the amplification product.

This presentation will impact the forensic science community because the SE33 locus is listed among the highly recommended loci to be included as part of the expanded CODIS core loci and because of the expectation that this locus will increase international compatibility to assist law enforcement data sharing efforts.[1]

Concordance between new and existing STR kits is of paramount importance when querying historical data stored on National DNA Databases or when sharing information across international borders. As such, one of the most important aspects of any multiplex STR development project is a comprehensive investigation into the impact any changes to existing primer sequences may have on comparison of new and existing results. SE33 is one of the most informative markers in forensic use due to its high power of discrimination. During the course of developing a multiplex STR kit several SE33 primer designs were screened with one primer pair yielding a high frequency of discordant alleles when compared to the AmpFlSTR® SEfiler Plus™ PCR Amplification Kit. This discordance was mostly specific to samples of African descent with an estimated frequency of 5.1% and was a result of a mobility shift of approximately +0.84nt. The sequence analysis of the affected alleles revealed that the only difference from the wild type sequence was a SNP outside of the SE33 repeat but within the amplicon of this particular set of experimental primers. In total, three different SNPs were all within 9nt of each other, each of which could cause the mobility shift individually. A computer model generated with the Mfold software predicted a region of secondary structure that encompassed the SNPs. This secondary structure was a stem-loop structure and the SNPs affecting the electrophoretic mobility of the amplicon fell within the stem portion of the structure. In order to characterize this region further the wild type SE33 sequence region was cloned into plasmid DNA. Site directed mutagenesis on this DNA revealed that mutations within the stem portion affected the mobility of the amplicon whereas mutations introduced immediately outside or within the loop portion of the stem-loop structure did not affect the mobility of the amplicon. Thermostability measurements using an oligonucleotide containing either the wild type sequence or sequences containing each one of the three SNPs demonstrated that the oligonucleotides containing the SNPs had significantly lower Tms when compared to the wild type sequence. These experiments strongly suggest that the polymorphic region contains a secondary structure that, when disrupted due to the presence of a variant SNP, results in mobility shift relative to the wild type sequence. To overcome this problem, the SE33 primers used in the final configuration of the multiplex STR kit avoided the amplification of this polymorphic region yielding in turn results highly concordant with the SEfiler Plus™ Kit.

Reference:

1. Harris et al., Expanding the CODIS core loci in the United States, Forensic Sci Intl., (2011) *in press*.

SE33, Stem-Loop, STR

 ** Presenting Author*