A67 Development of a Scorpion-Based Multiplex qPCR Assay for Pre-Screening Mixture Detection

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After attending this presentation, attendees will gain an understanding of the use of tri-allelic SNPs in mixture identification, how tri-allelic SNPs can be incorporated into existing qPCR multiplexes using Scorpion probes, and the importance of developing a pre-screening mixture detection assay for use in forensic casework.

This presentation will impact the forensic community by providing results of attempts to develop a qPCR multiplex capable of human quantitation, PCR inhibition, and mixture detection via tri-allelic SNPs. This work presents steps toward a pre-screening mixture assay that could help alleviate the low-level mixture deconvolution and interpretation issues faced by all forensic laboratories.

Mixture analysis of genetic profiles can be challenging for even the most experienced forensic DNA analysts. The interpretation and deconvolution of mixtures is only further complicated when the sample being analyzed is categorized as touch or contact DNA because one or both contributors' peaks often fall below the analytical threshold. Unfortunately, in the current workflow of forensic laboratories, mixture detection is not possible until the final interpretation of genetic profiles. Traditionally, forensic DNA mixtures are identified by the presence of three or more STR alleles at two or more loci. This identification method is not desirable because it is the last step in the workflow. The sample may have already been processed or consumed in such a way that analysts cannot go back and increase the input of DNA to STR amplification without significant modifications to the SOP. Previously, D1S80 and tri-allelic SNPs were evaluated for their ability to identify mixtures in a qPCR assay using the Lonza FlashGel® system for detection of alleles. Non-specific priming and gel resolution of heterozygous alleles, however, were problematic using this system. The development of a new Scorpion-based, 6-dye qPCR system for pre-amplification mixture detection could help alleviate these issues and improve both the mixture deconvolution and low-level DNA difficulties faced by forensic laboratories. Early knowledge of a mixture can provide an analyst with information that could alleviate sample consumption concerns, identify whether or not amplification input should be adjusted, and decide if surface swabs from the same item should be combined. The human DNA quantitation step provides an appropriate place to incorporate such an assay because it occurs early in the workflow and is easily multiplexed. The information allows analysts to meet the optimal DNA input range for amplification in an effort to avoid interpretation issues in the final genetic profiles. A molecular marker capable of incorporation into existing quantitation multiplex kits such as the Qiagen Investigator™ Quantiplex kit are tri-allelic SNPs. Tri-allelic SNPs are small in size, which is important in degraded samples, but also polymorphic enough to identify the presence of two individuals in a sample. Successful incorporation of tri-allelic SNPs into a quantitation multiplex requires an appropriate primer, probe, and detection system. Duplex Scorpion primers will be utilized and designed for tri-allelic SNPs rs5030240 and rs4540055 so that the primer covers the polymorphic region at the 3' end. Primers are tagged with a fluorescent molecule on the 5' end with each color corresponding to a particular SNP allele. This approach, under the correct amplification conditions, will allow for preferential amplification of the primer that is complementary to the SNP compared to the mismatched primers. Mixture detection will be possible with the interpretation of amplification curves using the Rotor-Gene Q thermal cycler, which has seven color channel detection capabilities. Initially, primer sets will be created for rs5030240 and rs4540055, and will be optimized separately in single source samples using the ABI 7500 thermal cycler. Next, primers will be multiplexed into a single amplification reaction and tested in single source samples using the ABI 7500. If successful, the primer sets will then be optimized using the Rotor Gene Q and eventually incorporated into the Qiagen Investigator™ Quantiplex kit. This multiplex will then be used to identify fabricated mixture samples and results compared with detection using the ABI 3130 genetic analyzer. It is expected that fabricated mixture samples will be identified using this system and that significant progress towards a multiplex capable of human DNA quantitation, assessment of PCR inhibition, and mixture detection will be made.

Mixtures, Tri-Allelic SNPs, qPCR

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