EVALUATION OF POWERPLEX® FUSION AT THE FULL, HALF, AND QUARTER REACTION VOLUMES

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The efficiency of reference sample processing for databasing and paternity purposes has been greatly increased by the development of direct amplification systems. DNA samples can be collected and stored on non-treated matrices, such as the Bode Buccal DNA Collector ™, until sample processing is required. The Promega Corporation has recently released a new direct amplification kit encompassing 24 loci. PowerPlex ® Fusion was designed for direct amplification of samples collected on both treated and non-treated collection paper. Direct amplification of reference samples eliminates the extraction and quantification steps encountered during routine DNA analysis as an effort to save time and costs in the laboratory.

This presentation will describe the studies with PowerPlex® Fusion to obtain optimal results from samples collected utilizing the Bode Buccal DNA Collector ™, a non-treated matrix, at varying reaction volumes. The amplification reaction volumes analyzed in this experiment were 25µl (Full Reaction), 12.5µl (Half Reaction), 6µl (Quarter Reaction), and 15µl. A total of one hundred (n=100) self-collected samples obtained in either 2010 or 2011 (2-3 years old at time of testing) were utilized in this experiment. Collectors from 2010 and 2011 were chosen as they may be more indicative of a sample encountered during routine databasing rather than a fresh sample collected a few days prior to testing.

This presentation will display the optimized procedures for cell lysis, reaction mix components, thermal cycling parameters, and 3130XL injection conditions. Amplification reaction mix components were optimized to overcome potential inhibitory effects of the lysis solution. As the reaction volume decreased, the relative concentration of the lysis solution in the amplification reaction increased which may cause inhibition. These optimized procedures resulted in a first pass success rate of >80% for all reaction volumes tested. The highest first pass success rate (95%) was observed when utilizing the half reaction (12.5µl) protocol. In addition to displaying first pass success rates for each reaction volume, interlocus balance information for each will also be discussed.

While eliminating the extraction and quantification steps in an effort to save time, it can also lead to additional amplification reactions and costs as normalization does not occur. Cellular deposition varies from individuals resulting in either excessive or inadequate samples. Eating, drinking, medicine intake, health status, and non-cooperation during sample collection will all affect the amount of cells deposited onto the collection paper. This variation in cellular deposition can either lead to over or under amplification if the procedure is not optimized. Optimizing the amplification parameters to account for sample variation reduces re-sampling and re-amplification. This reduction in reprocessing can result in an improvement in the overall processing efficiency of the laboratory.

Direct amplification of reference samples can provide a time efficient method for obtaining complete genetic profiles but can be cost prohibitive. Utilizing one of the reduced volume reaction procedures demonstrated in this poster can greatly reduce the cost per amplification reaction. This may allow for the process to not only be time efficient but also cost efficient.