# UTILITY OF AMPLIFICATION ENHANCERS IN THE ANALYSIS OF MIXED DNA SAMPLES

Alexander Bethea, Clarissa Trogdon, Linda Le, Steven Lee
Forensic Science Program, San Jose State University, San Jose, CA 95192

Mixture interpretation is a multi-step process that involves identifying that a sample is a mixture, designating alleles, determining if two or more individuals are involved, inferring relative ratios of the different contributors, comparing all possible allelic combinations, and finally determining the donor genotypes with reference sample comparisons (Butler 2005). Deconvoluting mixtures is complicated when donor alleles are not balanced. Imbalanced allelic ratios may be due to non optimal conditions, such as the presence of inhibitors, degradation and/or low amounts of sample with less than 100pg (Alonso 2004) resulting in stochastic effects.

Sexual assault cases are among the most common analyzed in forensic DNA laboratories and in most of these cases, mixtures are encountered. The number of completed cases has dramatically increased since the utilization of short tandem repeats and the implementation of the Forensic DNA backlog reduction program (http://www.dna.gov/funding/backlog-reduction/ and Shrestha 2006), however, the challenges invoked with mixtures have persisted.

Challenges presented by mixtures include the presence of external contamination, those that contain more than 2 contributors, low quantity imbalanced donor ratios and damage to the templates at different levels. One or more of these confounding factors may lead to poor amplification, imbalance in peak heights within and among loci and allelic drop out.

A relatively new commercial product, PCRBoost, (Biomatrica San Diego, CA), has been explored by other scientists and has shown promise in its ability to overcome some of the challenges in DNA analysis. Previous studies have shown that PCRBoost has the ability to recover alleles from low quality and low quantity single source DNA samples containing inhibitors.

The hypothesis of this study is that amplification of low quantity mixed DNA samples with PCRBoost will enhance recovery of alleles and maintain or enhance the allelic balance representative of the donor ratios amplified.

In order to test this theory, mixtures of male and female DNA were made with the total amounts ranging from 0.25ng to 2.5ng. Within each set of these amounts, replicate mixtures of 10:0, 9:1: 8:2, 7:3, 6:4, 5:5 and visa-versa were made. All samples were quantified using qPCR with the Quantifiler kit, amplified using the Ampfistr Identifiler kit, and analyzed with the ABI 310 Genetic Analyzer and Genemapper (Applied Biosystems, Foster City, CA).

Initial results were unexpected. Preliminary results suggest that at the higher concentrations of template DNA (greater 0.8 ng), PCRBoost was able to enhance the balance of alleles at different loci, but the overall peak heights decreased as compared to the same samples amplified without PCR boost. This data suggests that PCRBoost used in its current form may not enhance results for *all* DNA mixtures, but only those below a certain threshold. Although further research is needed, initial data demonstrated that DNA samples below 0.8 ng/ul increased in peak height and had enhanced allelic balance. Results on low quantity DNA mixtures with commercial PCRboost and additional formulations of PCR boost as well as amplification of non-probative case samples are underway.

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