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Commentary on: Foran DR, Gehring ME, Stallworth SE. The recovery and analysis of mitochondrial DNA from exploded pipe bombs. J Forensic Sci 54;1:90–4.

Sir,

Foran et al. (JFS 54:90–94) describe mitochondrial DNA (mtDNA) analysis of handled pipe bomb components. The practices used in their experiments are inappropriate in forensic casework.

Ninety-four of 114 PCR amplifications of the swab samples in this experiment yielded insufficient or no amplification product for DNA sequence analysis after 38 cycles of mtDNA amplification. These samples were subsequently processed further: a 1 μL subsample of first-round PCR product, whether it was visible on a yield gel or not, was placed into a second nested amplification of 24 cycles, giving a total of 62 cycles of amplification. With this approach, every swab produced an amplification product. Due to tight controls applied to handling of materials prior to sampling, and because the investigators knew the profiles of all contributors, every amplification product should have been assigned to a donor. However, the investigators recovered 10 profiles (28% of the samples) that could not be assigned. Therein lies the problem of nested PCR. The use of nested PCR can yield amplification product from contaminant molecules rather than the target DNA, particularly when the amount of target DNA is minimal. If a contaminant does become detectable through nested PCR, it is impossible to discriminate between the contaminant, a handler, or someone who may have handled the materials some time well before bomb preparation. In addition, PCR artifacts may result due to stochastic effects that occur when amplifying low levels of DNA.

Previous descriptions (1,2) of the use of nested PCR in the examination of mtDNA have also demonstrated increased risk of contamination and elevated background noise. In fact, Gryzbowski et al. (2) state that "...the nonreproducibility of the results…suggest that some of those mutations might be artifacts resulting from specific conditions of nested PCR…," "…specific conditions of nested PCR favor the occurrence of PCR replication errors…," and "…the exclusion of nested PCR from the techniques employed in forensic casework would be a more conservative approach…." Brandstatter and Parson (3) also found that higher quality electropherograms were obtained from direct sequencing when compared with nested PCR products. The ancient DNA and forensic DNA communities have been aware of these potential problems for nearly two decades, and the use of more than 40 cycles of PCR amplification in mtDNA analysis and 34 cycles of PCR amplification in short tandem repeat (STR) analysis has been rejected by the broad forensic community.

In addition, while Foran used "control samples" that were not handled by any subjects participating in the experiments, there is no indication that simple reagent blank extraction negatives were run through the comparable 62 cycles of PCR, or if so, what they yielded. The authors do not report the results of the PCR negatives described in the experimental methods. While the control bomb samples gave "nonsense" data, as opposed to negative results, there is no description of what these sequences consisted of, or that they were investigated for a possible origin.

The negligible difference in the nested fragment sizes (256 bp vs. 283 bp) seems unlikely to explain the difference in obtaining results for HV1 and HV2. However, first round amplification products were significantly different in size: 403 bp (HV2) versus 333 bp and 266 bp (HV1). It is unlikely that forensic evidence samples could routinely and successfully be amplified in a 403 bp amplicon. In fact, standard forensic practices such as the use of smaller amplicon sizes and even use of mini-primers might have been suitable for this evidence without nested PCR. While mixtures, with their overwhelming interpretational challenges, might have been obtained on some samples, the use of smaller amplicons might have yielded single profiles on some samples that were not successful at 38 cycles with the larger amplicons. However, given the sensitivity of mtDNA analysis and our experience with its use on touched objects, we would predict a high incidence of detecting mixtures on touched casework items, which have likely been exposed to and handled by numerous individuals.

Furthermore, it is well known in both the forensic and global science community that the peaks generated by Dye-Terminator sequencing chemistry are not quantitative. With current technology, the practice of attempting to assign peaks to an individual when a mixture is present in a sequencing trace is limited, especially in a circumstance such as this one where experiments have been performed on DNA amplified under conditions sure to induce stochastic effects. Additionally, only a single-strand of sequence data containing high levels of background noise was presented by the authors, illustrating the problems of interpreting such data.

Finally, it should be noted that the authors soaked or cleaned the bomb components in a 10% bleach solution prior to perform experiments. While a controlled study should first be performed with the cleanest samples possible, this study shows that the cleaning steps taken in these experiments still gave erroneous results. Given that bomb components obtained from a true crime scene would have a far greater potential for contamination than that seen in these experiments, the problems seen here would be exacerbated under real world conditions. The authors also reported an "individualizing success rate, given the closed population," as 50%. An analysis performed at this sensitivity cannot assume a closed system and the reported 28% of profiles that were unassignable in these experiments demonstrate that this type of testing cannot individualize the source of evidentiary items. In fact, the great strength of mtDNA analysis is its ability to exclude individuals as potential sources of evidence.

The investigators state that DNA quality is the relevant factor for obtaining results. As the fragment sizes here are similar to those of small STR amplicons, we maintain that copy number is one critical factor in obtaining successful results. When copy number is a critical feature, the ability to amplify a single contaminant molecule with 62 cycles of PCR to a detectable level will result in meaningless outcomes. Another critical factor in obtaining DNA profiles from any type of evidence is the absence of inhibitors. The probable presence of explosive residue and its impact on the PCR cannot be overlooked as an important parameter in the analysis of postblast DNA. The assertion that this study "holds more promise than any technique that has preceded it" for identification of IED assemblers should be viewed with extreme skepticism.

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