HOW LOW CAN YOU GO? A SYSTEMATIC APPROACH TO DEVELOPING THE MOST SENSITIVE DNA-STR PROCEDURE

Alex Sinelnikov, Karl Reich, Independent Forensics

For a variety of reasons, both legal and criminalistic, forensic practice is demanding more DNA profiles from ever smaller (in terms of DNA content) samples. This trend includes testing 'gun swabs', swabs from bullets (fired and unfired), exploded bomb parts (including IEDs), zipper pulls, door knobs, hammer handles, and even latent ridge impressions (fingerprints). Many of these kinds of samples fail to provide useful DNA profiles using current methods.

Deficiencies in the current approach to processing minute samples include inefficient sampling of physical evidence, poor recovery of biological material from the swabs themselves, poor recovery from solid phase DNA purification methods (lyse, bind, wash, & elute), and deliberately disregarding more than 90% of the multiplex PCR reaction for CE analysis.

We have devised solutions for each one of these deficiencies by critically examining the steps used to (i) recover biological materials, (ii) release DNA from recovered cells and debris, (iii) purify DNA away from PCR inhibitors, and (iv) electrokinetically inject dye-labeled amplified fragments during capillary electrophoresis. We have paid particular attention to liquid handling issues, tube transfers and recovery volumes which when appropriately economized, greatly reduce losses for the entire procedure.

We have developed a wetting / digestion solution that recovers biological materials from both non-absorbing surfaces (computer mice, bullets) and absorbing materials (fabric, swabs) with almost quantitative efficiency. Subsequent digestion and release of both nuclear and cell-free DNA is accomplished at elevated temperatures with strong detergents.

The released DNA is purified by subtraction through a spin column filled with a proprietary resin: inhibitors, detergents and proteases are retained by the resin and DNA suitable for multiplex PCR is recovered with an efficiency of >80%.

We have used AmpliconRx™ as a post-PCR purification and concentration for the entire PCR reaction as this further increases (by ~20X) the CE signal.

When combined into a complete protocol, this method of collection, extraction, purification and post-PCR greatly increases the sensitivity of obtaining DNA profiles. We have obtained reproducible DNA profiles from single fingerprints, single bullets and small areas of worn clothing; items that are generally beyond the limit of current forensic DNA methods.

We estimate an overall recovery of at least 70% of input DNA – well above any other published procedure; we consistently obtain DNA profiles from a few dozen nucleated cells from a variety of objects, including latent ridge detail, bullets, handled objects and evidence tape. A full description of the method and results obtained are described.