B9 Obtaining DNA-Short Tandem Repeat (STR) Profiles From Evidentiary Samples With Extremely Limited Amounts of DNA

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After attending this presentation, attendees will have learned about a systematic approach to obtaining DNA-Short Tandem Repeat (STR) profiles from samples with very limited amounts of DNA. Forensic science practitioners will have learned about an optimized method for: (1) the collection of small biological samples; (2) the extraction of DNA from these samples; (3) the purification of DNA from such samples; and, (4) the post-Polymerase Chain Reaction (PCR) purification and concentration of amplicons. The final goal of this sequence of techniques is to obtain DNA profiles from the smallest possible forensic samples; this study has fully optimized the individual steps and introduced a method that utilizes the entire PCR reaction for electrokinetic injection on Capillary Electrophoresis (CE) instrumentation.

This presentation will impact the forensic science community by describing the procedure that can be used to successfully obtain DNA-STR profiles from very challenging forensic samples (sometimes referred to as "touch DNA" samples) like single fingerprints, single bullets, and small areas of worn clothing.

For a variety of reasons, both legal and criminalistic, forensic practice increasingly demands 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 Improvised Explosive Devices (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. This is primarily due to losses of biological material during the collection, DNA extraction, and purification steps. Another contributing factor in failing to obtain a useful DNA profile is that only a fraction (typically <6%) of the multiplex STR PCR reaction is used for CE analysis. The procedure discussed here corrects all of these deficiencies.

Specifically, the protocol includes the following materials and methods. Larger objects (e.g., aluminum cans) and absorbing surfaces (e.g., clothing) are swabbed with regular-sized swabs. Small objects (e.g., individual fingerprints, bullets, etc.) are swabbed with user-produced mini-swabs. Mini-swabs are made by deliberately truncating the swab head to approximately ¼-½ of the regular cotton swab. Regular cotton swabs were wet with 50µL and mini-swabs with 10-20µL of collection buffer containing a low concentration of detergent. Following collection, regular swabs were saturated with 70µL and mini-swabs with 40µL of lysis buffer that includes detergent and Proteinase K. Swabs were incubated at 56oC for one hour. The lysate was collected by centrifugation in a spin-basket and loaded onto a column filled with polyamide resin. DNA was purified on the column at 4,000 Relative Centrifugal Force (RCF) for two minutes. Purified DNA was either amplified directly or concentrated three- to four-fold under vacuum centrifugation. Following PCR amplification using a commercial forensic multiplex kit (the use of additional PCR cycles is not recommended), an AmpliconRx™ kit was used for post-PCR purification and concentration of the entire PCR reaction, as this further increases (by up to approximately 20 times) the final CE signal.

The current study shows that when combined into a complete protocol, this integrated procedure for collection, extraction, purification, and post-PCR clean-up, concentration significantly increases the sensitivity of obtaining DNA profiles from low-template (touch DNA) samples. Specifically, the limit of detection of this method was tested by applying progressively smaller amounts of a control DNA solution to a non-absorbent surface, allowing the deposition to dry, and then collecting and processing as described. These experiments demonstrated that full profiles (26 alleles) could be reproducibly obtained from as little as 62.5pg of starting DNA material on a non-absorbent surface.

Testing of this method has continued using developed and undeveloped fingerprints on a variety surfaces. Examples of some successfully profiled items that will be presented are: (1) three poorly developed fingerprints deposited on an aluminum can — full profile (28 alleles); (2) a single well-developed fingerprint on a plastic surface — 26 out of 27 expected alleles; and, (3) an unfired bullet, loaded into a magazine, retrieved, and tested — 25 out of 28 expected alleles.

Touch DNA, Sample Collection, DNA Purification