

## **PROTEOGENOMIC ANALYSIS OF TOUCH SAMPLES TO GENERATE FORENSICALLY-RELEVANT RANDOM MATCH PROBABILITY VALUES IN THE ABSENCE OF DNA**

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Human touch samples represent an increasing fraction of forensic casework analysis each year. Unfortunately, these samples are also among the most difficult to analyze effectively, especially with respect to traditional DNA analysis. DNA present in touch samples is primarily extracellular and may be washed away prior to deposition (e.g., hand washing) or degraded in the environment (e.g., UV exposure). As a result, many touch samples return partial or negative profiles. Proteins represent a separate class of biomolecules in touch deposits that are both highly abundant and environmentally robust. The use of mass spectrometric-based proteomic analysis to identify genetically variable peptides (GVPs) has been recently demonstrated to provide forensically-relevant match probabilities to suspected individuals. However, these proof-of-concept studies have not taken implementation into a forensic casework laboratory into account. Here, we describe a complete casework-oriented proteomic analysis method which encompasses sample preparation, a core set of GVP markers common to touch samples, a targeted DNA sequencing approach for comparison to known individuals, and a GVP-specific random match probability (RMP) calculator specifically designed to use proteogenomic data sets. Critically, the sample collection and extraction procedure stratifies and differentially extracts DNA and protein fractions, enabling parallel DNA and GVP analysis. This capability promotes future casework laboratory implementation by positioning GVP analysis as a complimentary tool and enhancement to standard DNA analysis (as opposed to promoting GVP analysis as a replacement of current gold standard). The differential extraction approach generally yields equivalent amounts of DNA compared to traditional swab-based DNA collection and extraction (0.75 ng versus .96 ng, respectively), while also yielding an average of 54 GVP alleles per sample. Using our method, we have demonstrated that humans can be identified from touch samples generated using GVP data with RMP values ranging from  $10^{-5}$  to  $10^{-16}$ , even in samples where no corresponding DNA profile could be generated. Taken together, our novel sample preparation strategy and custom analysis method provides a transformative capability for the analysis of touch samples.