Engineering a New Future for Fingermark Sample Processing

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Why Engineer Anything?

- Humans are everywhere – why reinvent samples for R&D use?
- Regulatory Oversight
  - Protocol prep and approval, review, recruiting, and sample stability all create hurdles to the process
  - Issues related to collection process – low diversity in sample set, slow process
- Sample variability
  - Variability between individuals
  - Variability within an individual
- Can we engineer artificial samples to improve the quantity and quality of human forensic research?
  - Can these work across key sample types of interest to the community?
  - Can these support emerging techniques (e.g., protein analysis)
  - Can these replace human samples altogether?

hDNA Analysis from Shell Casings

- Gun violence is a significant issue
  - 1.2 firearms for every 1 person in the U.S.[1]
  - 13,958 people died of firearm homicide in 2018[2]
  - Approximately 73% of all homicides involve firearms[2]

[1]Small Arms Survey - Small Arms Survey reveals: More than one billion firearms in the world. *www.smallarmssurvey.org*. Retrieved 14 February 2019.

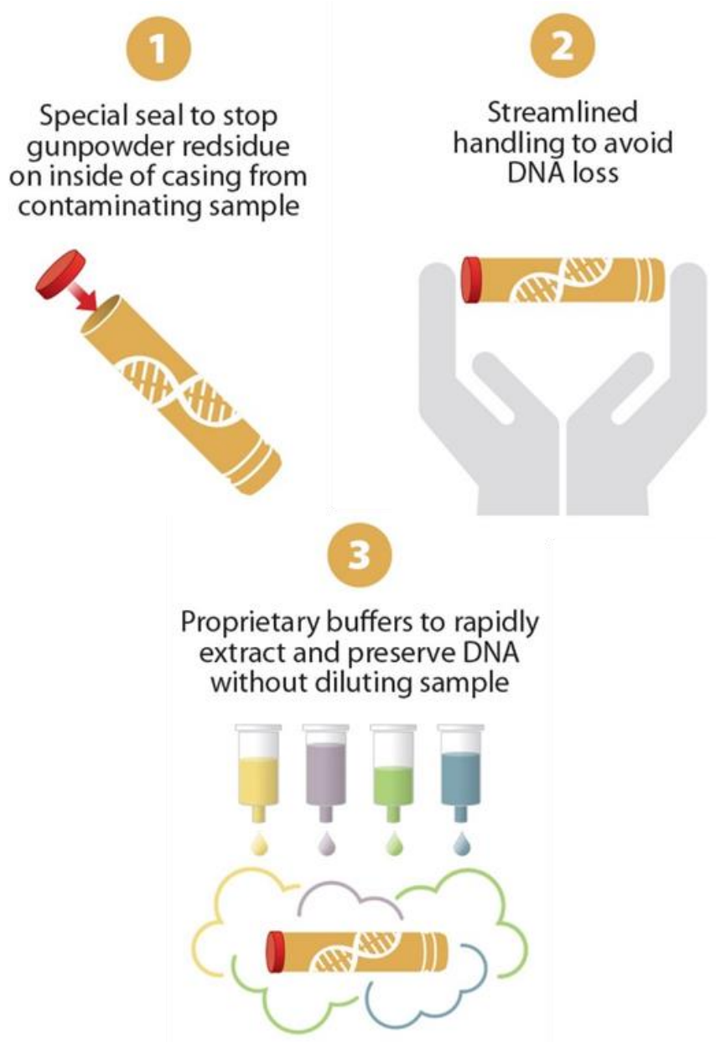
[2]10 Leading Causes of Injury Deaths by Age Group Highlighting Violence-Related Injury Deaths, United States – 2018 (PDF). Injury Prevention & Control, CDC.

hDNA Analysis from Shell Casings

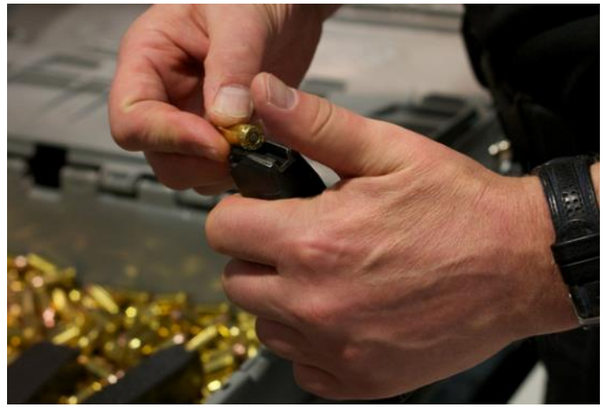
- Shell casings present a hostile environment for DNA
  - Touch (trace) deposition
  - Sample age
    - Time since loading
    - Time since firing
  - Heat and pressure during firing
  - Metal ions and reactive chemical species
  - Contamination
  - Inhibitors (GSR)

Optimized Shell Casing Collection Method

- Typical shell casing processing methods involve swabbing, soaking in a buffer, or a mix thereof
- Our validated method, Forensic Recovery of Identity from Shell Casings (FRISC™), utilizes soaking and swabbing
- We are engineering novel methods to allow simplified handling, reduced risk of GSR contamination, shorter processing time, and separate extraction of skin protein

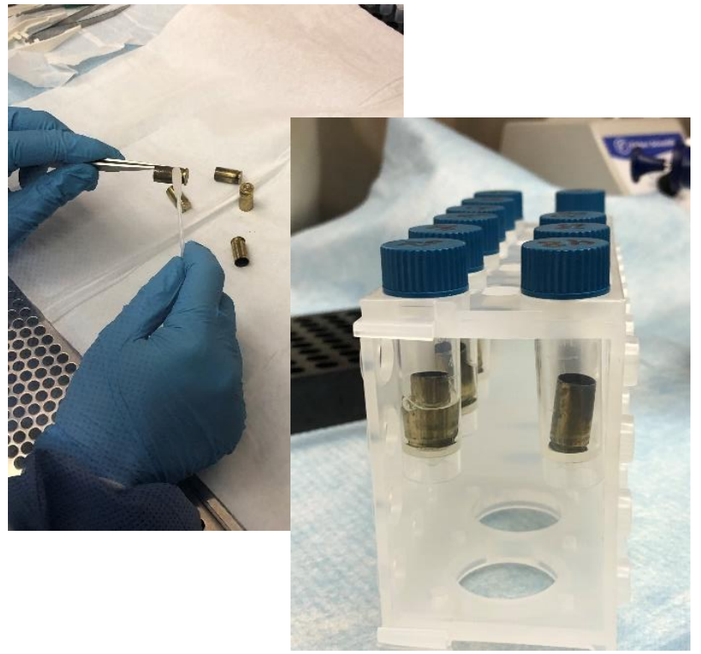


Experimental Overview



Enlisted three human donors to deposit touch samples on cartridges and load into decontaminated 9mm magazines

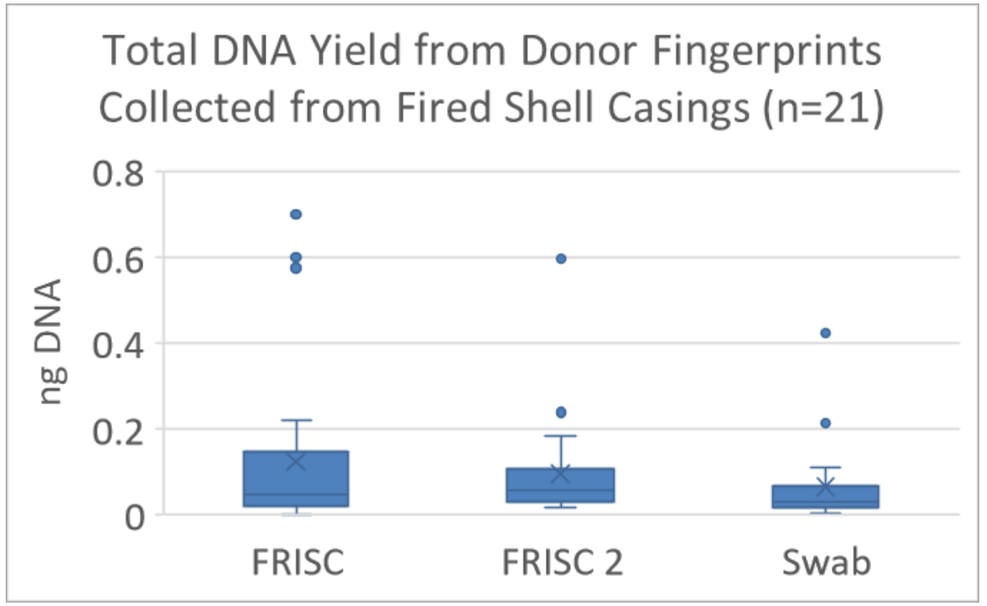
Fired and collected shell casings at a local firing range (typically seven replicates per donor)

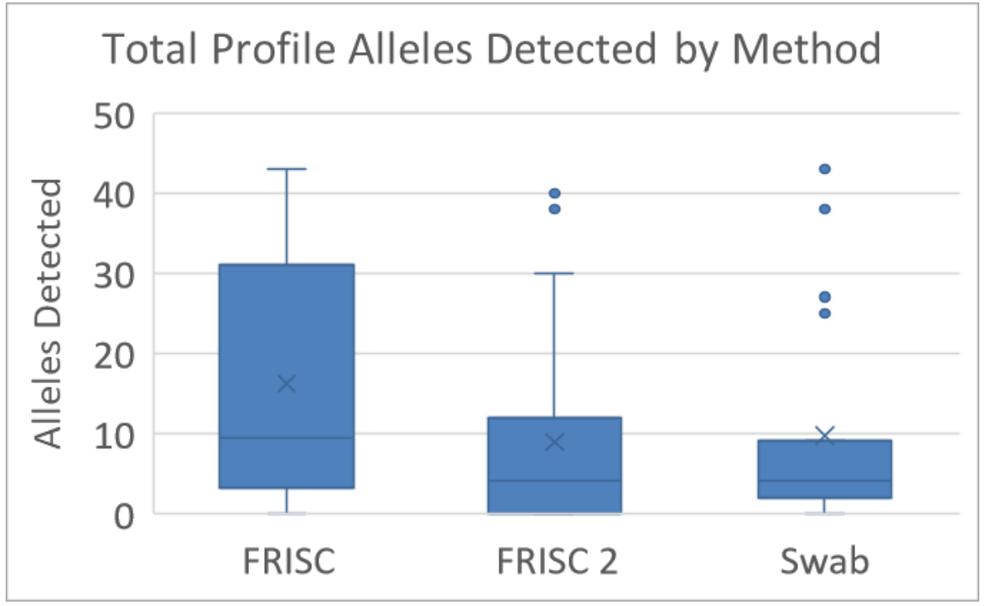


Collected samples using a standard swab method, SigSci's validated FRISC™ method, and the Gen 2 version version of the FRISC™ protocol

Shell Casing DNA Analysis

- Total DNA yields comparable and not statistically significant across methods
  - Complicated further by high quantity of outliers
- FRISC methods generally recover more total alleles than a swab-based approach
- FRISC produces CODIS-eligible profiles from fired shell casings 38% of the time with > 50% of samples suitable for comparison

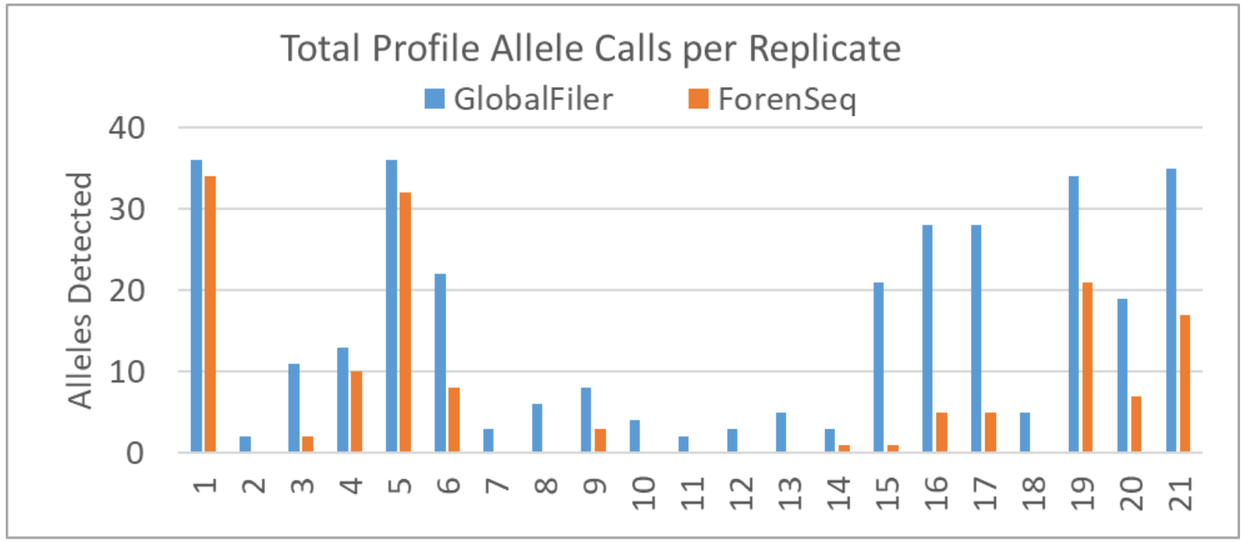


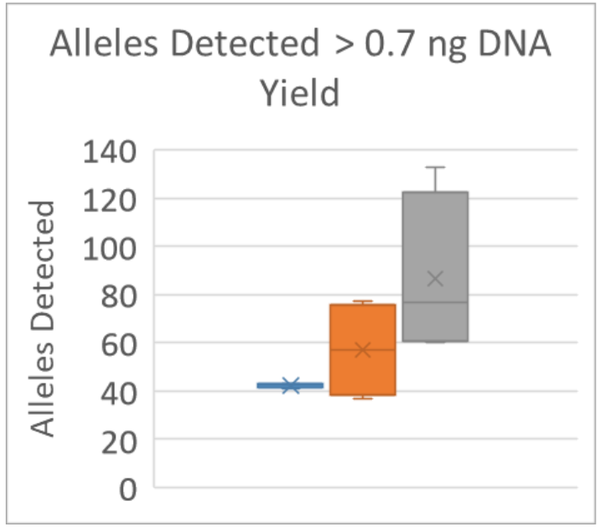


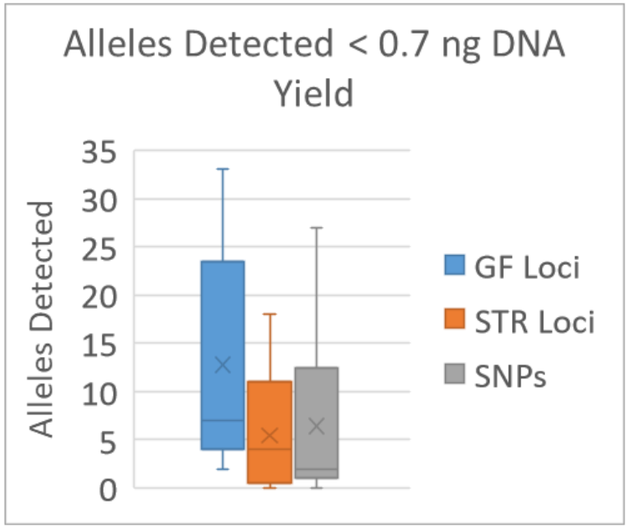
CE vs. NGS Analysis of Shell Casing Samples

- CE-based analysis shows better sensitivity across the 20 core CODIS loci
- Low DNA input seems to outweigh benefits from short amplicons for degraded DNA

- When sufficient DNA is available, sequencing produces significantly more genetic information
  - ForenSeq DPMB
- Variability between replicates is extreme





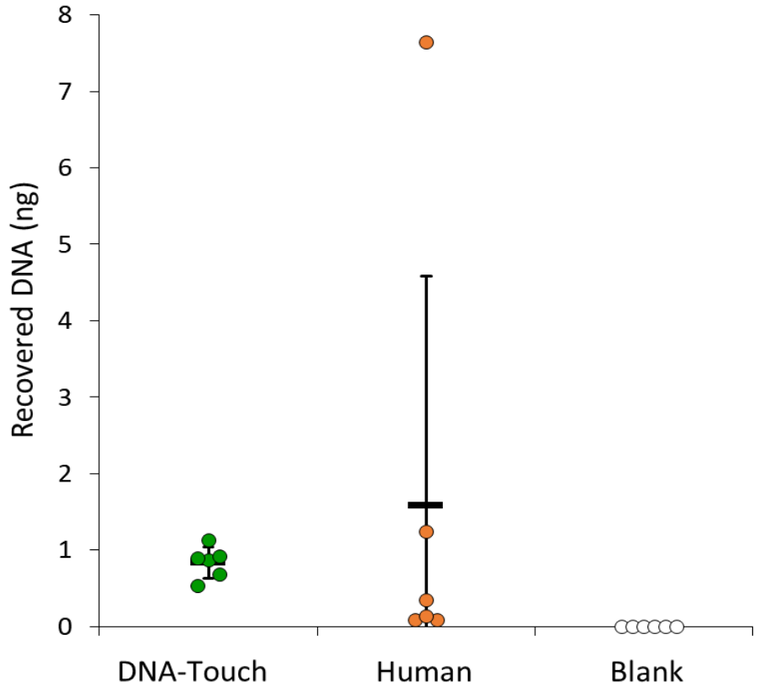


Our Solution: DNA Touch

- Standardized, simplified, scalable synthetic fingermarks
  - Reduces deposition variability
  - Permits quantitative calculation of DNA and protein yield
  - Enables greater statistical power during method development

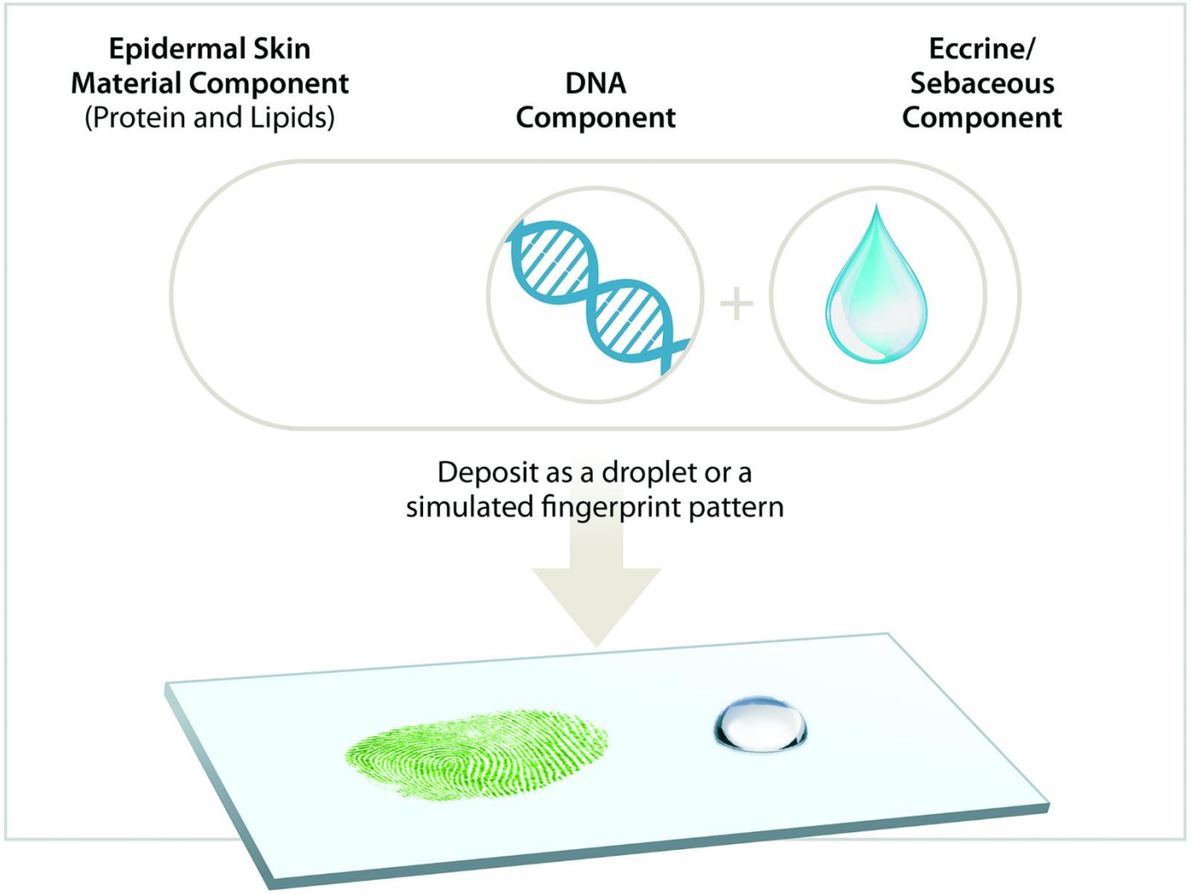
DNA-Touch™

Synthetic Fingermarks for Genomics Research

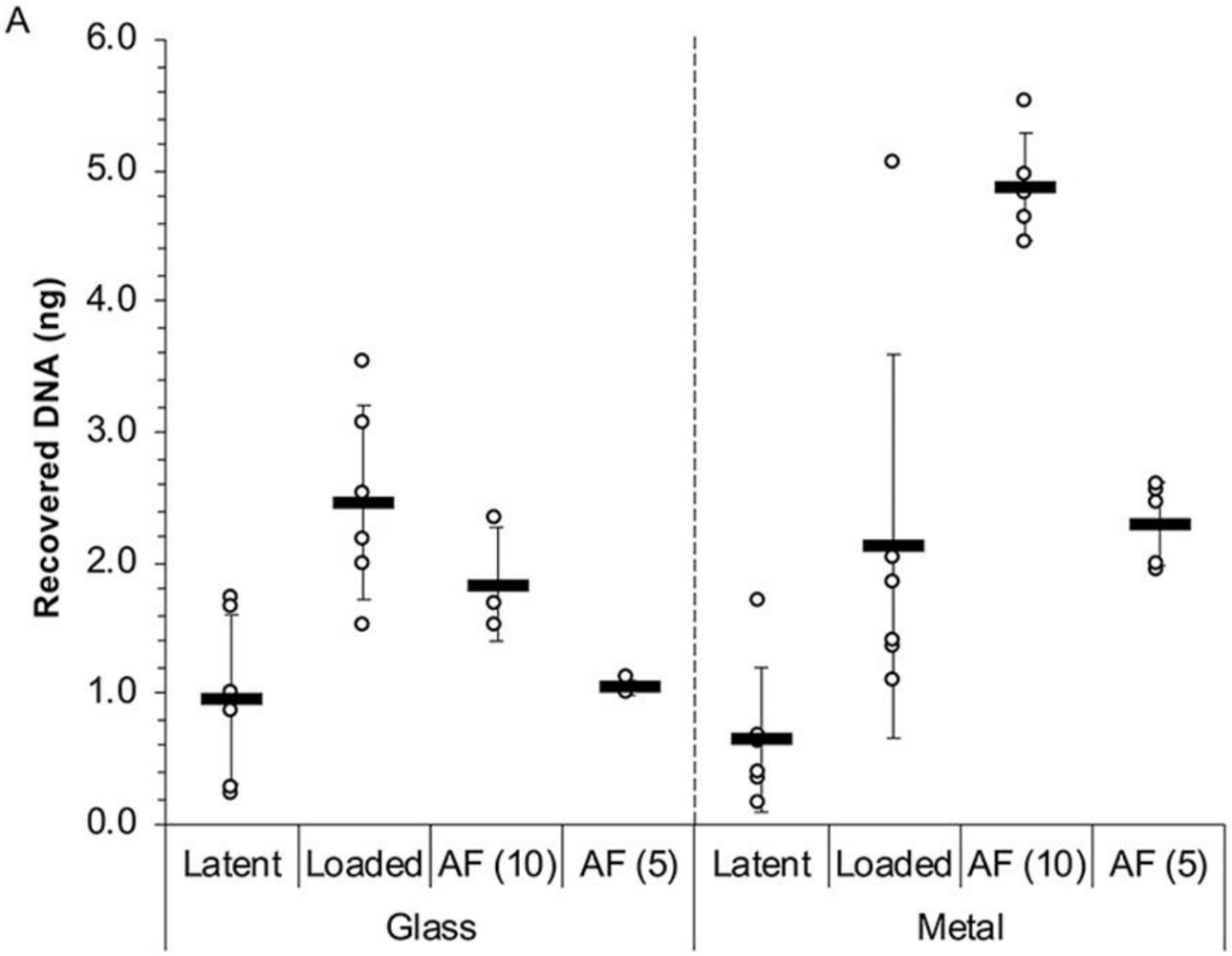


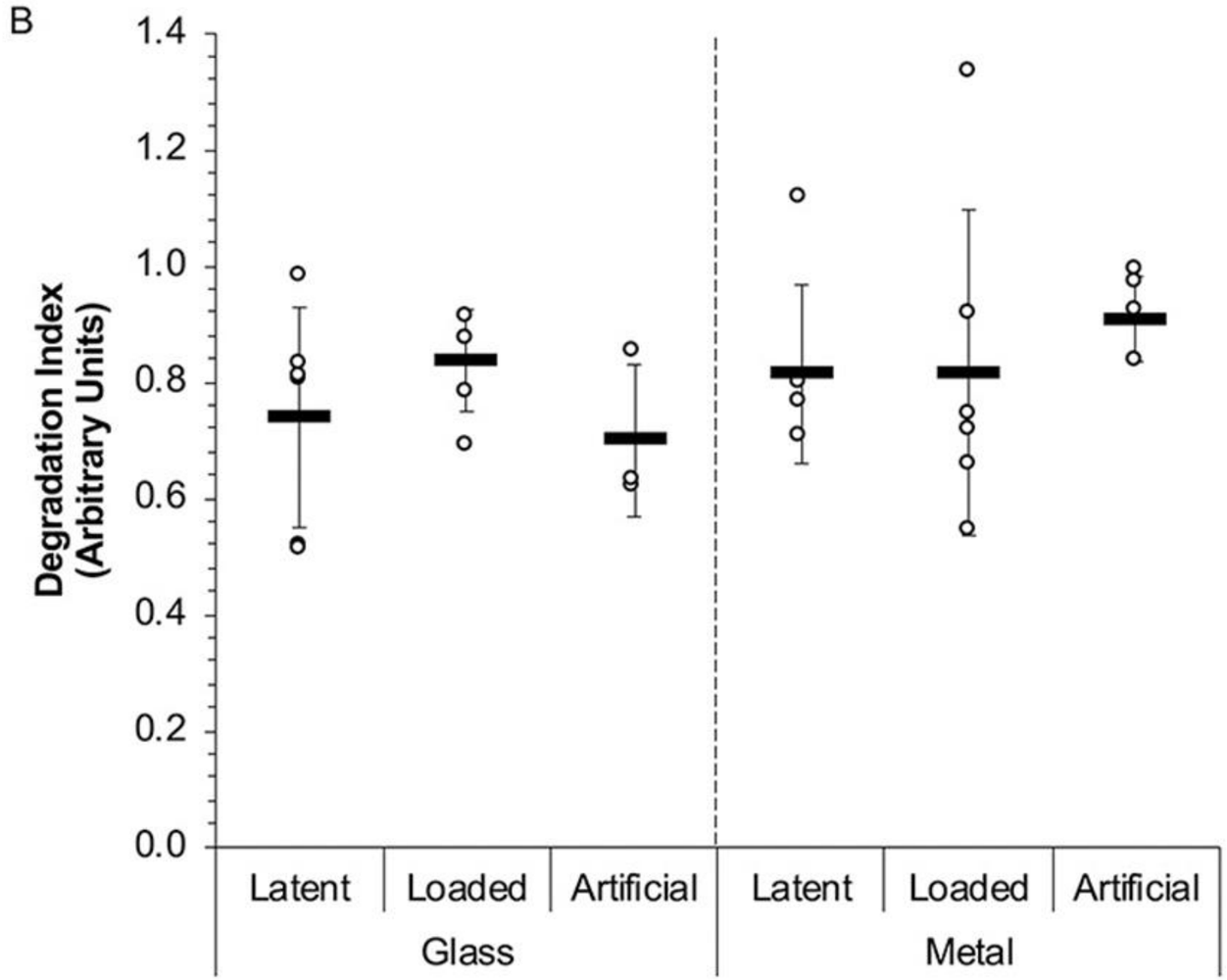
Artificial Print Composition

- Primary components of a fingermark:
  - Sebaceous oils
  - Eccrine secretions (e.g., sweat)
  - Extracellular DNA
    - Typically fragmented
    - Utilize well-characterized, commercially available gDNA sources



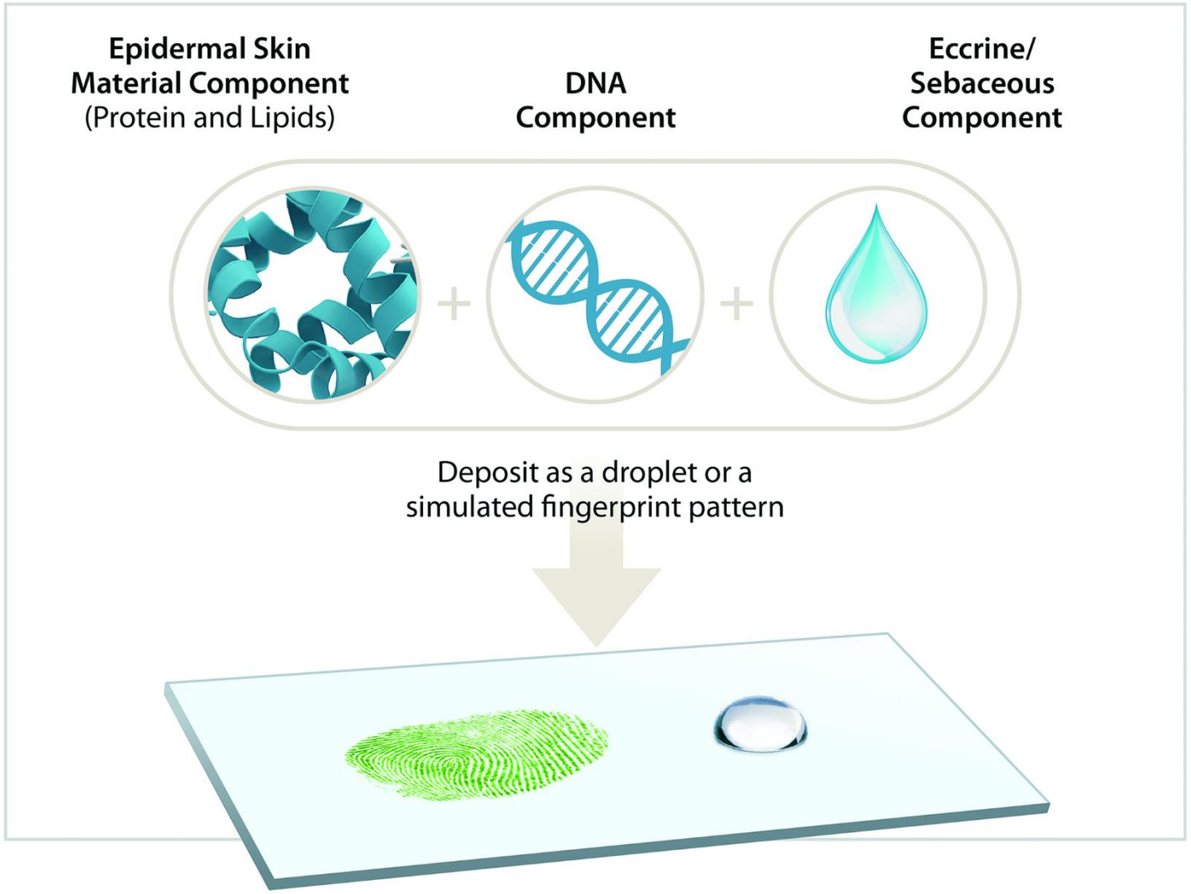
Comparison of Real and Artificial Touch Samples





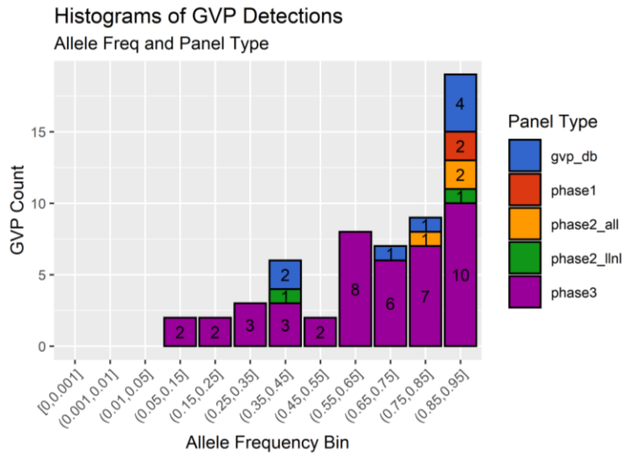
Artificial Print Composition

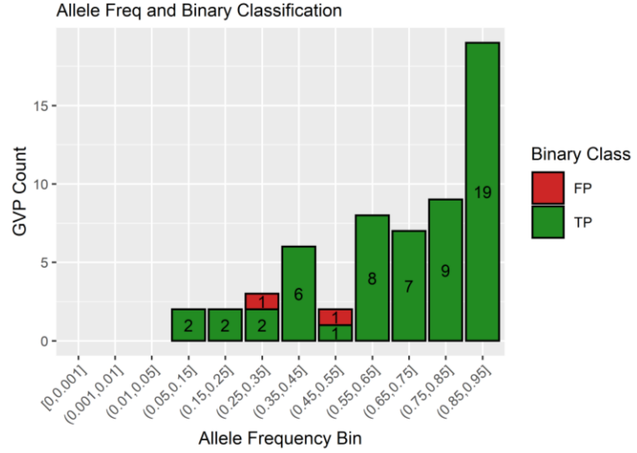
- Primary components of a fingermark:
  - Sebaceous oils
  - Eccrine secretions (e.g., sweat)
  - Extracellular DNA
    - Typically fragmented
    - Utilize well-characterized, commercially available gDNA sources
  - Keratinized epithelial cells
    - Typically anuclear
    - Can be obtained from volunteers or commercial biobanks

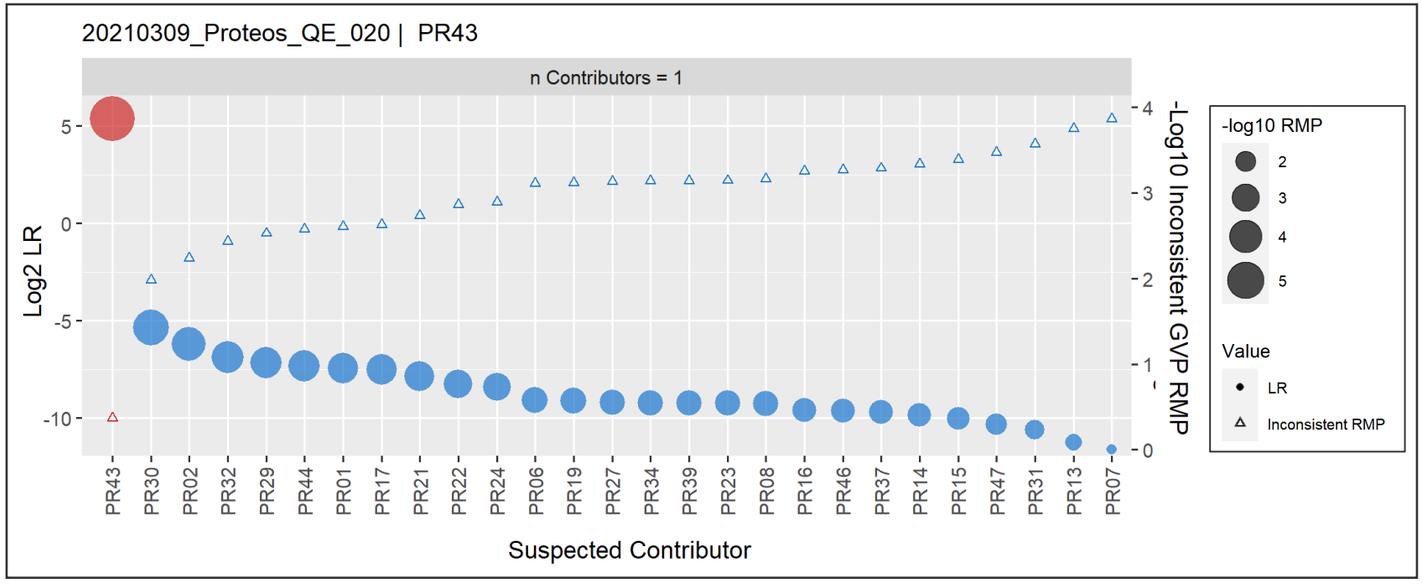


Forensic Identification using Protein Polymorphisms

- Utilizes protein sequencing to identify underlying SNPs in coding regions for human identification
- Protein polymorphism profiles can be compared to each other or to whole genome sequencing data for identification
- Individual PR43 example

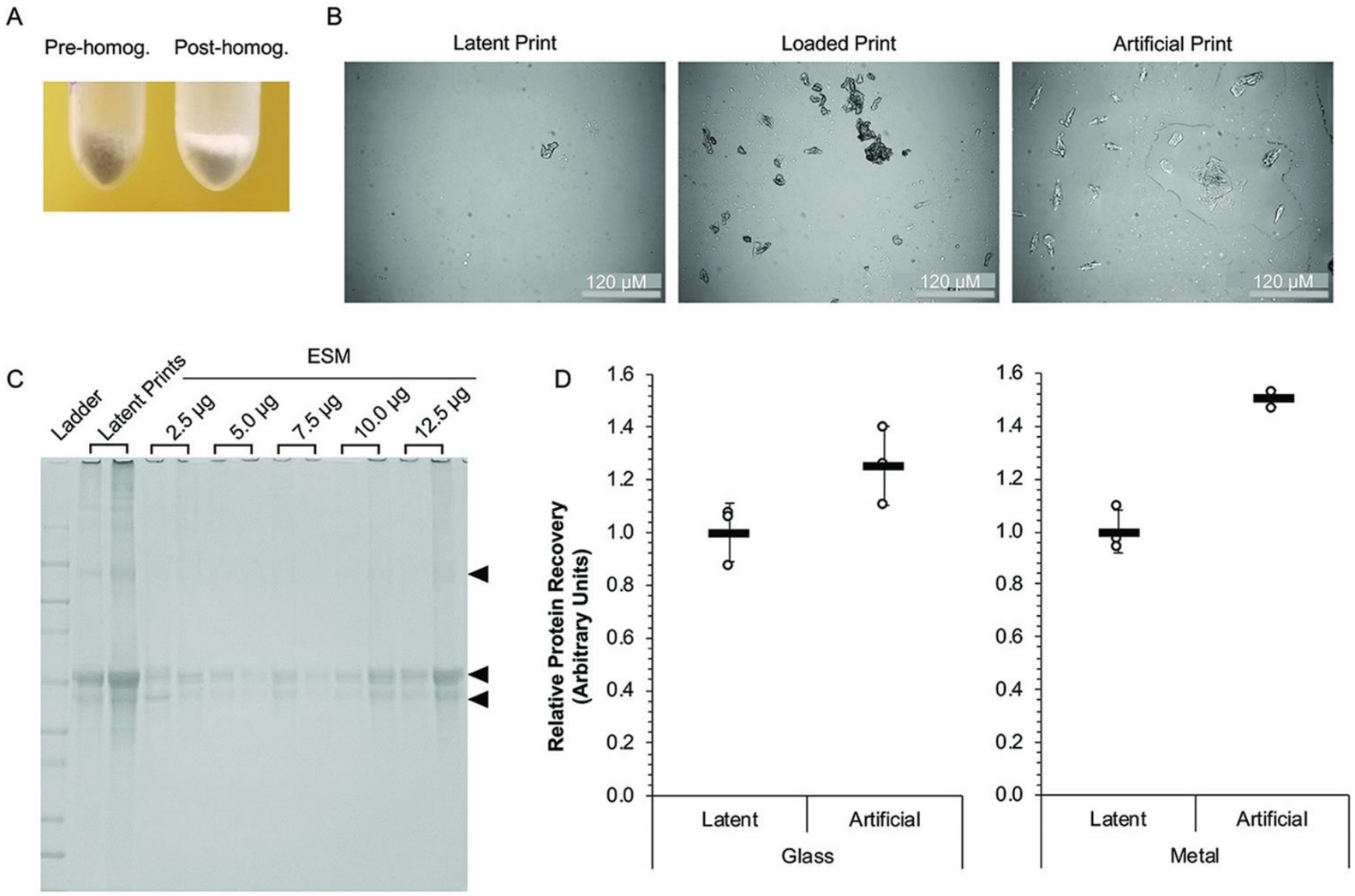






Incorporation of Skin Cells

- Skin cells collected from the palms of volunteers using a PedEgg
- Cells homogenized and added for formulation at appropriate concentration
- Enables parallel protein analysis, if needed
- Must consider DNA mixtures if applicable

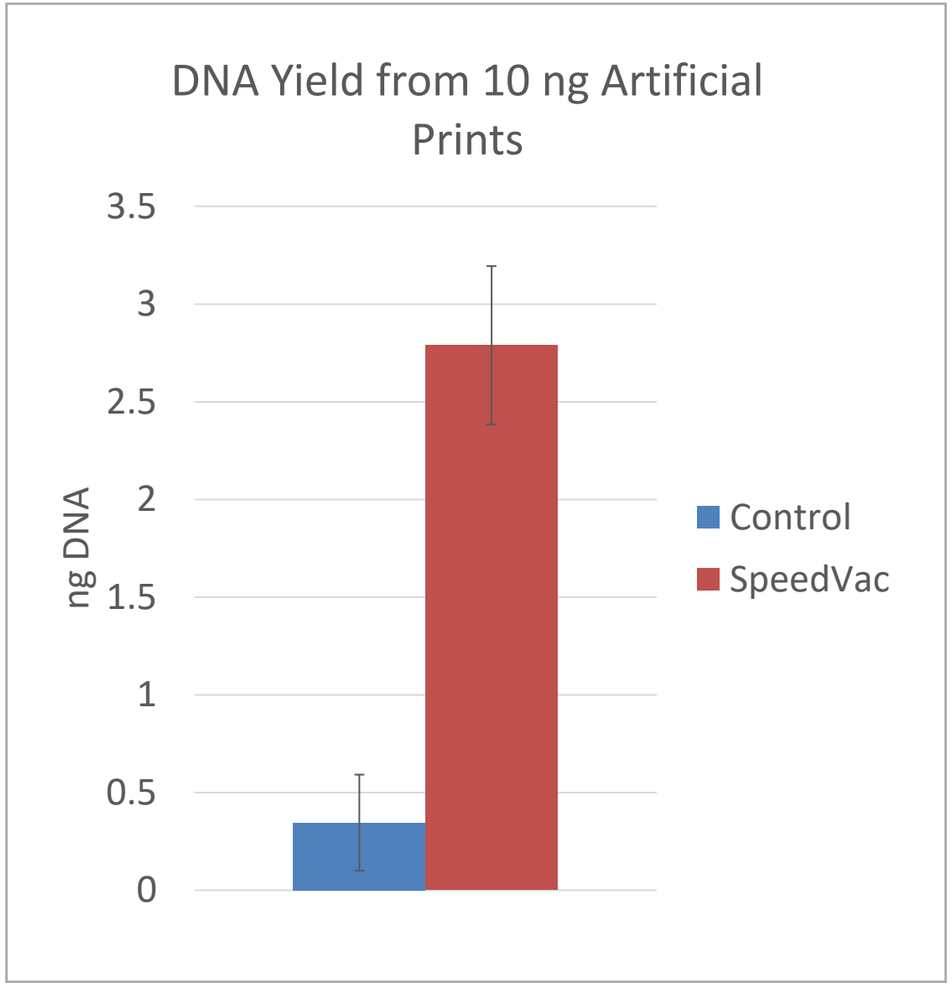


How to Turn Shell Casings Green

- Initial attempts to utilize DNA Touch on brass shell casings failed
- Apparent high levels of oxidation once artificial fingerprints had dried
- Attempts to recover DNA unsuccessful
  - Extremely low, if any, DNA yields from 10 ng artificial prints

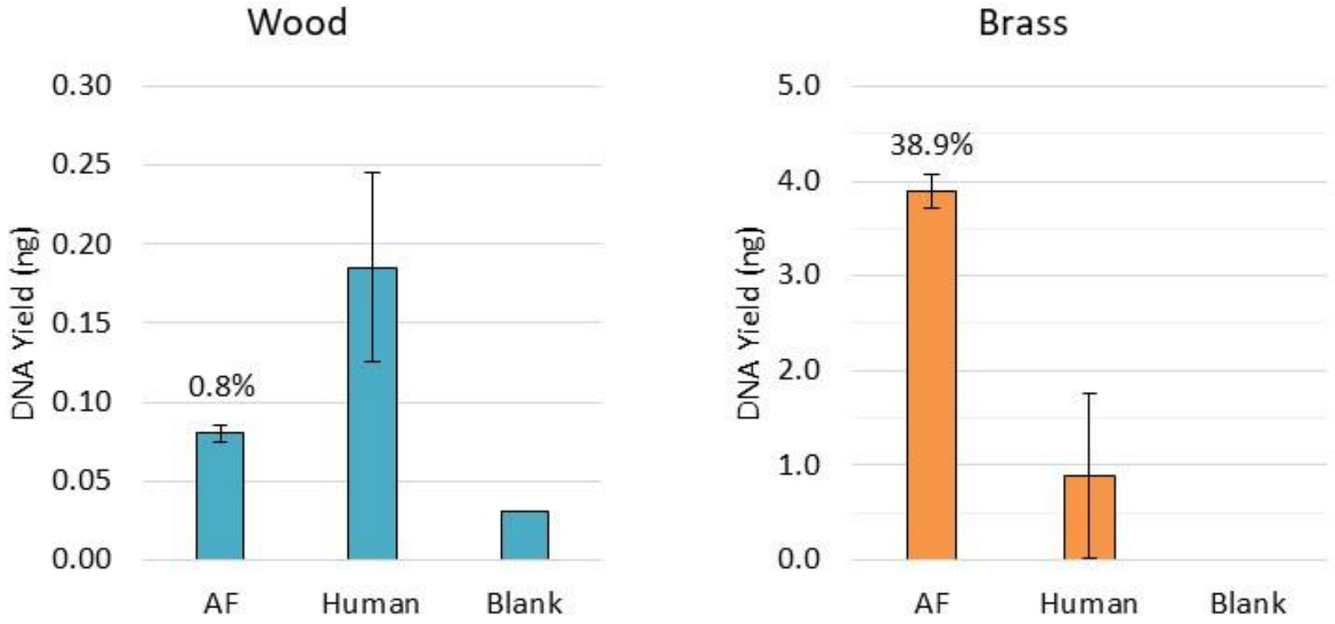
Rapid Drying to Improve DNA Recovery

- Can we minimize the time each sample spends exposed to the liquid stage of artificial fingerprint deposition?
- Artificial fingerprints with 10 ng gDNA on 9mm shell casings
- Placed into a speedvac (rotor removed) immediately following deposition to rapidly dry the sample
- Collected and extracted DNA and compared with artificial prints left to dry in ambient conditions
- Likely due to reduction in ROS formation
- Improved DNA recovery and lower DI



DNA Touch Print Pattern Visualization

- Artificial print DNA can be recovered from challenging surfaces
- No appreciable degradation across surfaces
- Recovery was surface variable
  - More porous surfaces are a current limitation
- Naked DNA shows especially poor compatibility with porous surfaces



DNA Touch Print Pattern Visualization

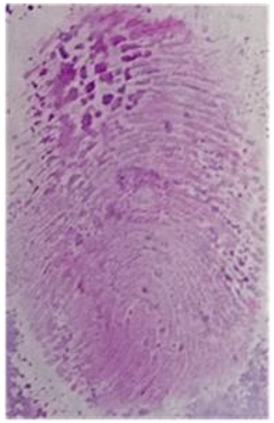
- Tested various imaging dyes/techniques on glass & tape
  - Cyanoacrylate fuming with fluorescent dyes (Rhodamine 6G/B)
  - Ninhydrin
  - Dusting powders (black & fluorescent)
- Print visualization successful on multiple surfaces
- Dusting powders failed to adhere

Glass

Unstained

Glass

Cyanoacrylate
Rhodamine 6G



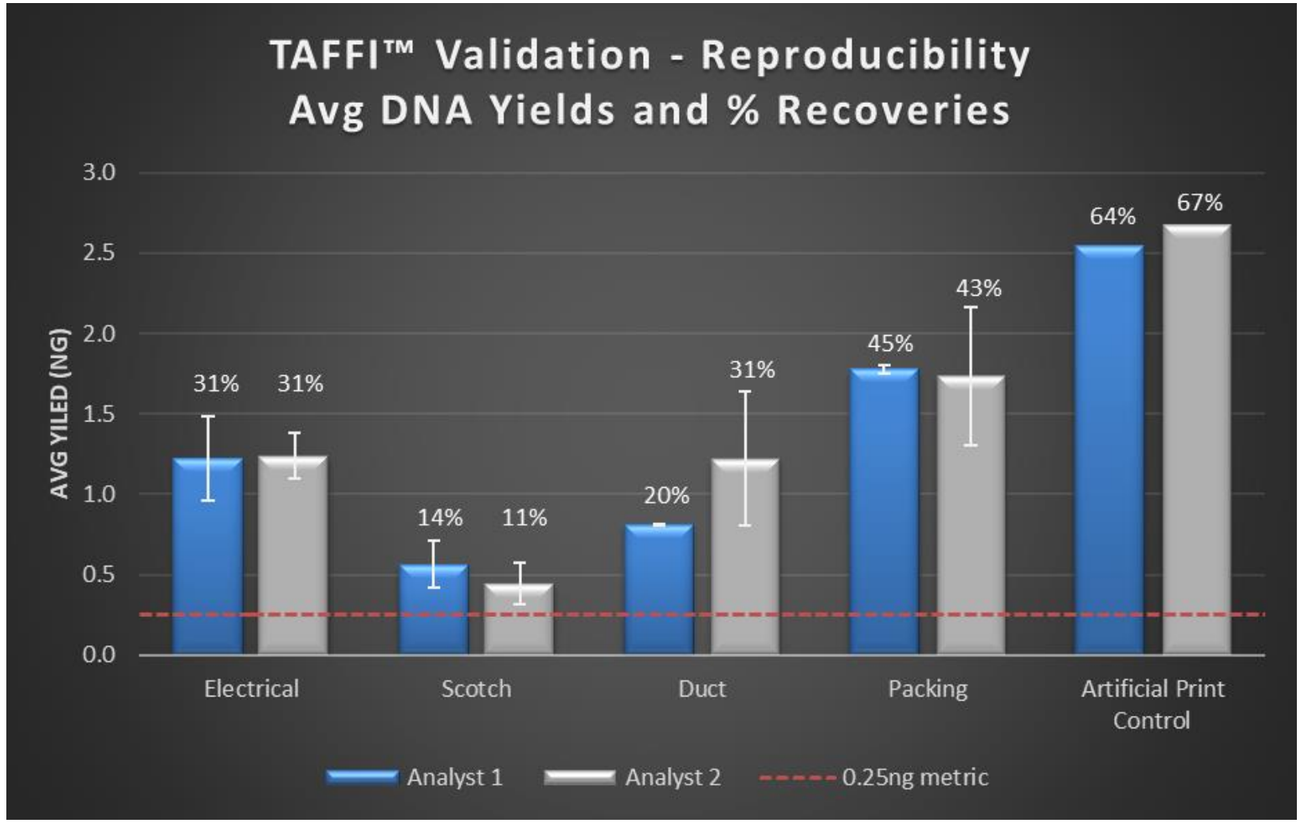
Ninhydrin

Electrical Tape

Cyanoacrylate
Rhodamine B

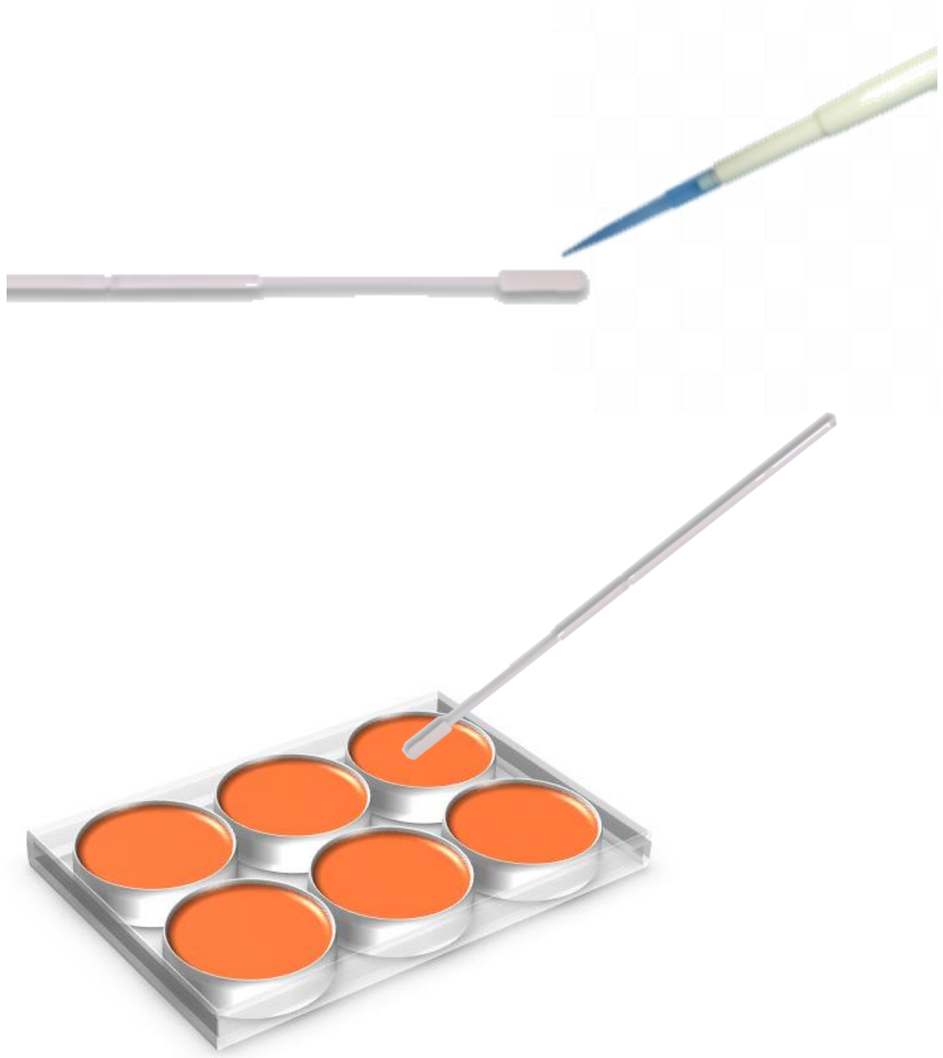
Extraction from Adhesives

- Artificial prints were placed on multiple types of tape
- DNA collected and extracted by two analysts
- No-collection control processed in parallel (DNA Touch placed in tube)
- Method showed robust, consistent results and similar yields across analysts/replicates



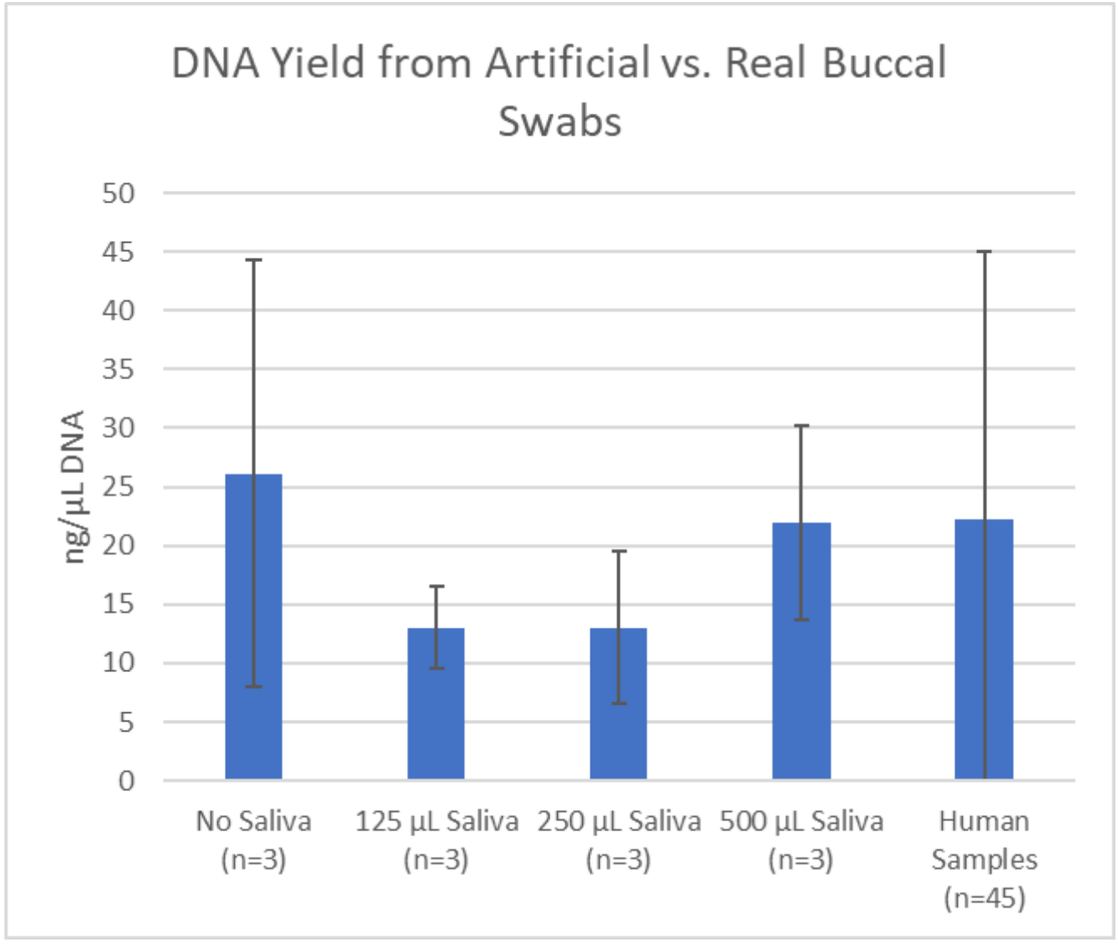
Synthetic Buccal Swabs

- Can we create realistic synthetic buccal swabs using human tissue culture?
  - Epithelial cells (A549)
  - Artificial saliva
  - Standard flocked swabs
- Multiple potential loading methods
  - Precise loading of the swab tip with a known number of cells
  - Direct swabbing of the plate to evaluate collection efficiency
- Various potential uses
  - Evaluation of collection tools
  - Assessment of differential extractions
  - Public health sample surrogates



Synthetic Buccal/Nasal Swabs

- Recovery from collections of artificial samples closely resembles actual buccal swabs collected in previous validation studies
- Recovery amount can be modulated, and more or less artificial saliva can be used
- Considering variability in collection, artificial samples still show lower variability than human samples



Conclusions

- Artificial samples successfully avoid additional regulatory steps and decrease variability associated with human samples
- Artificial samples do not perfectly recapitulate human signatures
  - Relative performance must be evaluated on the matrix of interest
- Artificial samples are a supplement to, not a replacement for, human samples
  - Goal is to dramatically reduce the number of human samples required for verification or validation

Path Forward

- Continue to integrate artificial prints into R&D studies

- Improve and characterize artificial print samples across a wide range of matrices and conditions
  - Metal, porous (paper, wood, etc.), and non-porous surfaces
  - Consider stability of artificial prints vs. human samples over time or in challenging conditions (e.g., heat, direct sunlight)

- Go back in time and repeat all the relevant studies
  - Highlights the need to develop accurate, synthetic standards now to position current and future studies for success

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