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A Proteos Program Retrospective: Establishing Protein Sequencing for Forensic Analysis

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Outline

- IARPA Proteos Program Overview
- Technical Approach
- Project Highlights
- Limitations
- Future Research Considerations

IARPA PROTEOS Program

- Intelligence Advanced Research Projects Activity (IARPA)
- Seeks to Develop Novel Methods for Human Identification by Protein Sequencing of Touch Samples
- Develop Methods to Efficiently Co-Extract Protein and DNA in Parallel

Office of the Director of National Intelligence

IARPA

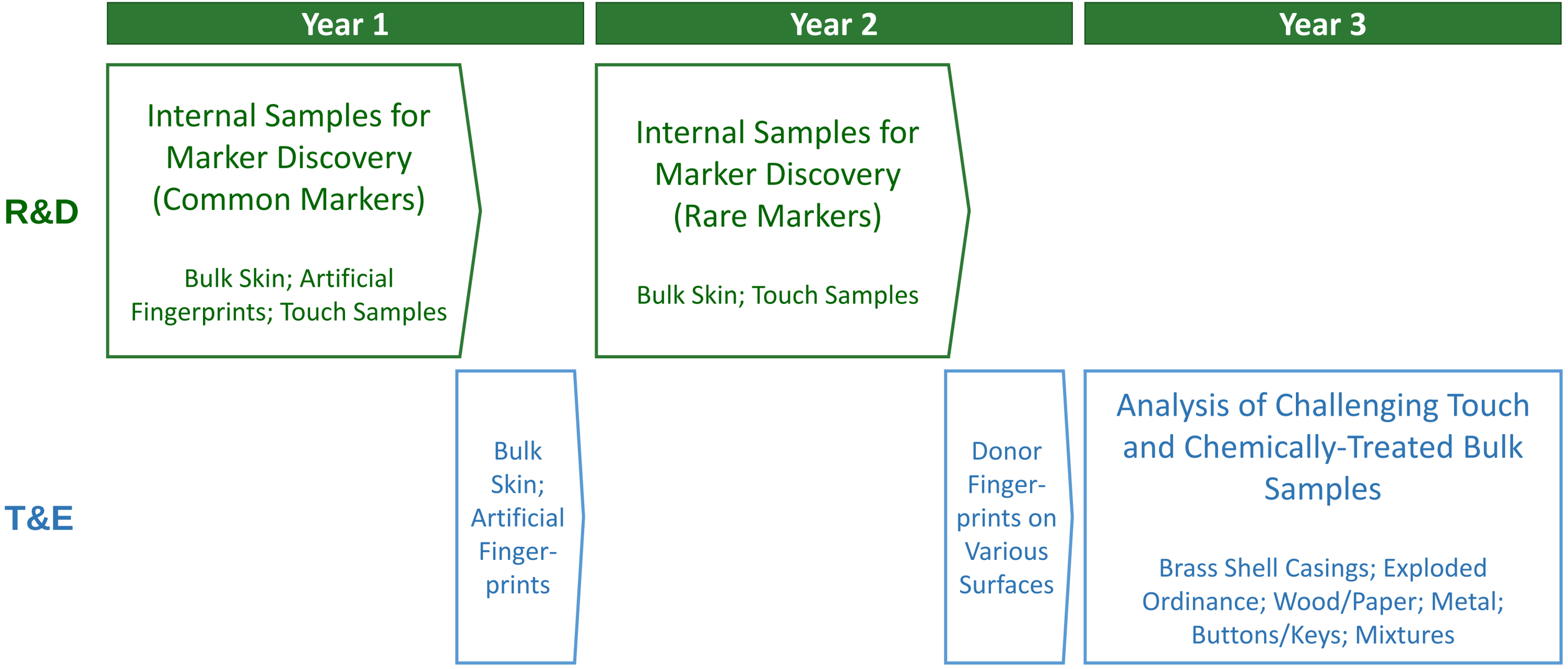
BE THE FUTURE

- SigSci teamed with UNT CHI and The Ohio State University for Proteos

THE OHIO STATE UNIVERSITY

CAMPUS CHEMICAL INSTRUMENT CENTER

IARPA PROTEOS Program

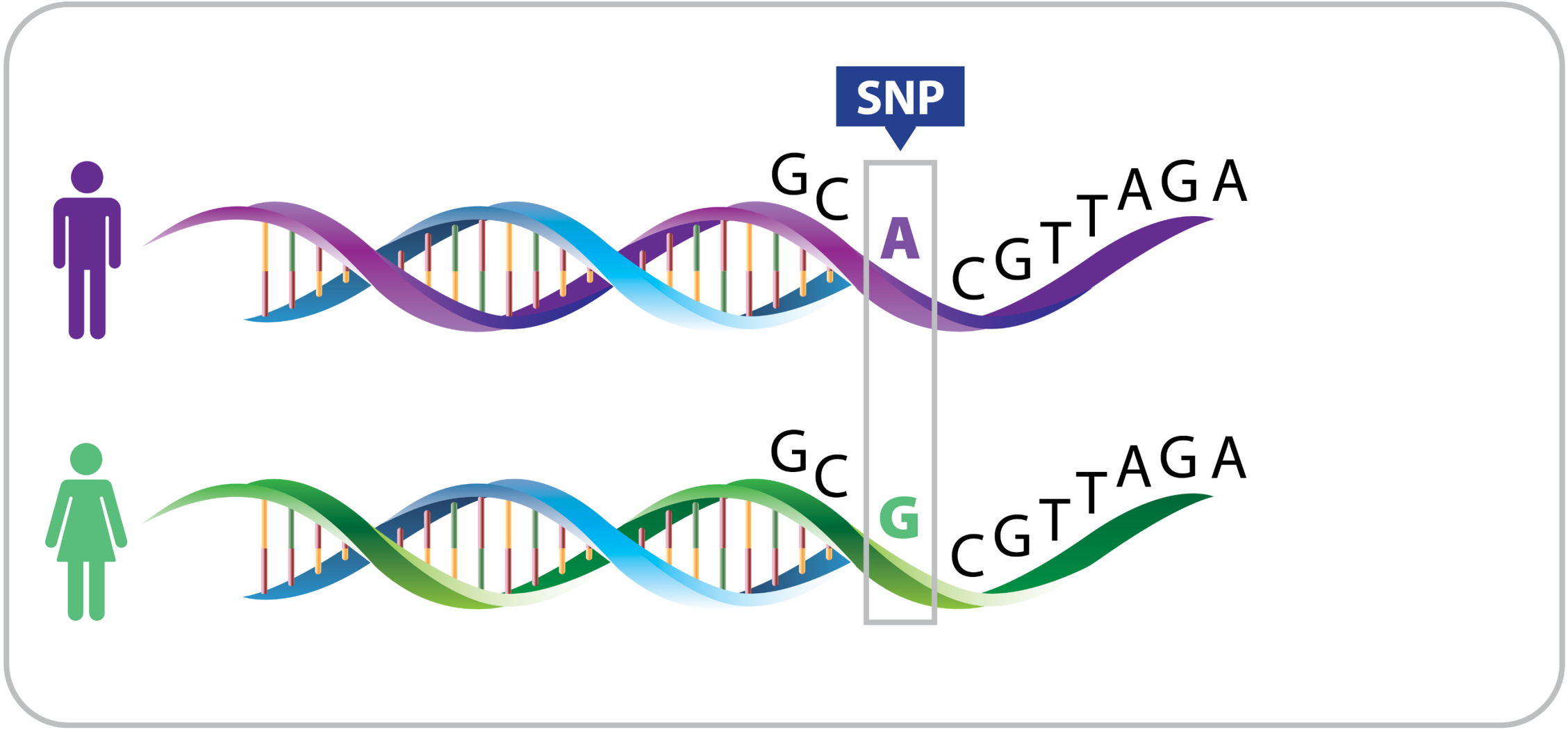


Challenges in Human Forensic Identification

Components of Touch Samples

- Ridge Pattern
- Sebaceous Fluids (Oils, Sweat)
- Extracellular DNA
- Keratinized Skin Cells

Current State of the Art – DNA Forensics



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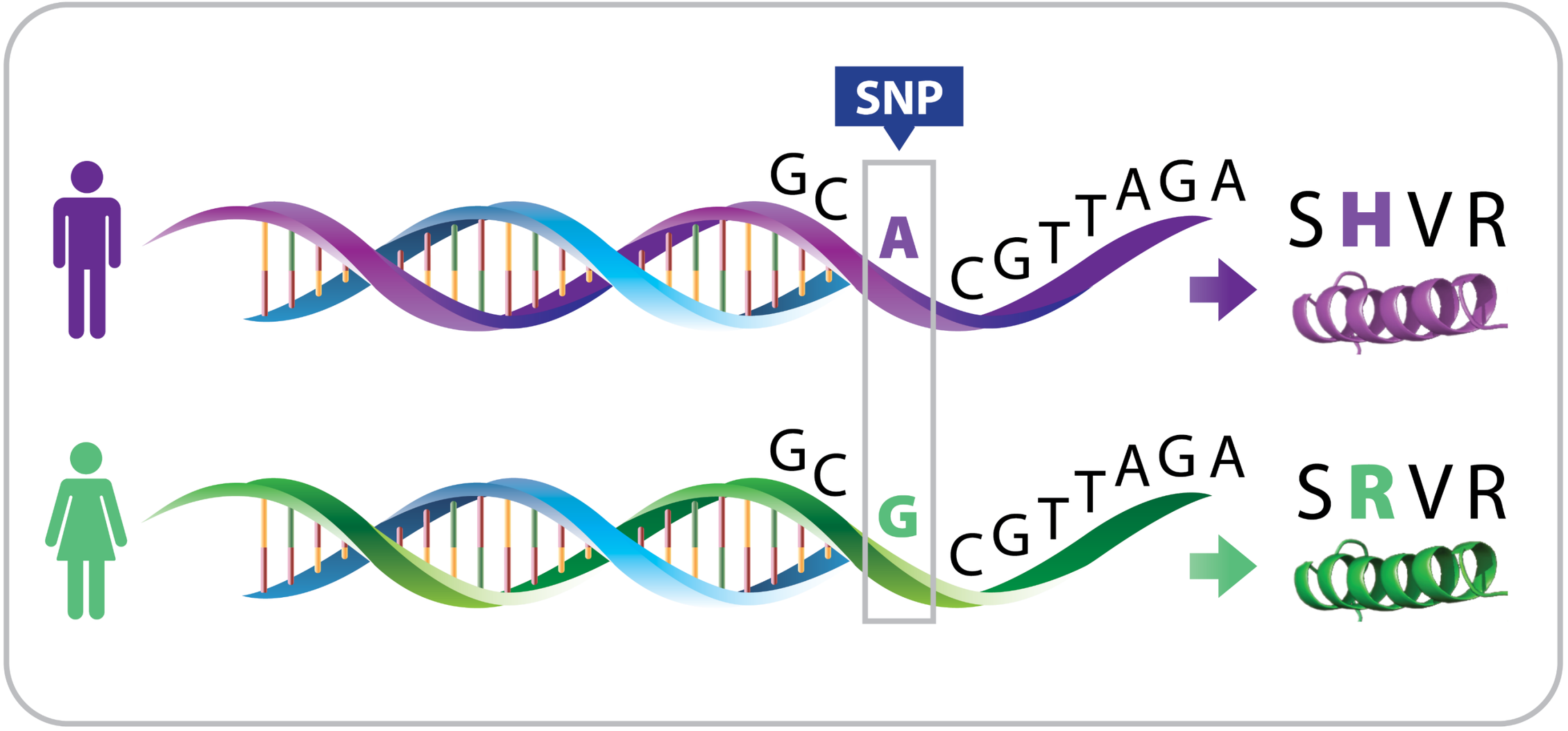


Thought Experiment…

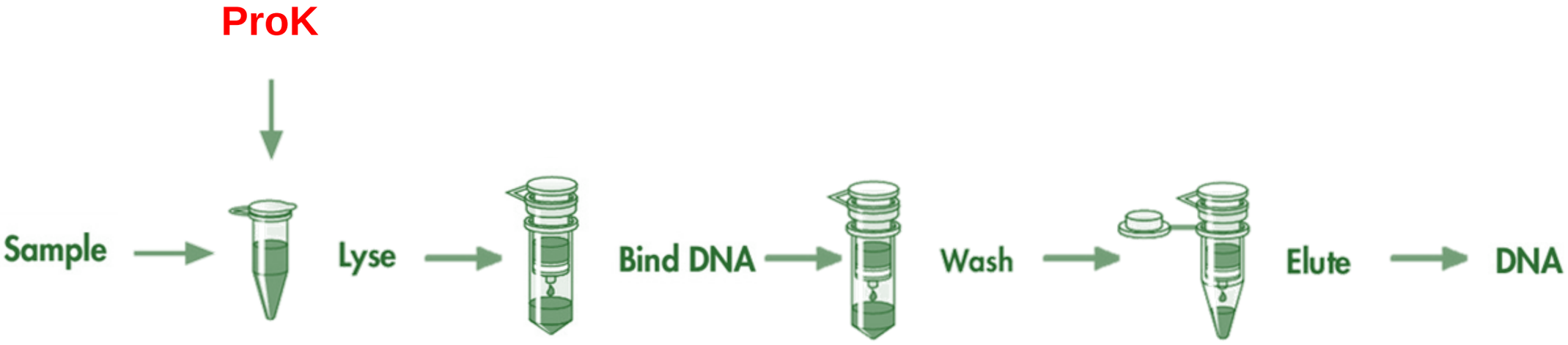
Let’s assume:

1. Protein sequencing has utility for forensic identification

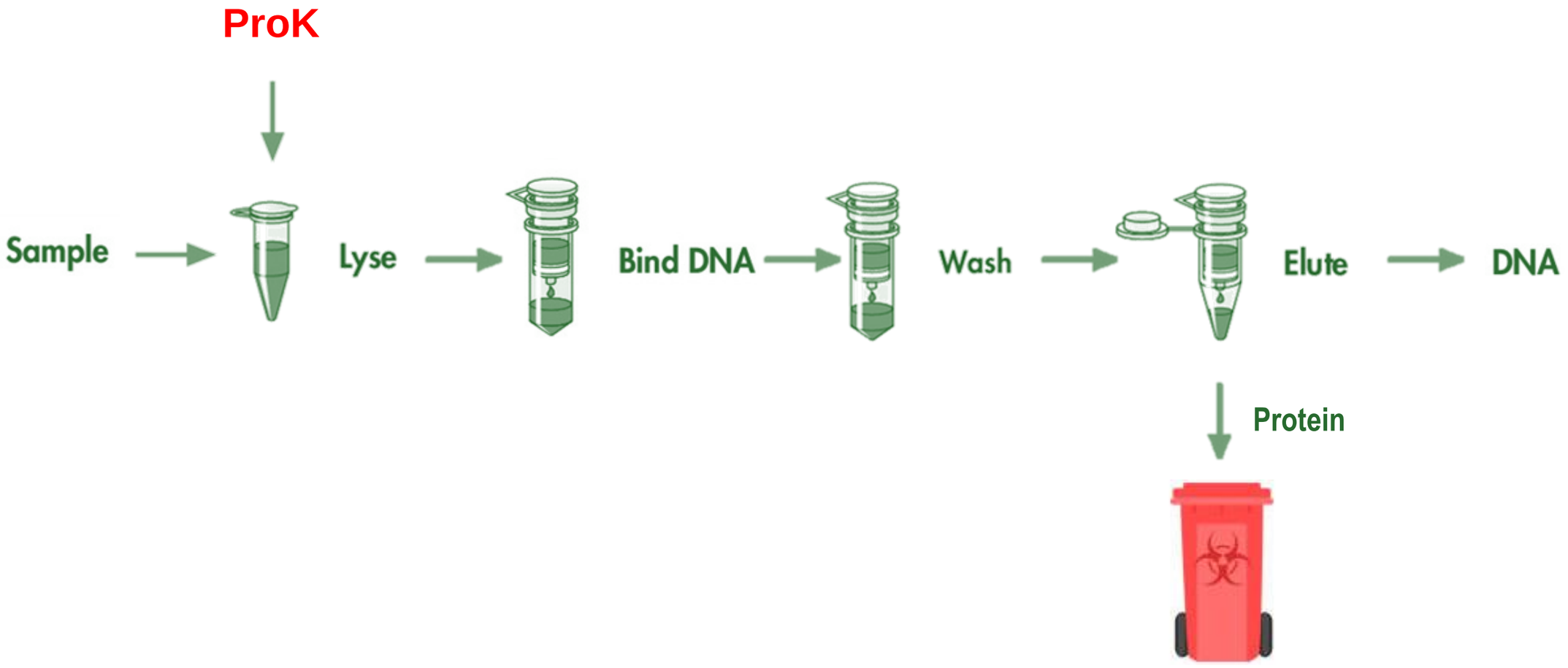
2. When you collect a touch sample, you’re collecting both DNA and protein

Extraction of Protein from Fingerprints

Extraction of Protein from Fingerprints

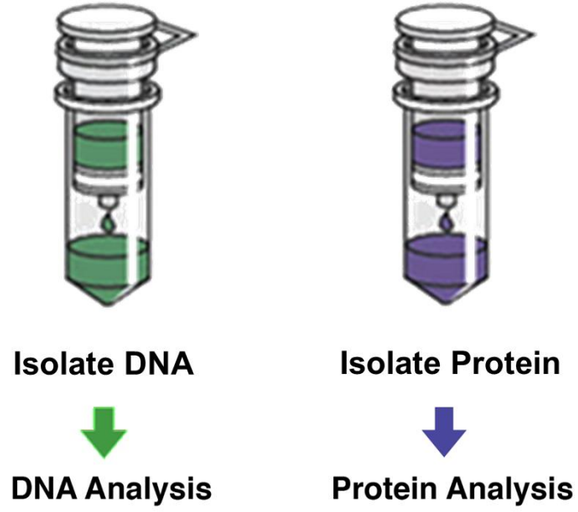


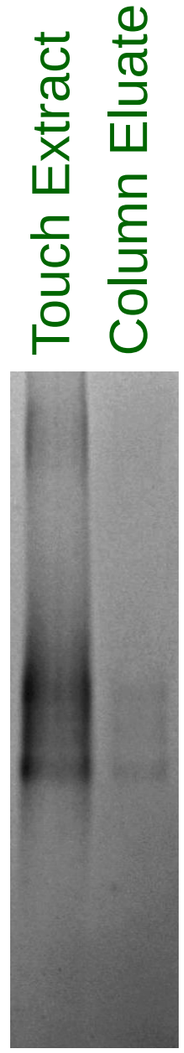
Extraction of Protein from Fingerprints



Protein Recovery from DNA Extraction Columns

- No significant protein detected in DNA column flow-through or washes
  - High salt concentration would complicate analysis
- Protein can be recovered from the column matrix following stringent denaturation/elution
  - Heat + DTT
- ~25% recovery of total protein in sample





Extraction of Protein from Fingerprints

1

Visualize Fingerprint

2

Solubilize and Collect

3

Bind DNA and Protein with DNA Investigator

4

Isolate DNA

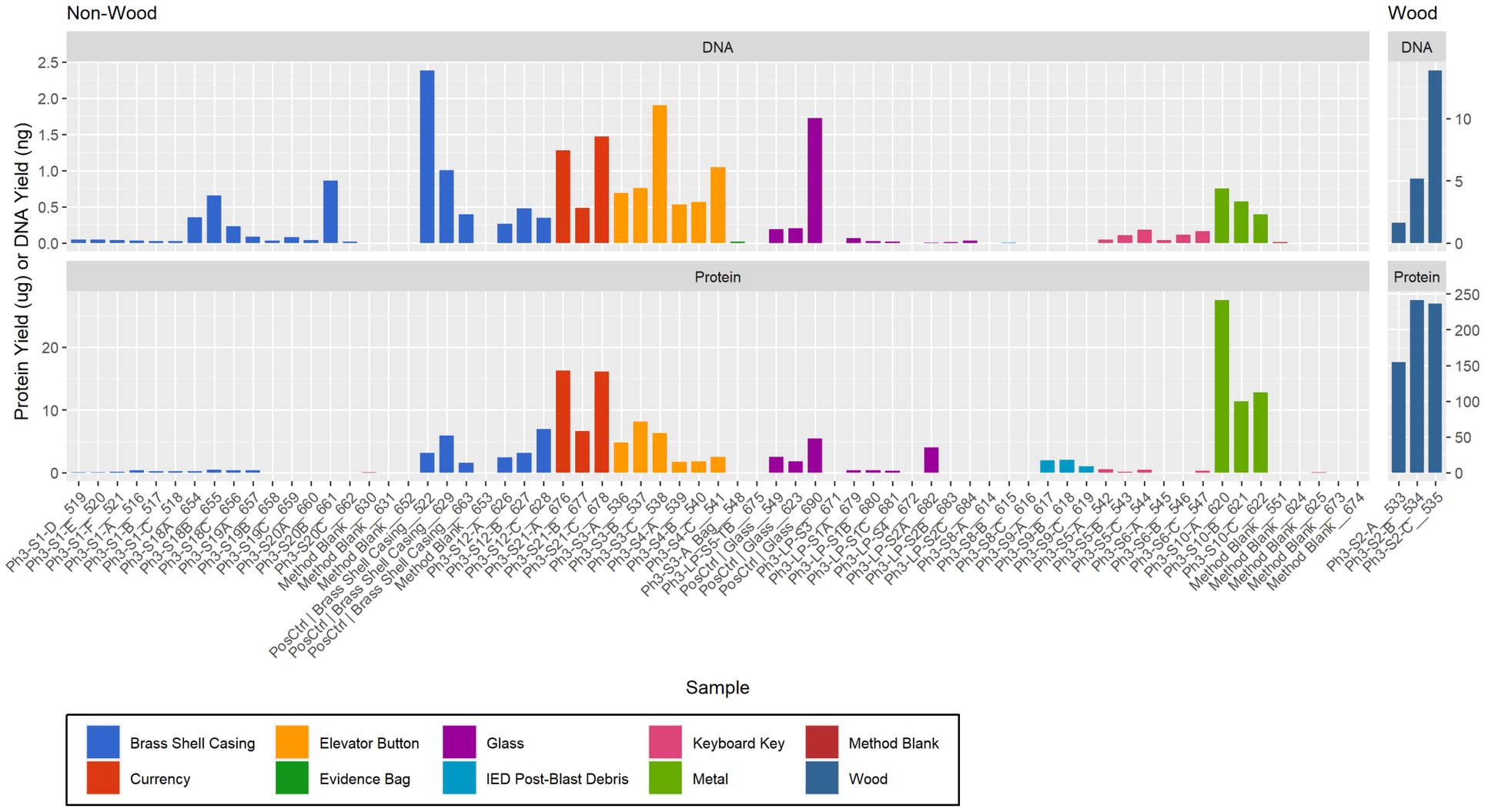
DNA Analysis

5

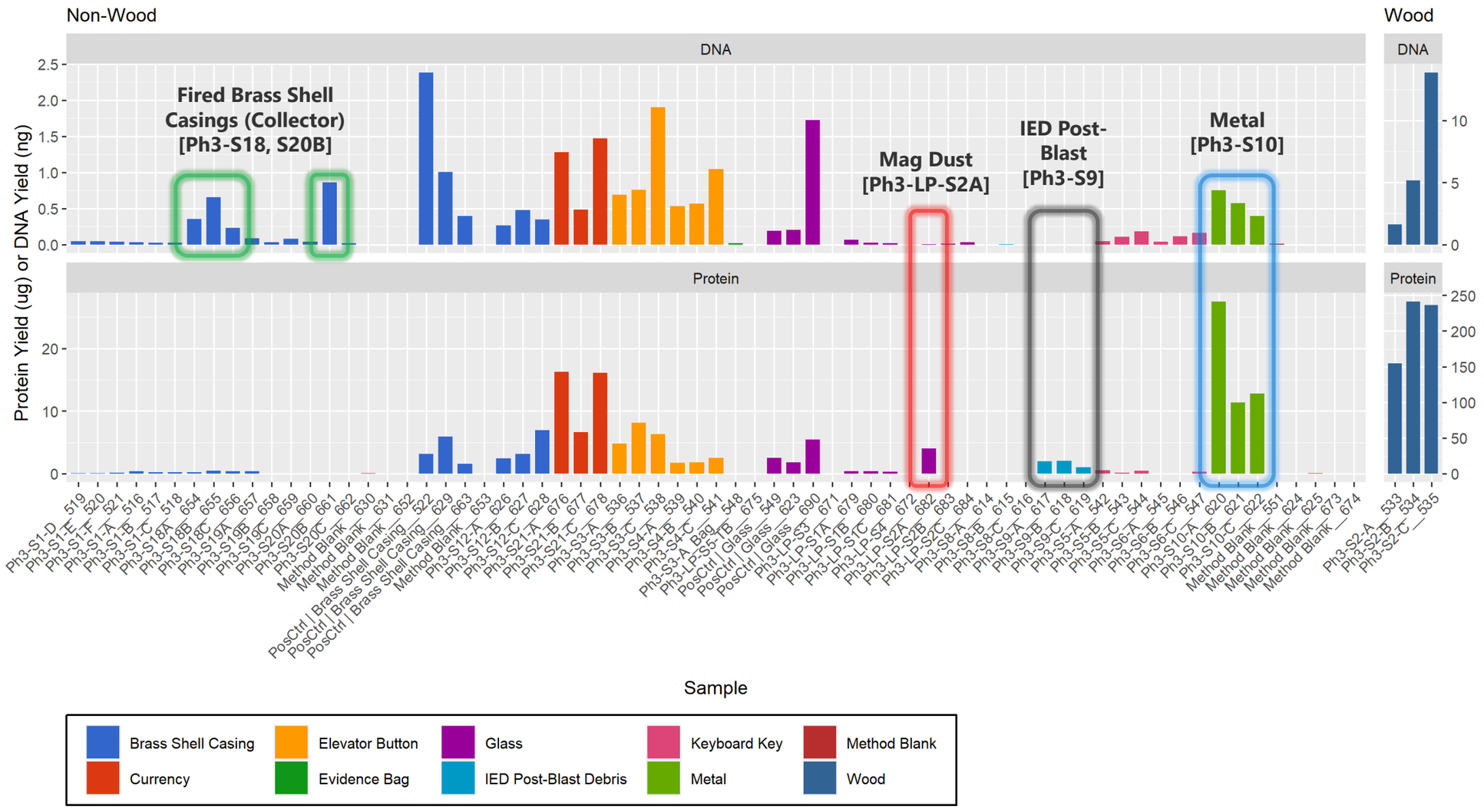
Isolate Protein

Protein Analysis

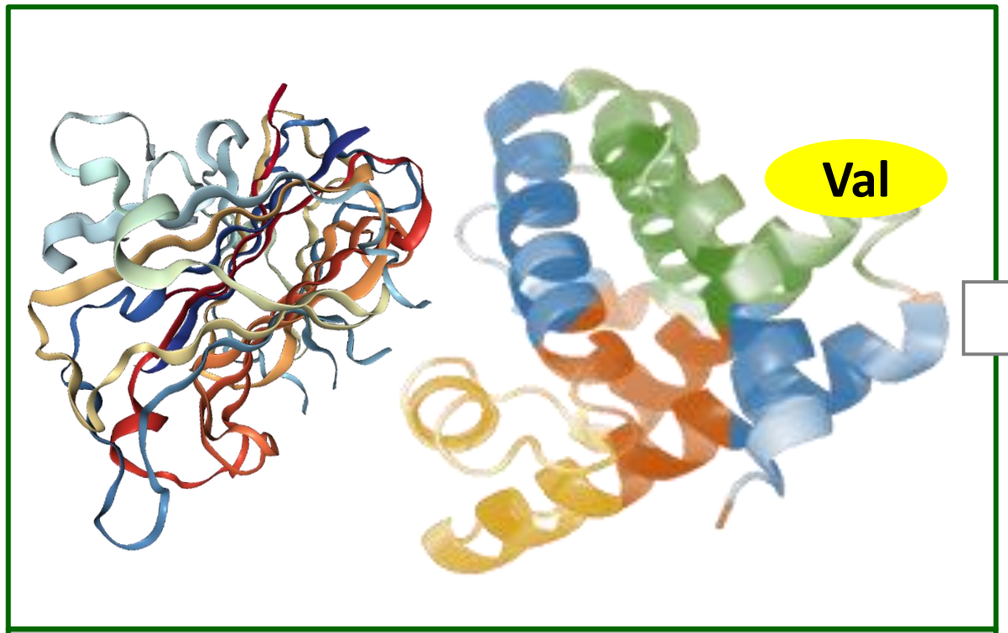
Correlation Between DNA and Protein Recovery



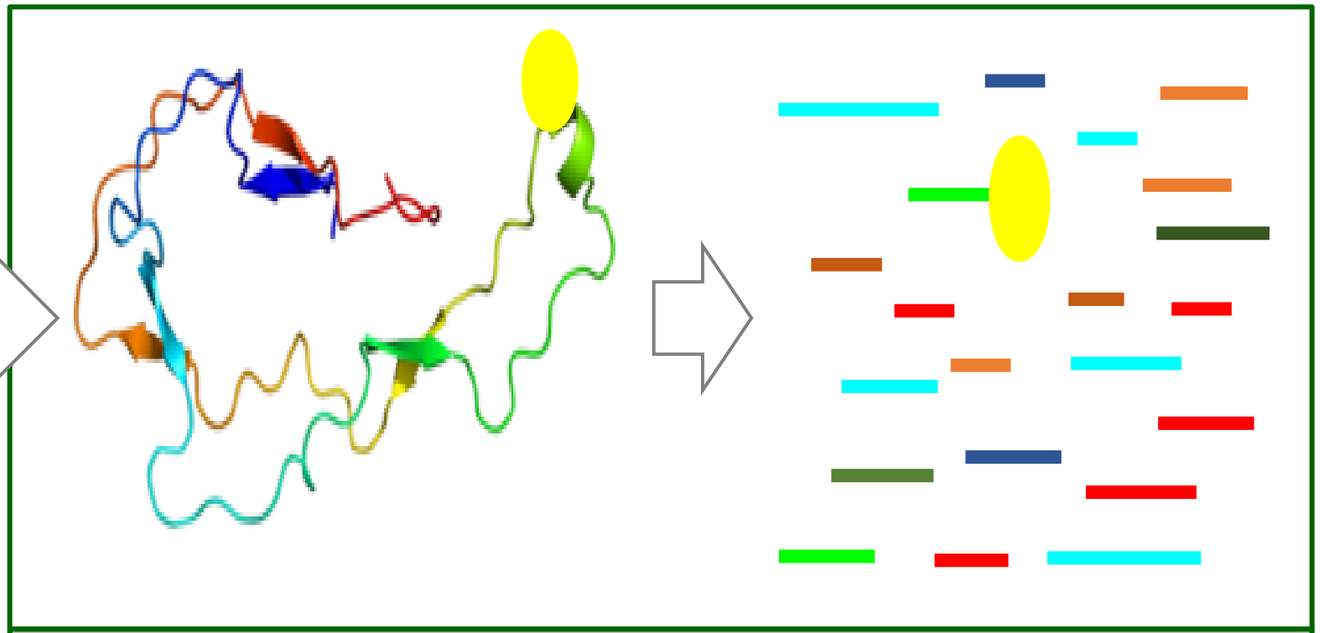
Correlation Between DNA and Protein Recovery



Proteogenomic Chemical Analysis



Extracted Protein



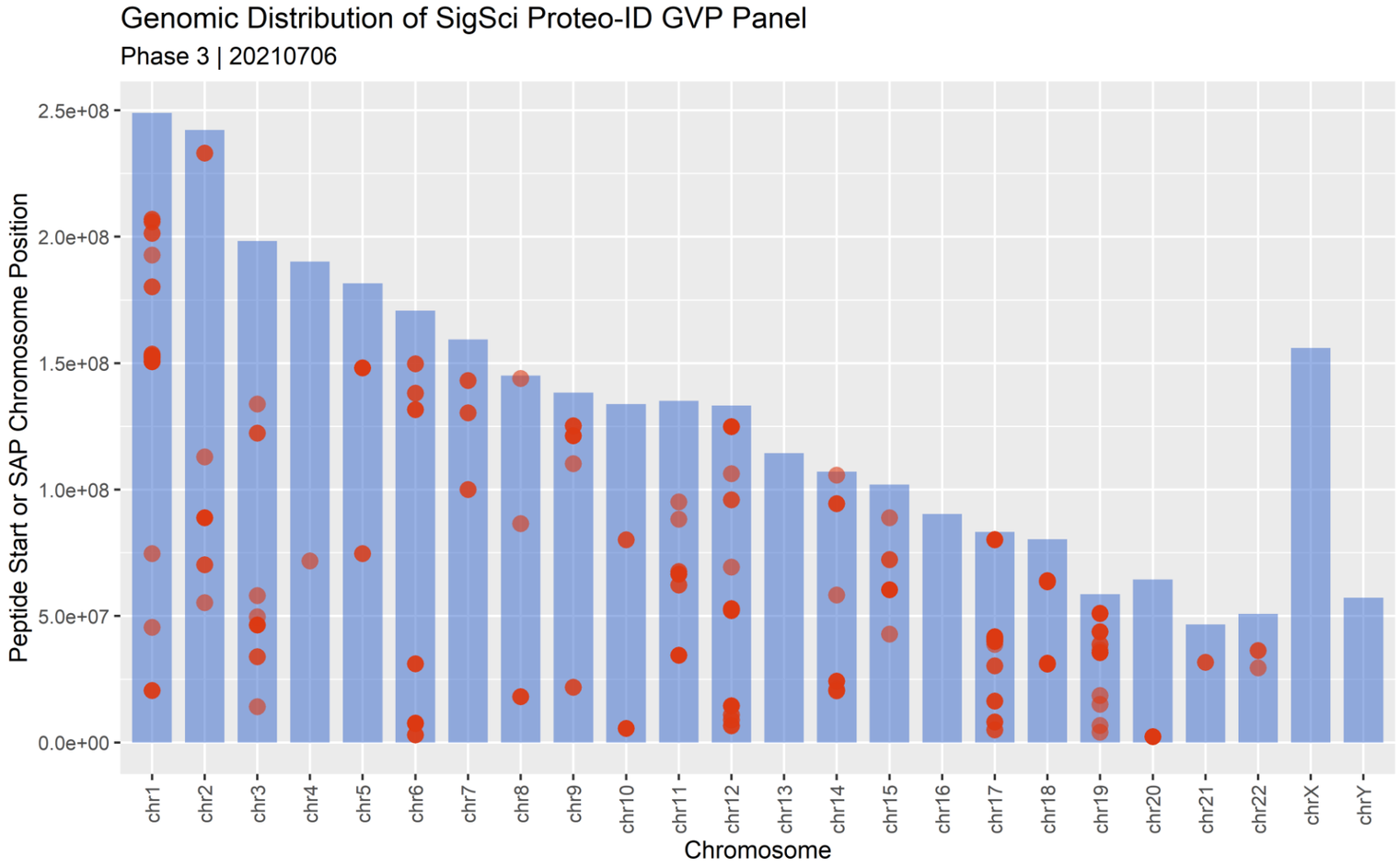
Enzymatically Digest Protein into Peptide Segments

Proteomic Analysis Method | LC-HRAM-MS2

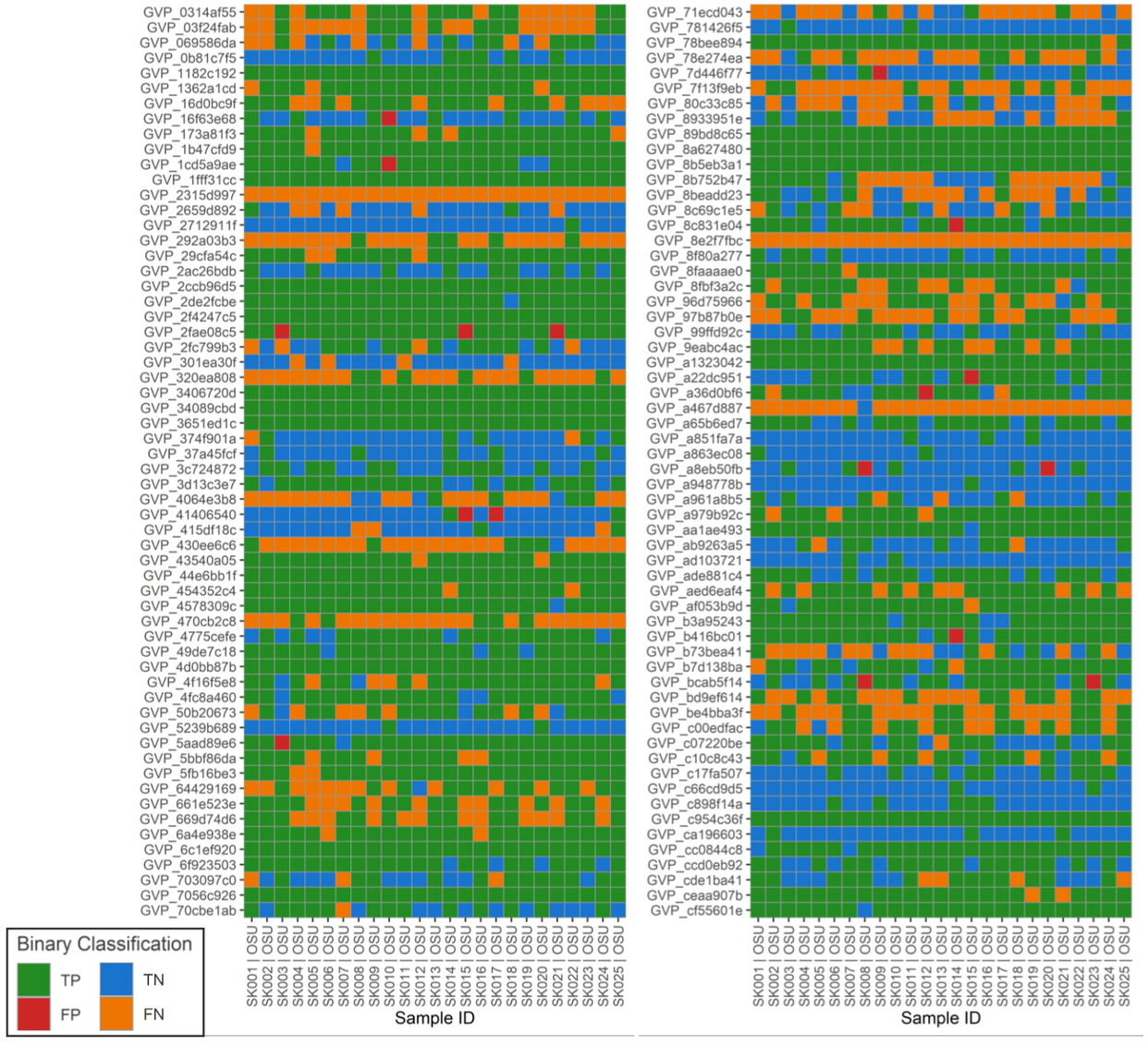
- Nano-LC
  - Thermo Scientific Ultimate 3000 RSLCNano
  - LC Run Time: 320 min (5 h gradient)
- High Resolution, Accurate MS2
  - Thermo Scientific Q Exactive Plus
  - Data Dependent MS2 or PRM Resolution = 17,500

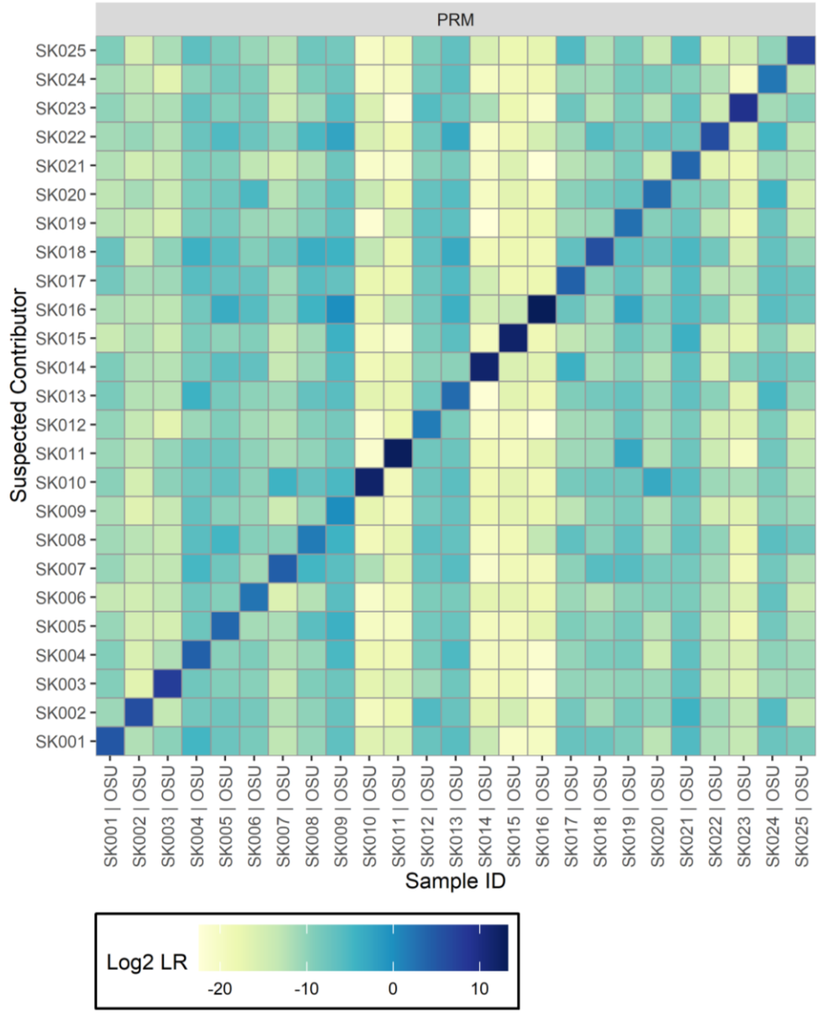
Genome-Wide Marker Distribution

Statistic	Value
Unique Proteins	445
Reference GVPs	281
Variant GVPs	191
Unique to 1 Donor	49
Unique to 1 T&E Donor	39
Unique to 1 SigSci Donor	42

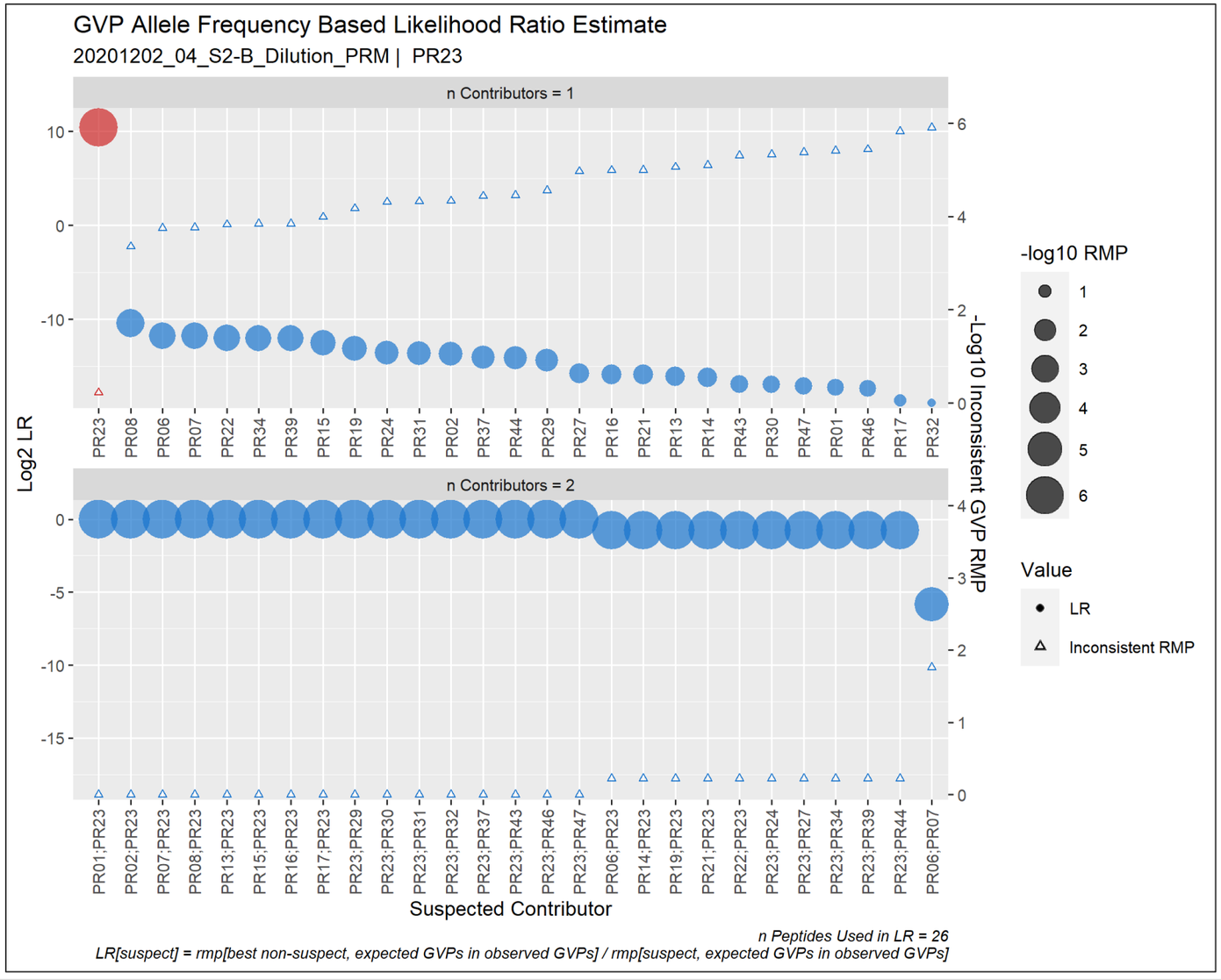


PRM Analyses of Internal Donors Using Common GVPs





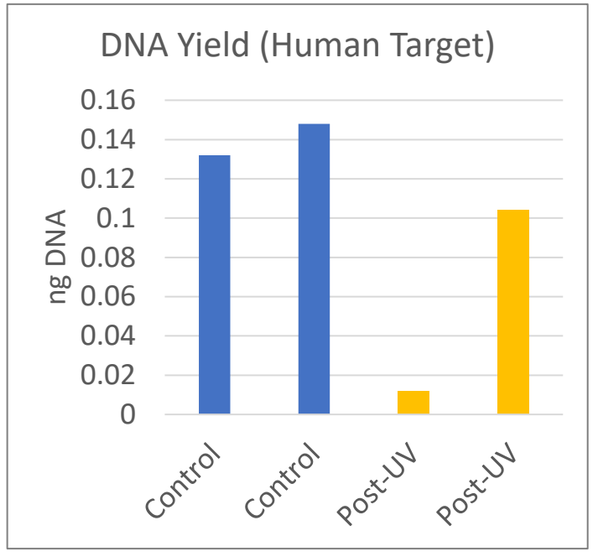
Simple Likelihood Ratio Calculations

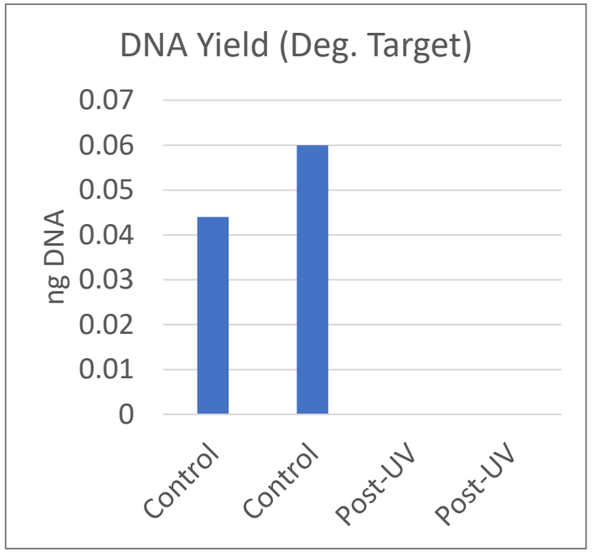


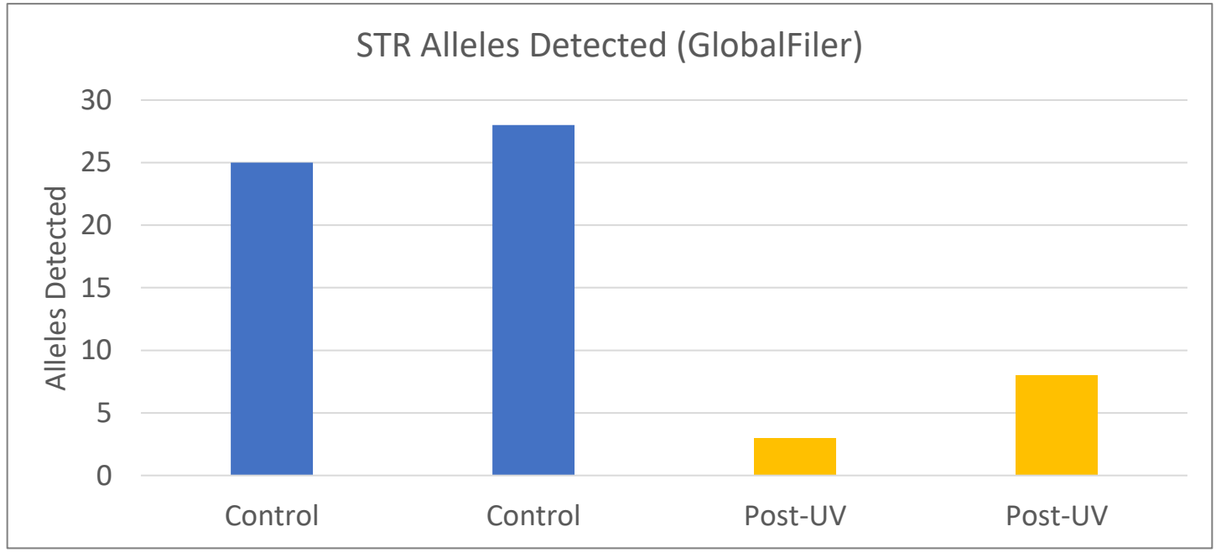
Project Highlights

DNA Degradation | Case Study

- Internal touch samples collected on plastic coupons
  - Controls (no UV)
  - UV treatment
  - Two replicates of each
- High degradation index
- Major decrease in STR alleles detected

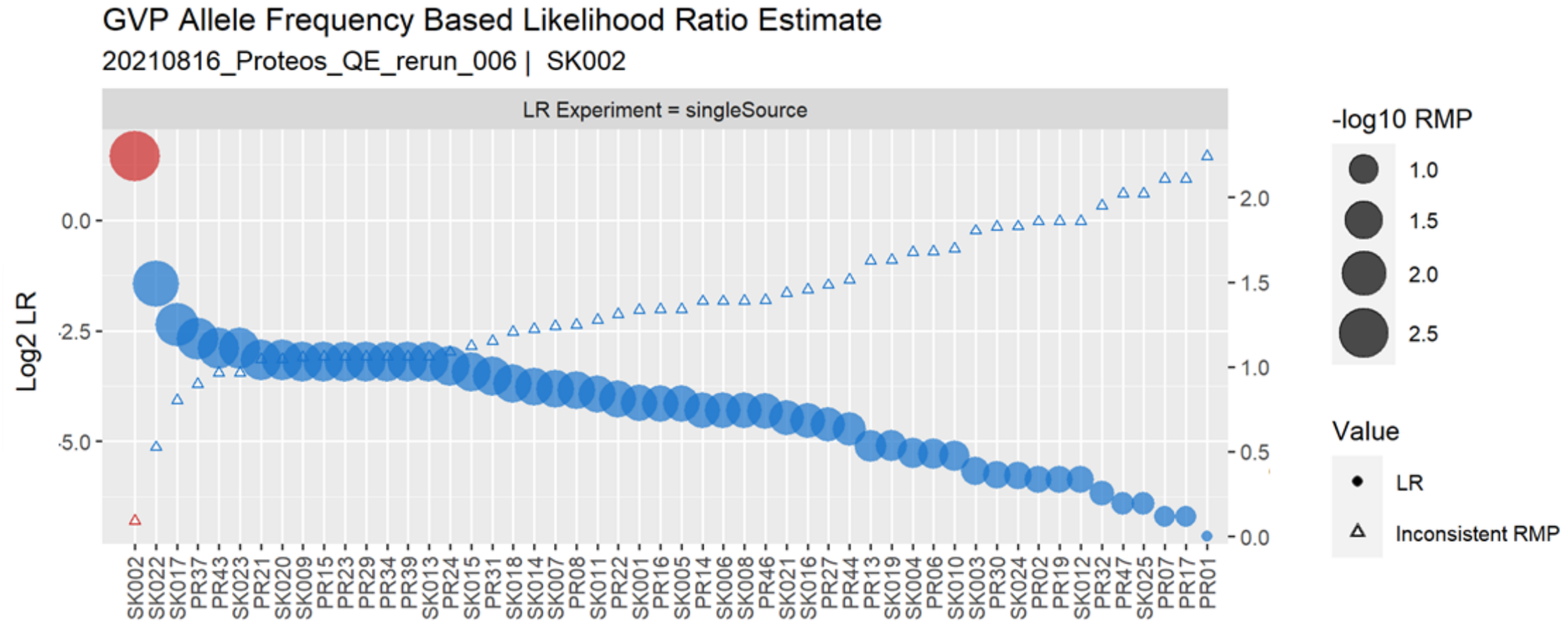






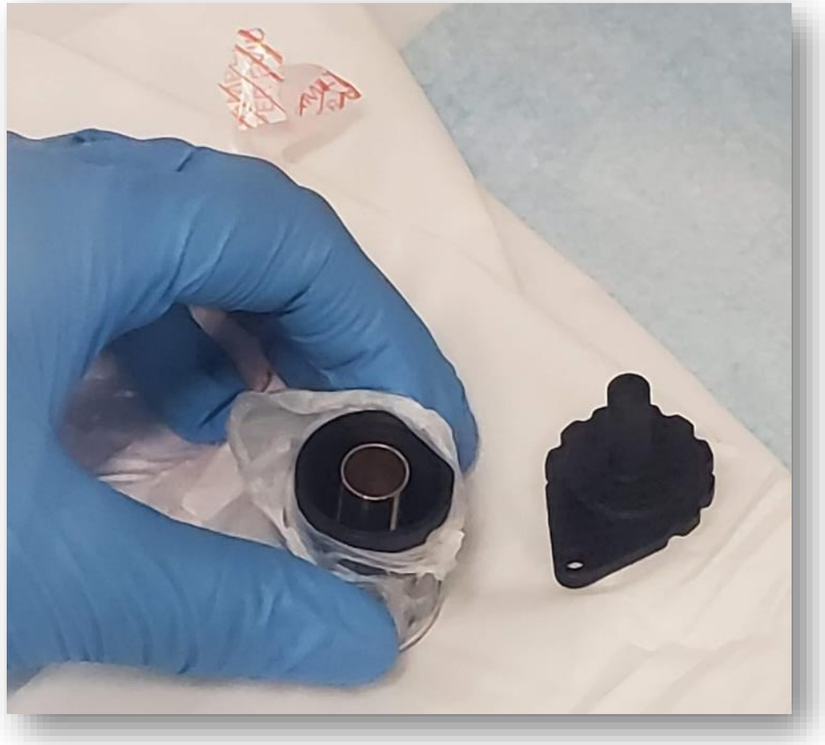
DNA Degradation | Case Study

- Following UV-exposure, 24 variable peptides identified
- Identified correct donor out of panel of 52 individuals



Brass Shell Casing | Case Study

- 9mm Brass Shell Casings (Blinded):
  - Three replicates
  - Question: Who is/are the contributor(s)?
- Contradictory results:
  - DNA:
    - PR02 = major contributor
    - Probable mixture, but insufficient data for comparison to any reference profiles
  - Protein
    - PR01 = major contributor
    - PR14 = minor contributor



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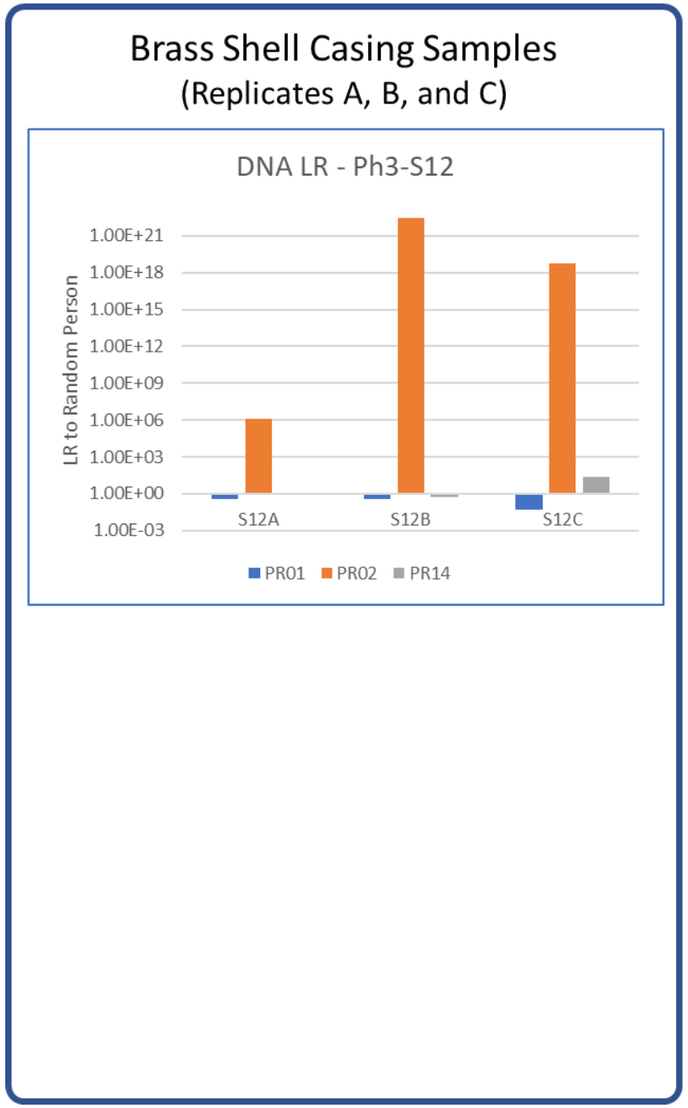

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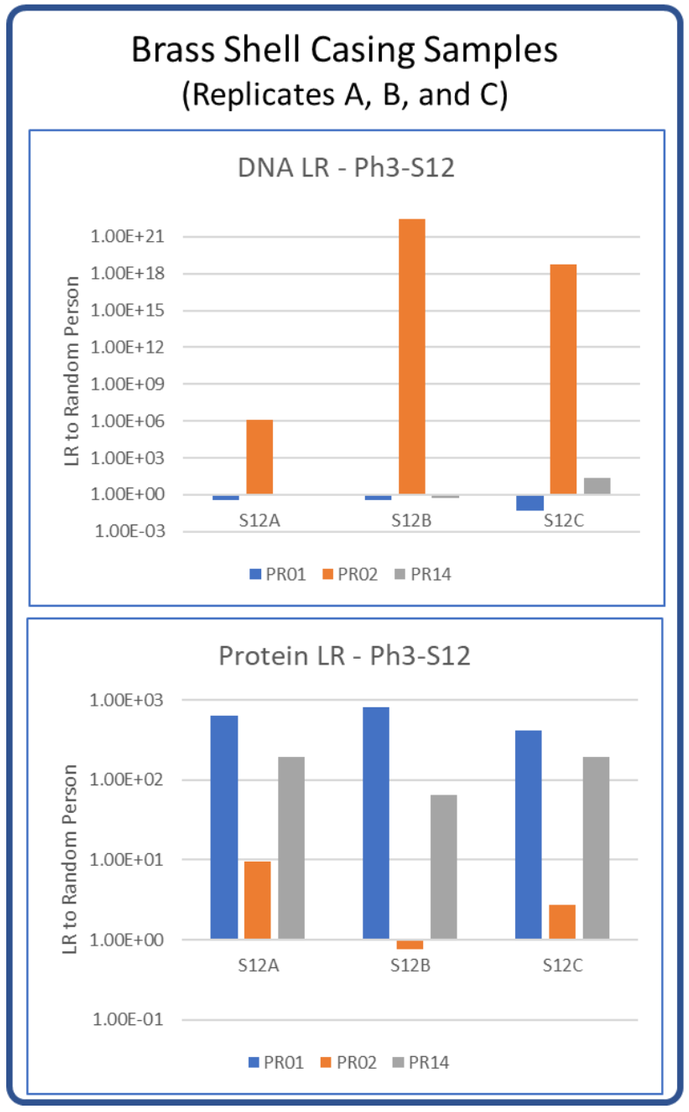

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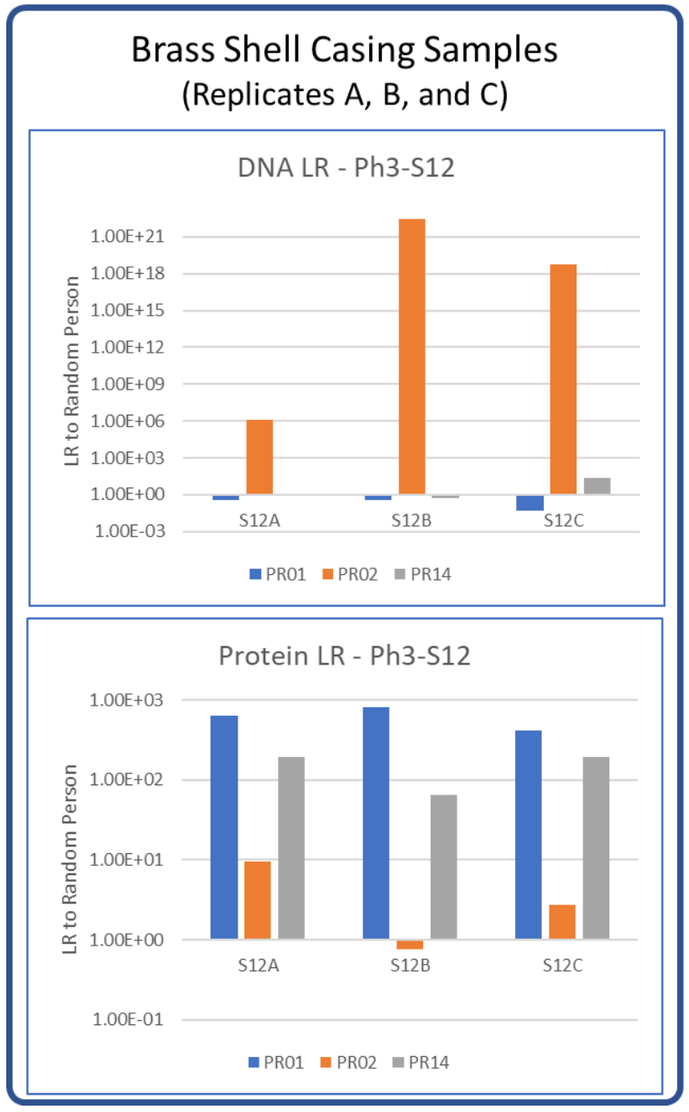
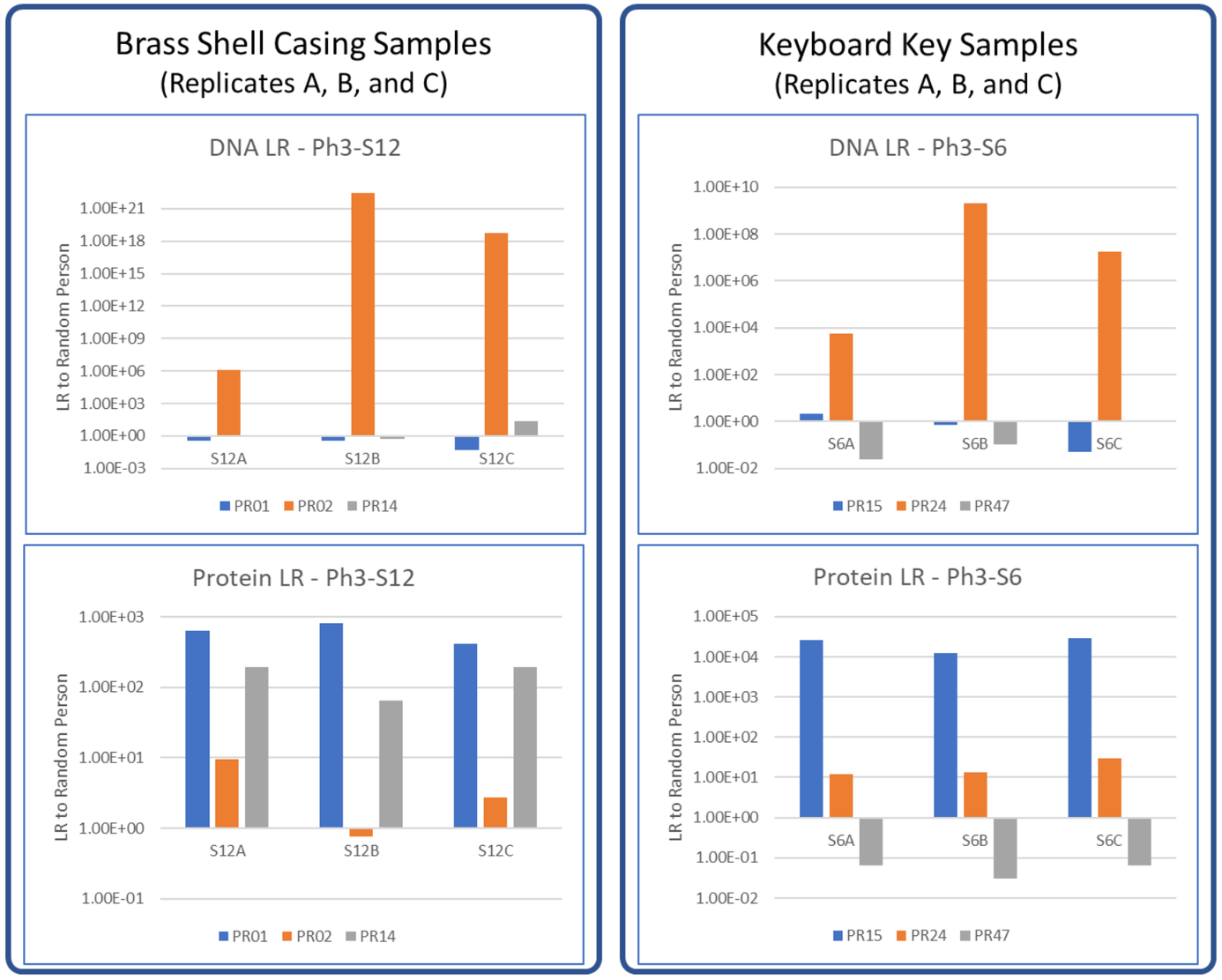


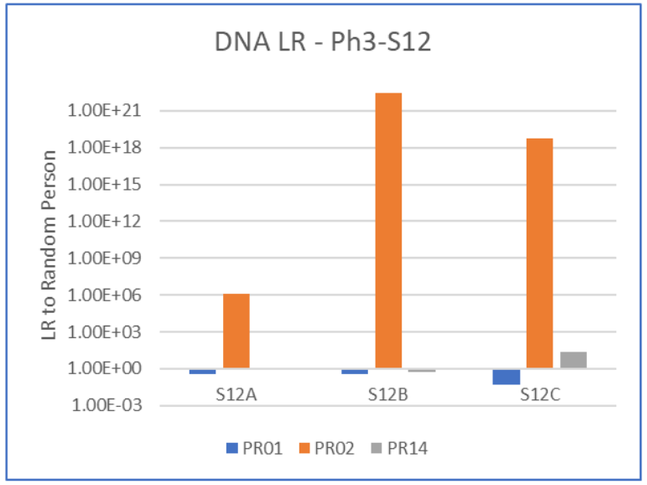
- Ground Truth:
  - Samples provided were mixtures
  - Three contributors: PR01, PR02, and PR14
- Contradictory results were actually complimentary
- How broad is the utility of combined DNA/protein analysis for mixtures?

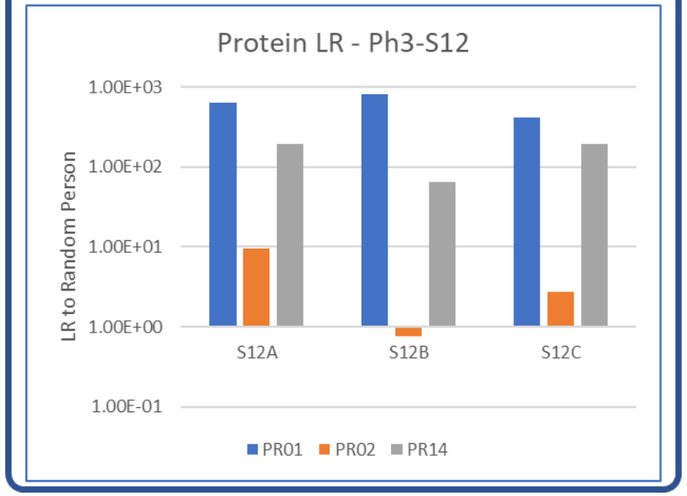
Brass Shell Casing | Case Study



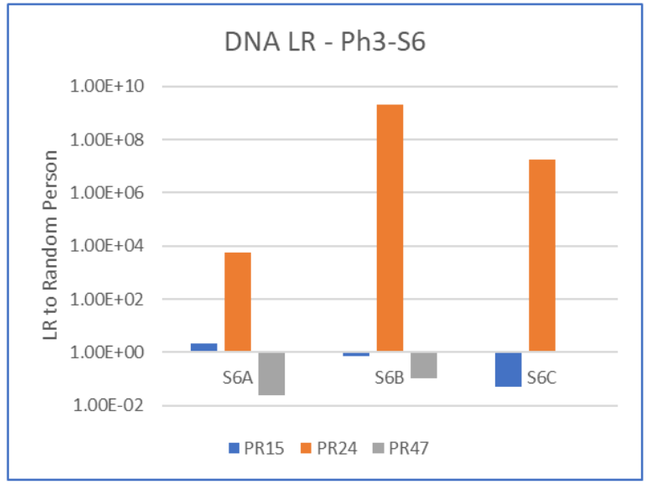
Brass Shell Casing | Case Study

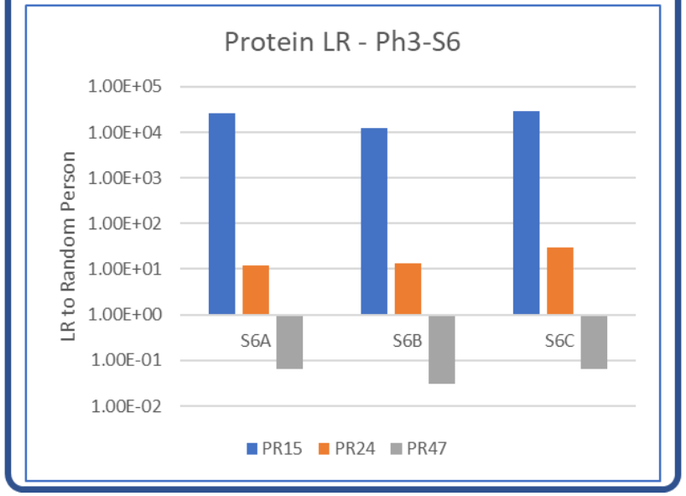
Brass Shell Casing Samples (Replicates A, B, and C)



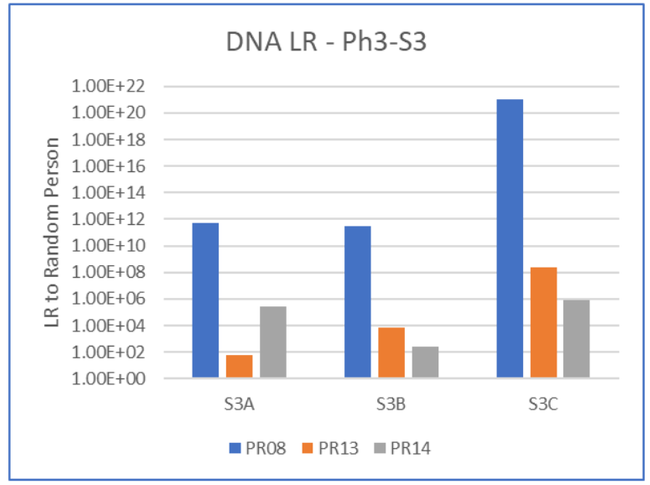


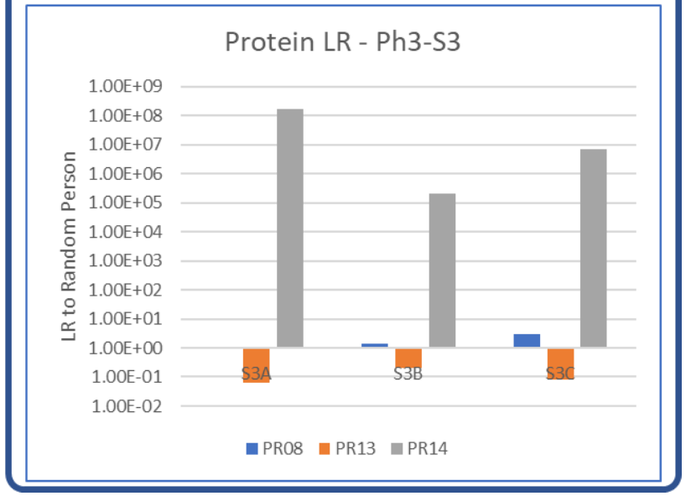
Keyboard Key Samples (Replicates A, B, and C)





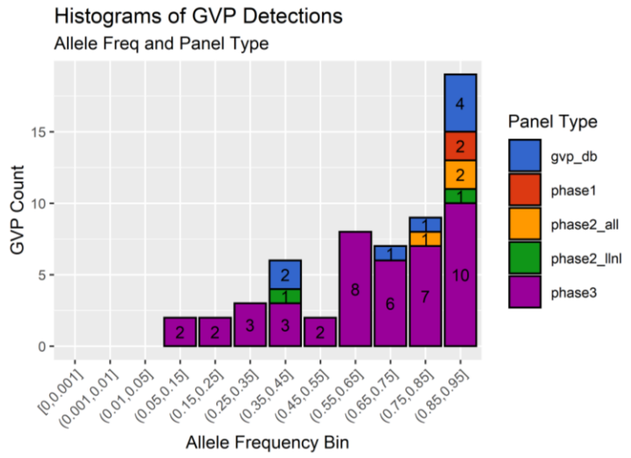
Elevator Button Samples (Replicates A, B, and C)

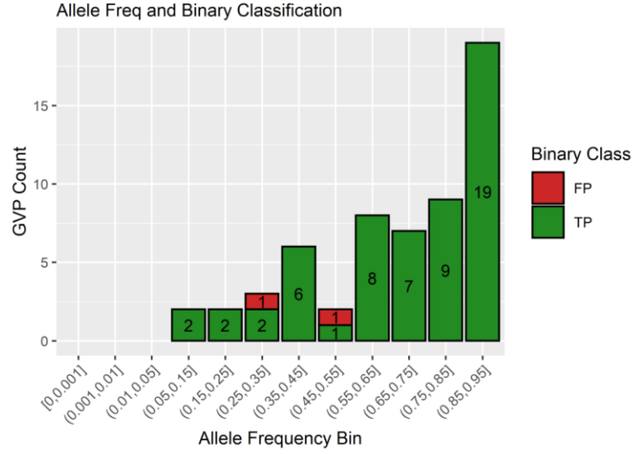


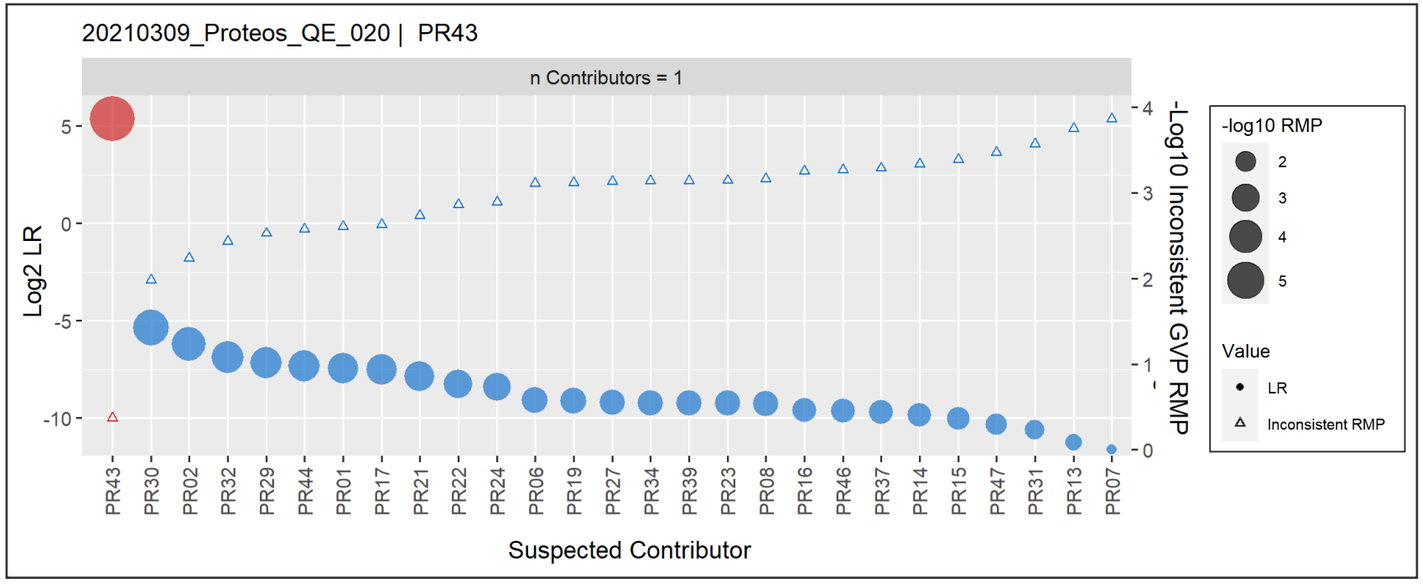


Identification of New Contributor with Known Exome

- New exome profile provided (PR43)
- Touch samples provided:
  - Three replicates
  - Question: Is PR43 present?
- Exome data processed, variable peptides identified
- Successful identification of new contributor





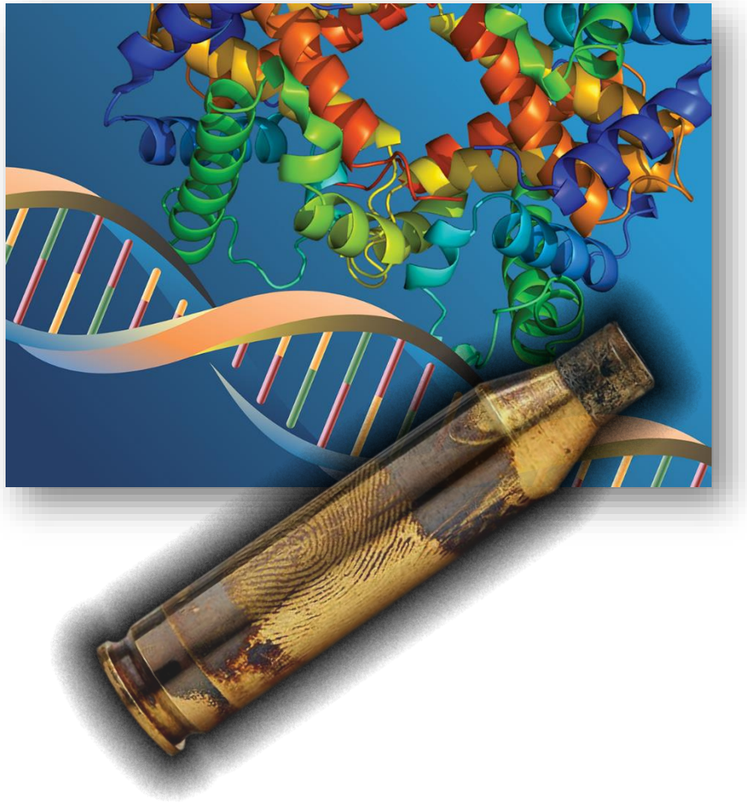


Current Challenges

- Sample collection and extraction methods not in complete alignment with most forensic labs
- Instrumentation
- No equivalent to CODIS for protein markers
  - Protein profiles can be compared to each other or a known exome/whole genome sequence
- Limited panel size and detectability can limit LR values
  - Even harder considering that some alleles may not be expressed or detectable
  - May be further complicated by kinship
- Rare protein markers
  - Extremely discriminating or just a false positive? How do you validate a marker you have never encountered?

Future Directions

- Collection and extraction method optimization
- Method transfer to operational labs and independent laboratory evaluation from beginning to end
- Validation on relevant sample types
- Collection of additional data on sample matrices with known contributor(s)
- Database development (structure, format, hosting, accessibility, content)



Acknowledgements

Project Co-PI
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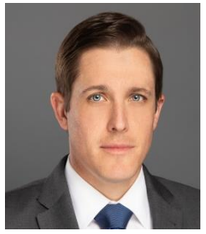

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