

Evaluation of Collection Methods for Direct Amplification of Touch DNA Samples

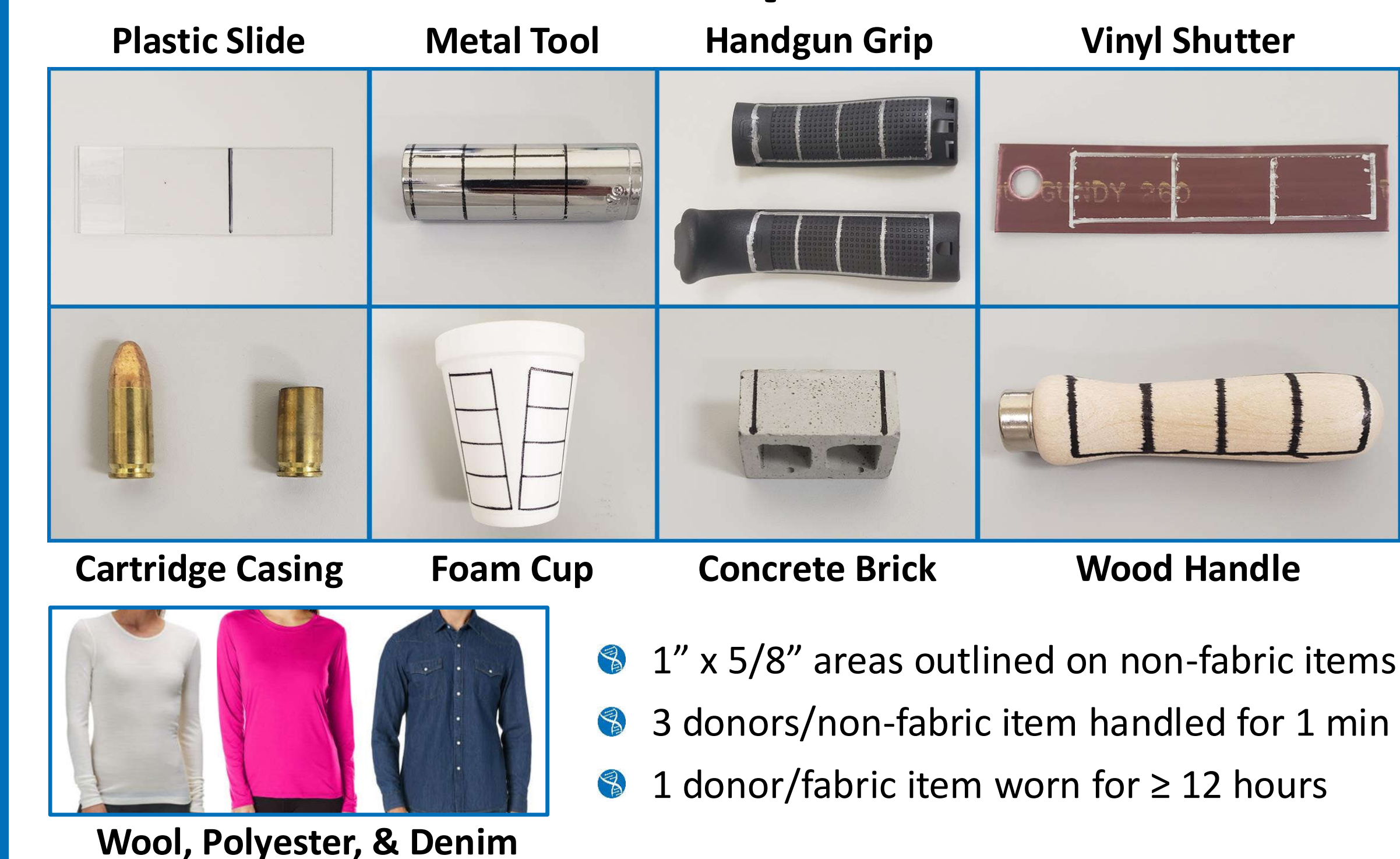
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Introduction

- Direct polymerase chain reaction (PCR) amplification is a sample processing method in which a swab or substrate punch is added directly to an amplification reaction without prior extraction or quantification.
 - May improve DNA profiles from low-yield touch DNA samples¹
 - Eliminates DNA loss that occurs during standard processing
- The amount of DNA available for direct PCR can be affected by the method used for DNA sample collection.
- Study objectives:
 - Evaluate touch DNA collection methods for direct PCR
 - Determine how DNA profile quality resulting from direct PCR compares to standard processing

Touch DNA Sample Substrates



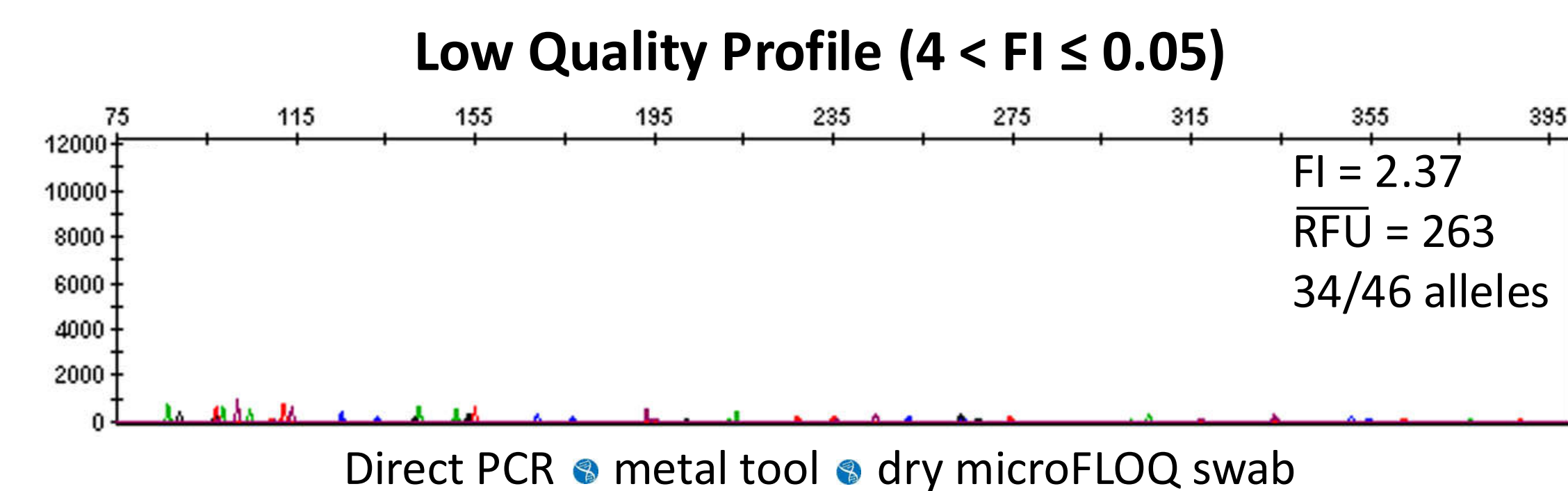
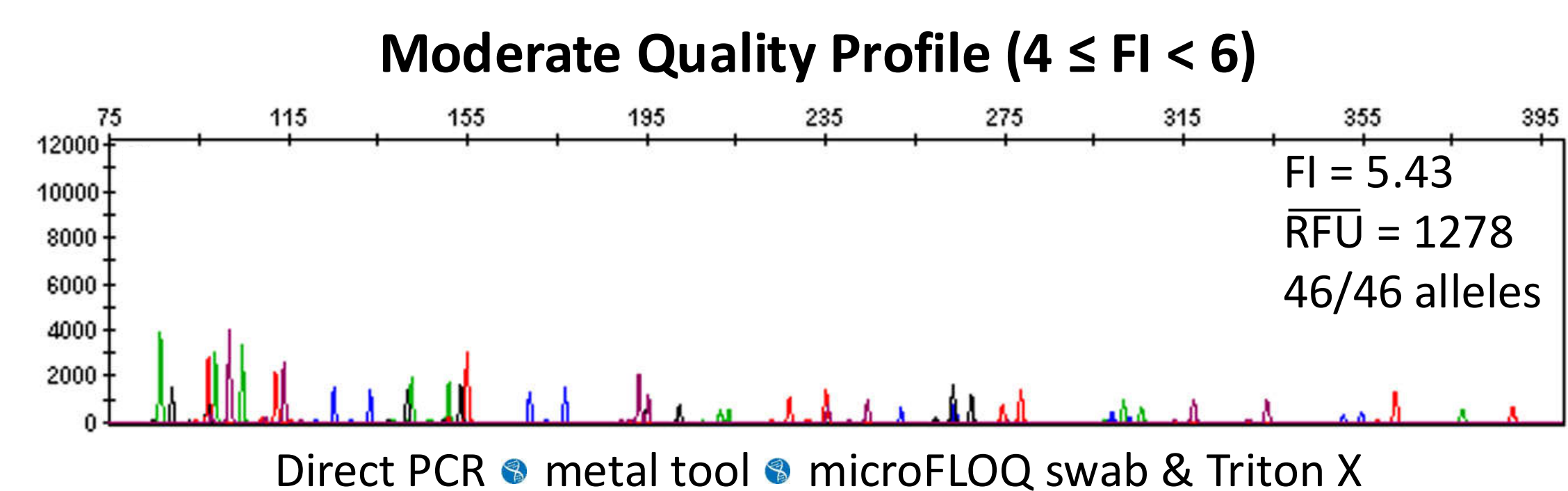
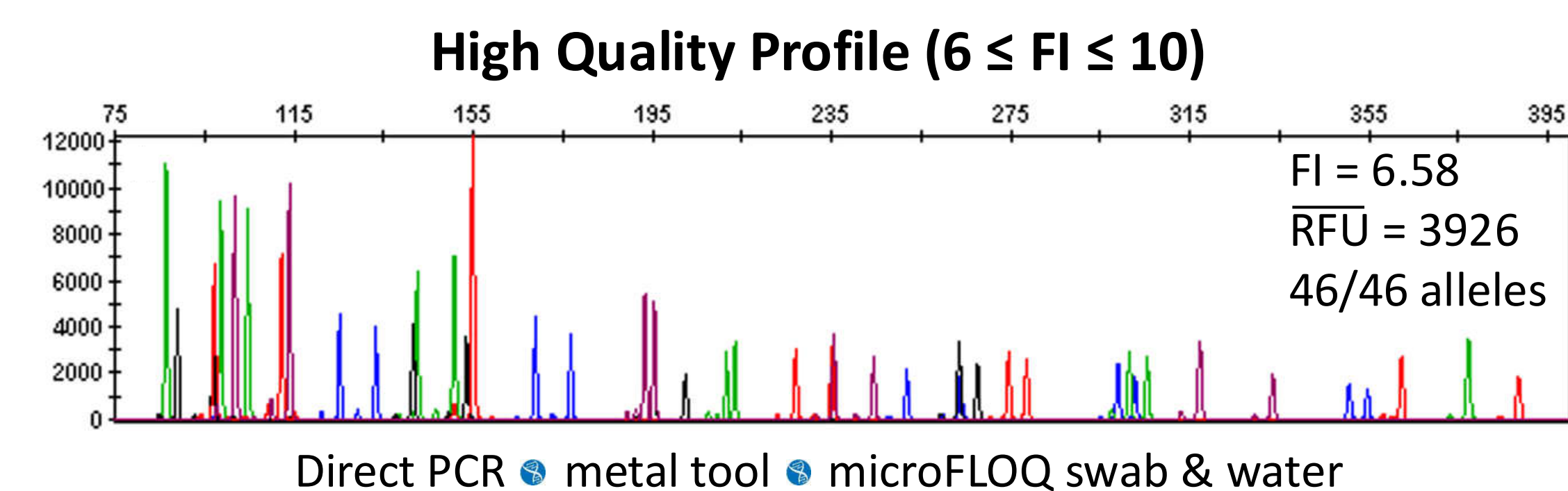
Methods

Collection Substrate	Diameter (mm ²)	Collection Method	Moistening Agent
Puritan [®] cotton swab	3.0	Swabbing	Sterile H ₂ O
Copan microFLOQ [®] swab	1.2-2.0	Swabbing	0.1% Triton X
Non-indicating FTA paper	1.2	Rubbing/scraping	Sterile H ₂ O
Fabric	2.0	Cutting	0.1% Triton X

Processing methods:	Process	Method
	Extraction	Qiagen Investigator [®] STAR™ Lyse&Prep Kit
	Quantification	Quantifiler [®] Trio
	Concentration	Microcon [®] DNA Fast Flow
	Amplification	GlobalFiler™
Eight replicates per processing method, donor, and collection method (n = 3376)	CE	3500xL Genetic Analyzer
	Data analysis	GeneMapper ID-X [®] v1.5
	Evaluation metric	Analytical threshold 125 RFU Stochastic threshold 600 RFU Forensic DNA profile index (FI)

STR Profile Quality

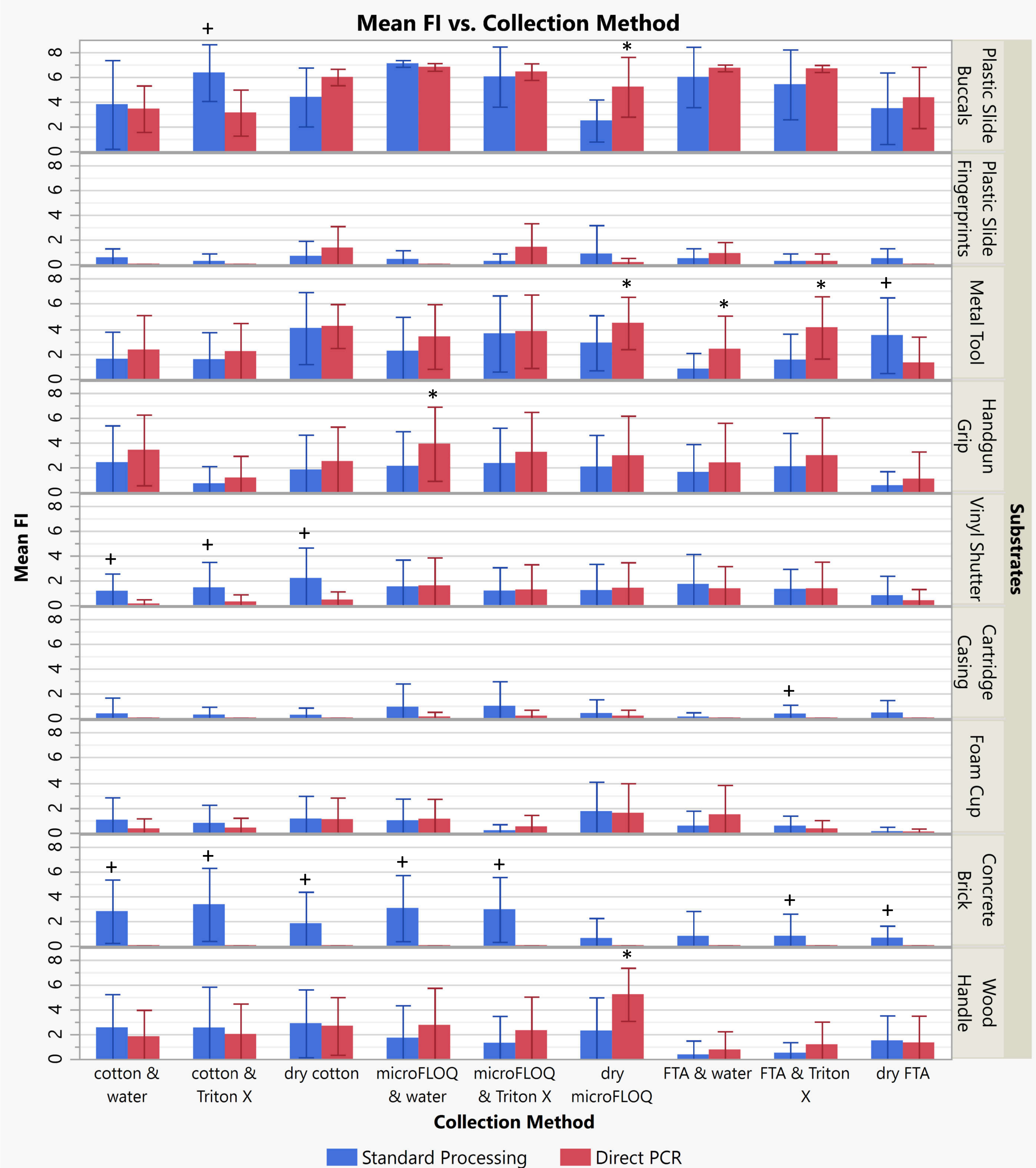
Forensic DNA Profile Index² (FI) values were assigned to each STR profile.



Results & Discussion

Mean Forensic DNA Profile Index – Non-fabrics

Mean FI was determined for each non-fabric substrate, collection method, and processing method.



- Direct PCR was unsuccessful for cartridge casings and concrete bricks due to PCR inhibition.
 - Cartridge casings are known to present challenges for DNA recovery⁵.
 - Concrete contains minerals that may negatively affect both PCR and CE. Amplification products from samples collected with cotton swabs & water and cotton swabs & Triton X resulted in ILS failure. Failed samples were diluted 1:10 in TE buffer and reinjected, after which the ILS peak heights were comparable to the positive control; however, no alleles were detected in the reinjected samples.

Collection Method Comparison

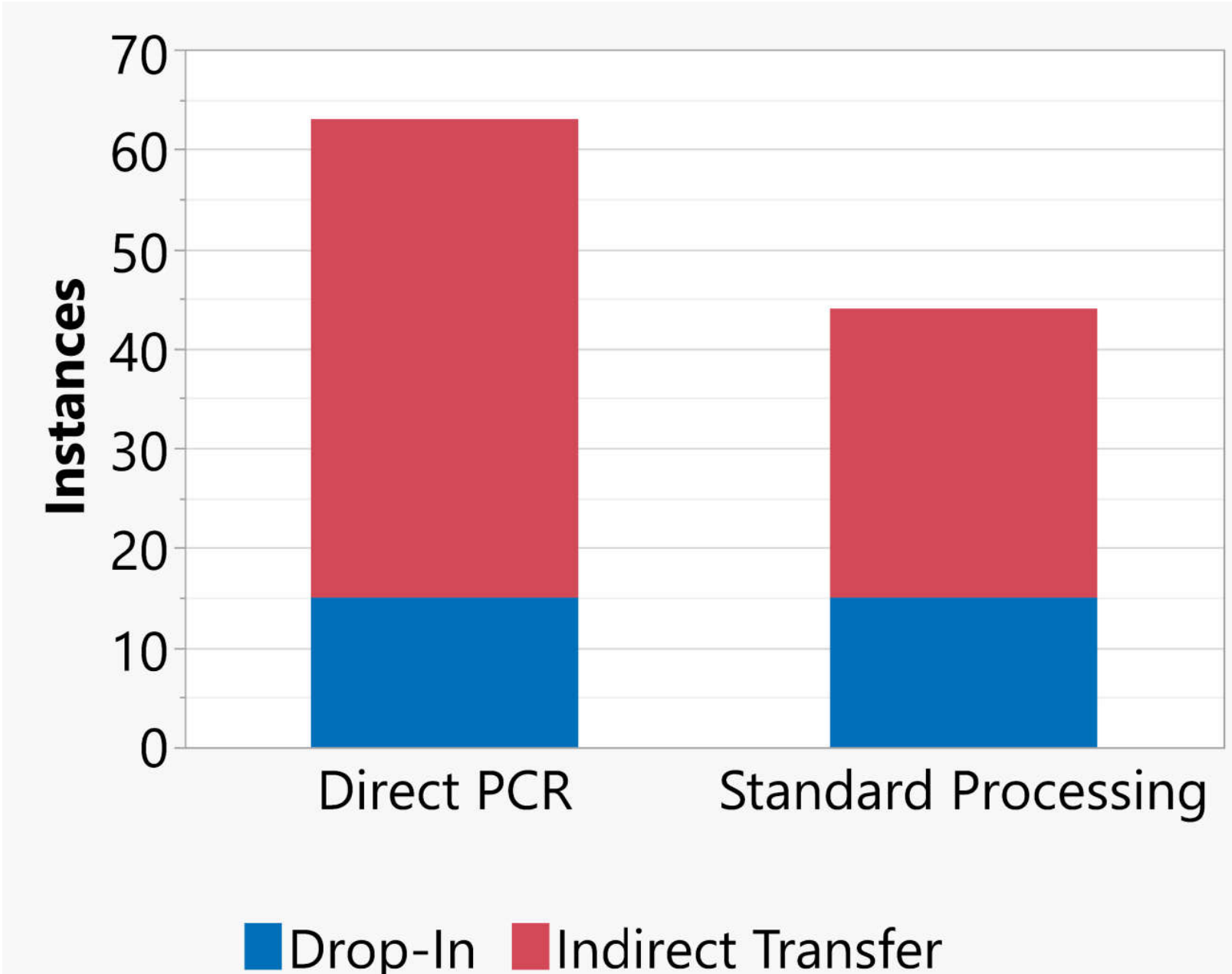
For each substrate and processing method, Tukey's HSD pairwise comparisons of FI were carried out between collection methods. The optimal method in each category produced significantly higher mean FI values than other collection methods. When two or more methods resulted in significantly higher FI values, the method that produced the highest mean FI was selected.

MicroFLOQ swabs are recommended for all substrates when performing direct PCR, whereas cotton or microFLOQ swabs are recommended for standard processing.

Substrate	Optimal Collection Method	
	Standard Processing	Direct PCR
Plastic slide (buccal cells)	microFLOQ swab & water	microFLOQ swab & water
Plastic slide (fingerprints)	dry microFLOQ	microFLOQ swab & Triton X
Metal tool	dry cotton swab	dry microFLOQ
Handgun grip	cotton swab & water	microFLOQ swab & water
Vinyl shutter	dry cotton swab	microFLOQ swab & water
Cartridge casing	microFLOQ swab & Triton X	microFLOQ swab & Triton X
Foam cup	dry microFLOQ	dry microFLOQ
Concrete brick	cotton swab & Triton X	N/A
Wood handle	dry cotton swab	dry microFLOQ

Allelic Drop-In & Indirect DNA Transfer

Non-donor alleles were observed in 3.2% of processed samples.



- 73% of drop-in events were attributed to indirect DNA transfer (alleles consistent with a cohabitating member of one donor's household).
- 78% of drop-in events involved three alleles or fewer.
- These events highlight the sensitivity of both the direct PCR and standard processing methods. Practitioners should be aware that mixture deconvolution may be needed.

Conclusions

- In general, direct PCR produced DNA profile results that were comparable to standard processing.
 - Direct PCR processing takes less time and is less expensive.
- MicroFLOQ swabs are recommended for direct PCR, whereas cotton or microFLOQ swabs are recommended for standard processing.
- Direct PCR success was highly dependent on the substrate from which samples were collected.
 - Direct PCR was not successful on denim, wool blend, 100% wool fabrics concrete bricks and fired cartridge casings. Further optimization of the PCR reactions may improve results.
- Direct PCR was successful for many substrates without using specialized amplification kits or modifying validated amplification SOPs.
- Regardless of the collection and processing methods used, all touch DNA samples contain highly variable quantities of DNA.

Future Research

- Evaluation of the effectiveness of direct PCR on swabs stored after collection would be valuable as forensic DNA evidence is not always processed immediately after collection.
- Re-sampling of areas previously consumed for Direct PCR to determine whether touch DNA remains for re-testing.
- PCR reaction optimization and additional post-PCR cleanup steps to improve the direct PCR results obtained from denim, wool, concrete bricks, and fired cartridge casings.
- Direct PCR with other STR megaplex kits, such as PowerPlex[®] Fusion 6C, for comparison to GlobalFiler.

References

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