Direct PCR of Forensic Evidence: Making the Case to Modify the Quantification Requirement

Abigail S. Bathrick, Anna C. Salmonsen, Jon M. Davoren

BODE TECHNOLOGY

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DIRECT PCR

Introduction

WHAT IS DIRECT PCR?

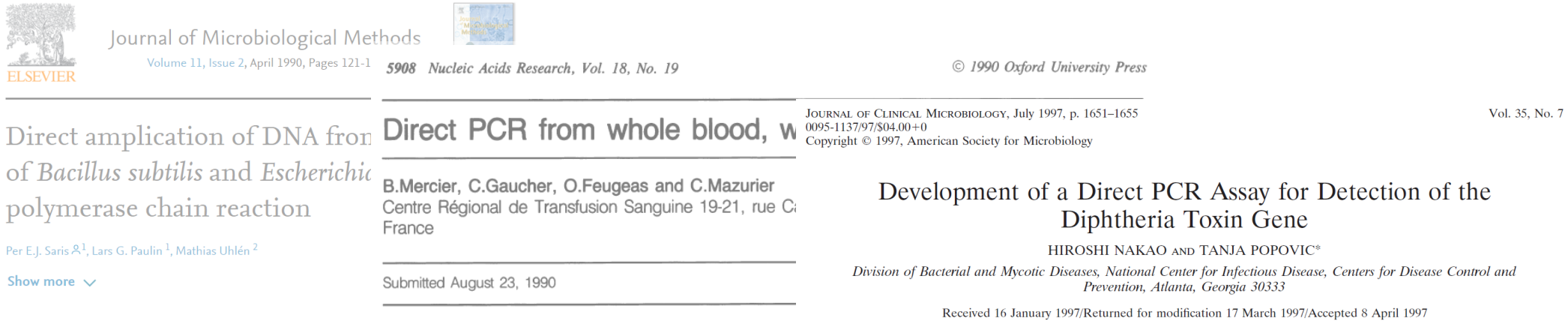
Direct PCR is a DNA processing method in which a sample is added directly to an amplification reaction without prior purification or quantification.

EARLY USE OF DIRECT PCR

NON-FORENSIC APPLICATIONS

Non-forensic applications – 1990s

- Colony PCR, a rapid screening method for large numbers of bacterial cells for a gene of interest
- Human leukocyte antigen (HLA) testing of whole blood
- Viral and bacterial pathogen detection in clinical specimens



ELSEVIER

Journal of Microbiological Methods

Volume 11, Issue 2, April 1990, Pages 121-1

Direct amplication of DNA fron
of *Bacillus subtilis* and *Escherichic*
polymerase chain reaction

Per E.J. Saris [1], Lars G. Paulin [1], Mathias Uhlén [2]

Show more

5908 Nucleic Acids Research, Vol. 18, No. 19

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Direct PCR from whole blood, w

B.Mercier, C.Gaucher, O.Feugeas and C.Mazurier
Centre Régional de Transfusion Sanguine 19-21, rue C
France

Submitted August 23, 1990

JOURNAL OF CLINICAL MICROBIOLOGY, July 1997, p. 1651–1655
0095-1137/97/$04.00+0
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Vol. 35, No. 7

Development of a Direct PCR Assay for Detection of the Diphtheria Toxin Gene

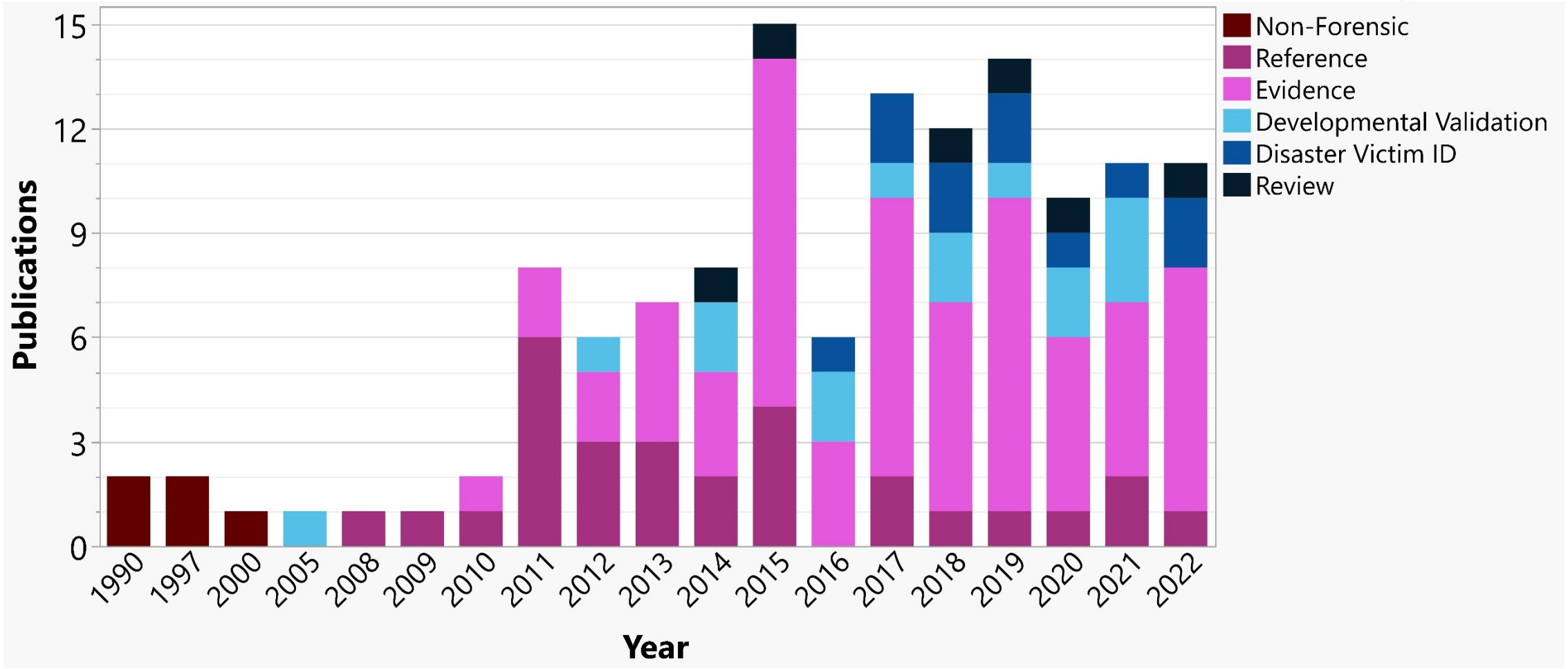
HIROSHI NAKAO AND TANJA POPOVIC*

Division of Bacterial and Mycotic Diseases, National Center for Infectious Disease, Centers for Disease Control and Prevention, Atlanta, Georgia 30333

Received 16 January 1997/Returned for modification 17 March 1997/Accepted 8 April 1997

DIRECT PCR FORENSIC INTEREST

PUBLICATION HISTORY



DIRECT PCR

Forensic Reference & Databasing Samples

FORENSIC APPLICATIONS

REFERENCES & DATABASING

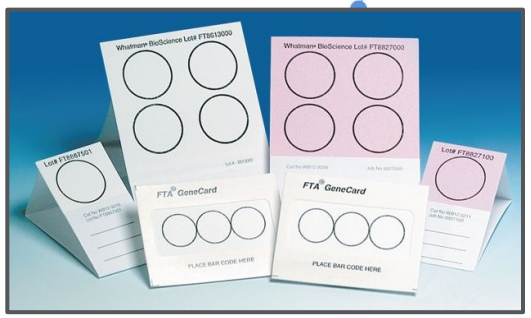
Forensic reference samples – mid-2000s

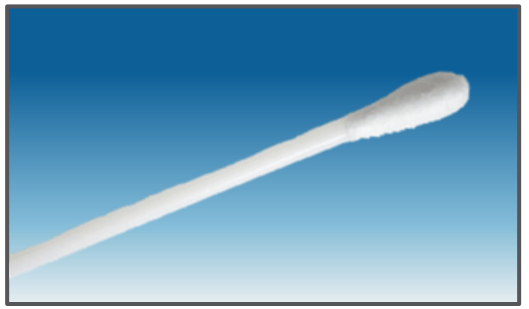
- Sample types: Blood, saliva, & buccal cells
- Substrates: FTA & non-FTA cards, swabs, Bode Buccal Collectors

FORENSIC APPLICATIONS

REFERENCES & DATABASING

In the U.S., direct PCR is used for casework reference & database samples.





FBI Quality Assurance Standards for Forensic DNA Testing Laboratories – Standard 9.4

The laboratory shall quantify or otherwise calculate the amount of human DNA in forensic samples prior to nuclear DNA amplification. Quantification of human DNA for casework reference samples shall not be required if a laboratory has a validated system demonstrated to reliably yield successful DNA amplification and typing without prior quantification.

DIRECT PCR

Forensic Evidentiary Samples

FORENSIC APPLICATIONS

EVIDENTIARY SAMPLES

Forensic evidentiary samples – 2010s

- Linacre A, Pekarek V, Swaran YC, Tobe SS. Generation of DNA profiles from fabrics without DNA extraction. Forensic Sci Int Genet. 2010;4(2):137-41.

Forensic Science International: Genetics 4 (2010) 137–141

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

ELSEVIER

Generation of DNA profiles from fabrics without DNA extraction

Adrian Linacre *, Vera Pekarek, Yuvaneswari Chandramoulee Swaran, Shanan S. Tobe

Centre for Forensic Science, WestChem, University of Strathclyde, 204 George Street, Glasgow G1 1XW, UK

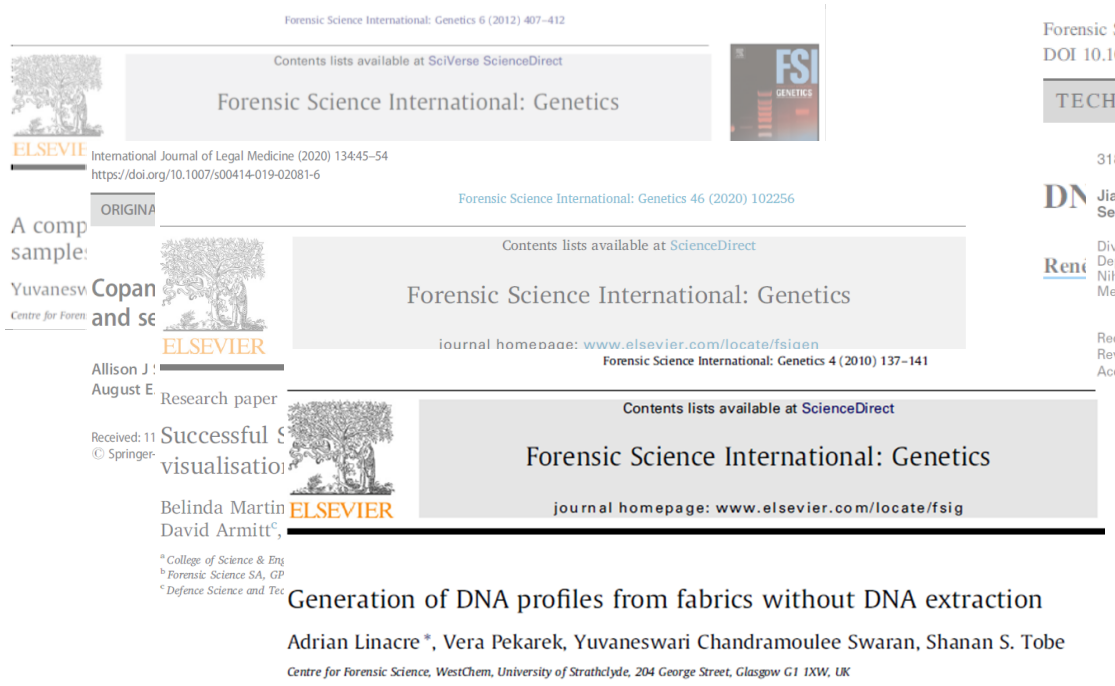
- Touch DNA
- Glass (cotton swabs & water)
- Fabric (cuttings)
- Direct PCR with PowerPlex 16 & AmpFℓSTR SGM Plus

FORENSIC APPLICATIONS

EVIDENTIARY SAMPLES

Collection & Sampling Method Evaluations

- Swab, cut, punch, tape-lift, etc.


Forensic Science International: Genetics 6 (2012) 407–412

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

International Journal of Legal Medicine (2020) 134:45–54
https://doi.org/10.1007/s00414-019-02081-6

Forensic Science International: Genetics 46 (2020) 102256

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

Forensic Science International: Genetics 4 (2010) 137–141

Research paper

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Generation of DNA profiles from fabrics without DNA extraction

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Forensic Sci Med Pathol
DOI 10.1007/s12024-016-9784-y

TECHNICAL REPORT

3188

Electrophoresis 2014, *35*, 3188–3192

Science & Justice 58 (2018) 303–307

Contents lists available at ScienceDirect

Science & Justice

Forensic Science International: Genetics 57 (2022) 102653

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

Exploring tapelifts as a method for dual workflow STR amplification

Belinda Martin [a,b,*], Duncan Taylor [a,b,c], Adrian Linacre [a,b]

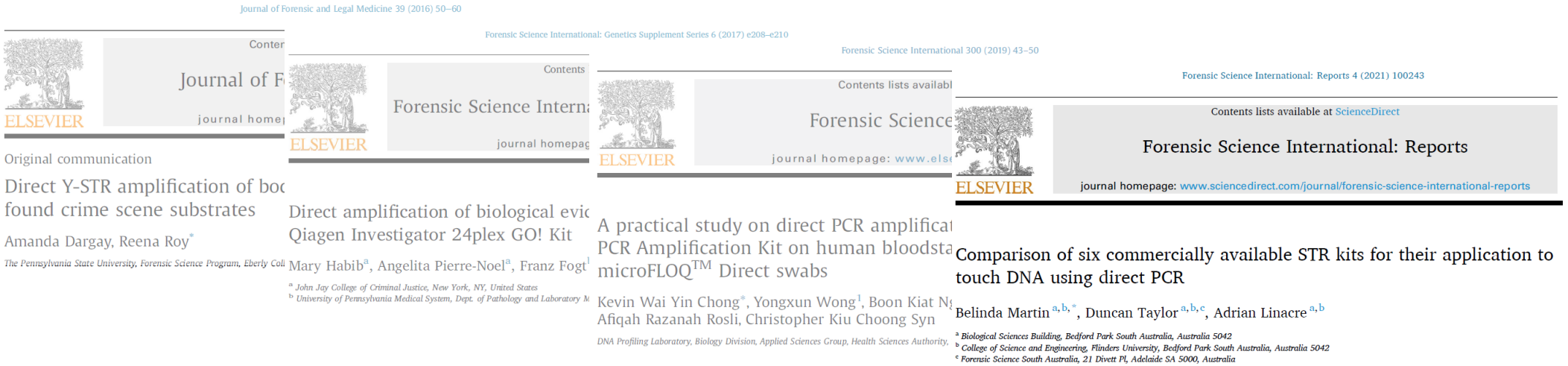
[a] Biological Sciences Building, Flinders University, Bedford Park 5042, Australia
[b] Biological Sciences Building, College of Science and Engineering, Flinders University, Bedford Park 5042, Australia
[c] Forensic Science South Australia, 21 Divett Pl, Adelaide, SA 5000, Australia

FORENSIC APPLICATIONS

EVIDENTIARY SAMPLES

Amplification System Evaluations

- Y-STRs: Yfiler™ Direct, Yfiler Plus, PowerPlex® Y 23
- Autosomal STRs: PowerPlex 16HS, Profiler Plus, PowerPlex 21, SGM Plus®, NGM, NGM Select™, GlobalFiler®, Identifiler® Plus, Identifiler Direct, VeriFiler™ Plus, Investigator® 24Plex QS

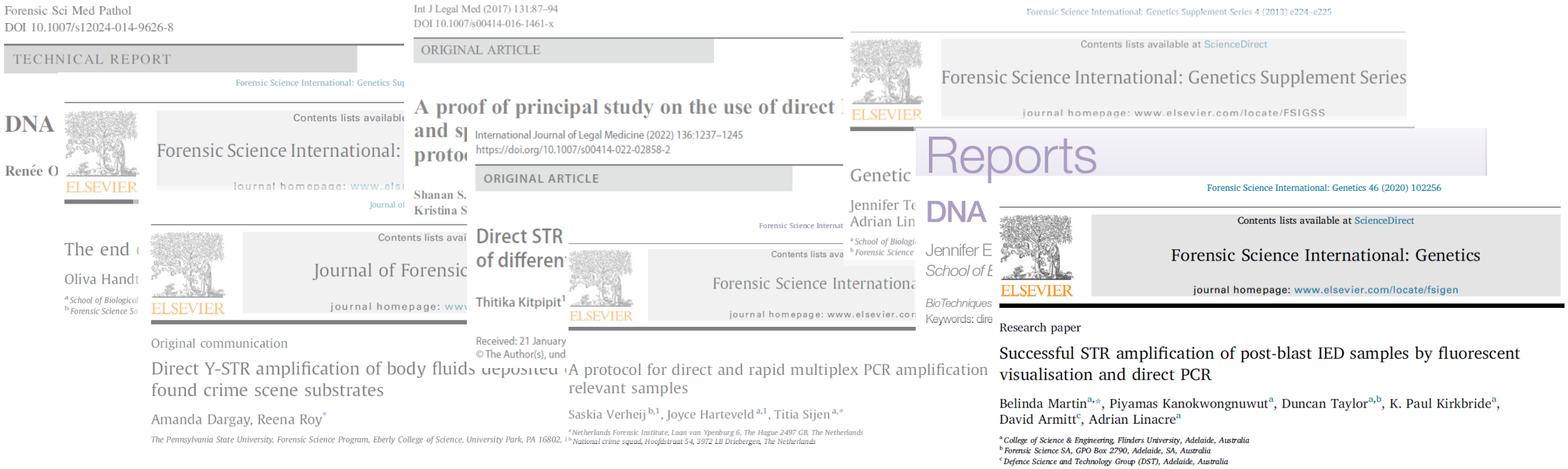


FORENSIC APPLICATIONS

EVIDENTIARY SAMPLES

Evidentiary Sample Type Evaluations

- Blood, saliva, and touch DNA/fingerprints on various substrates; semen; fingernails; hair



DIRECT PCR

Current Research

NIJ AWARD # 2019-DU-BX-0009

OVERVIEW

Direct PCR & Touch DNA Research at Bode

- NIJ-funded project; Jan 1, 2020 – Dec 31, 2022
- Evaluate different methods of collecting touch DNA from various substrates and perform direct PCR using amplification methods that were already validated for standard casework processing
- Research was divided into two phases
  - Phase I – direct PCR with GF; nine touch DNA collection methods evaluated on eight substrates; three worn fabrics also evaluated
  - Phase II – direct PCR with PPF6C; best collection methods selected, GF vs PPF6C comparison, 6-month time study, post-direct PCR re-sampling evaluation

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PHASE I SAMPLE PREPARATION

- 1” x 5/8” areas outlined on non-fabric items
- 3 donors/non-fabric item; handled for 1 min
- 1 donor/fabric item; worn for ≥ 12 hours

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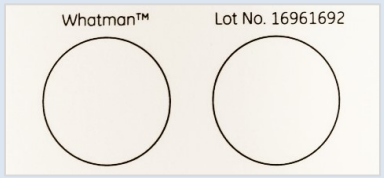
PHASE I SAMPLE COLLECTION

Collection Substrate	Diameter (mm^2)	Collection Method	Moistening Agent
Puritan® cotton swab 	3.0	Swabbing	Sterile H_2O
			0.1% Triton X
			None - dry
Copan microFLOQ® swab 	1.2-2.0	Swabbing	Sterile H_2O
			0.1% Triton X
			None - dry
Non-indicating FTA paper 	1.2	Rubbing/ scraping	Sterile H_2O
			0.1% Triton X
			None - dry
Fabric	2.0	Cutting	None - dry

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PHASE I SAMPLE PROCESSING

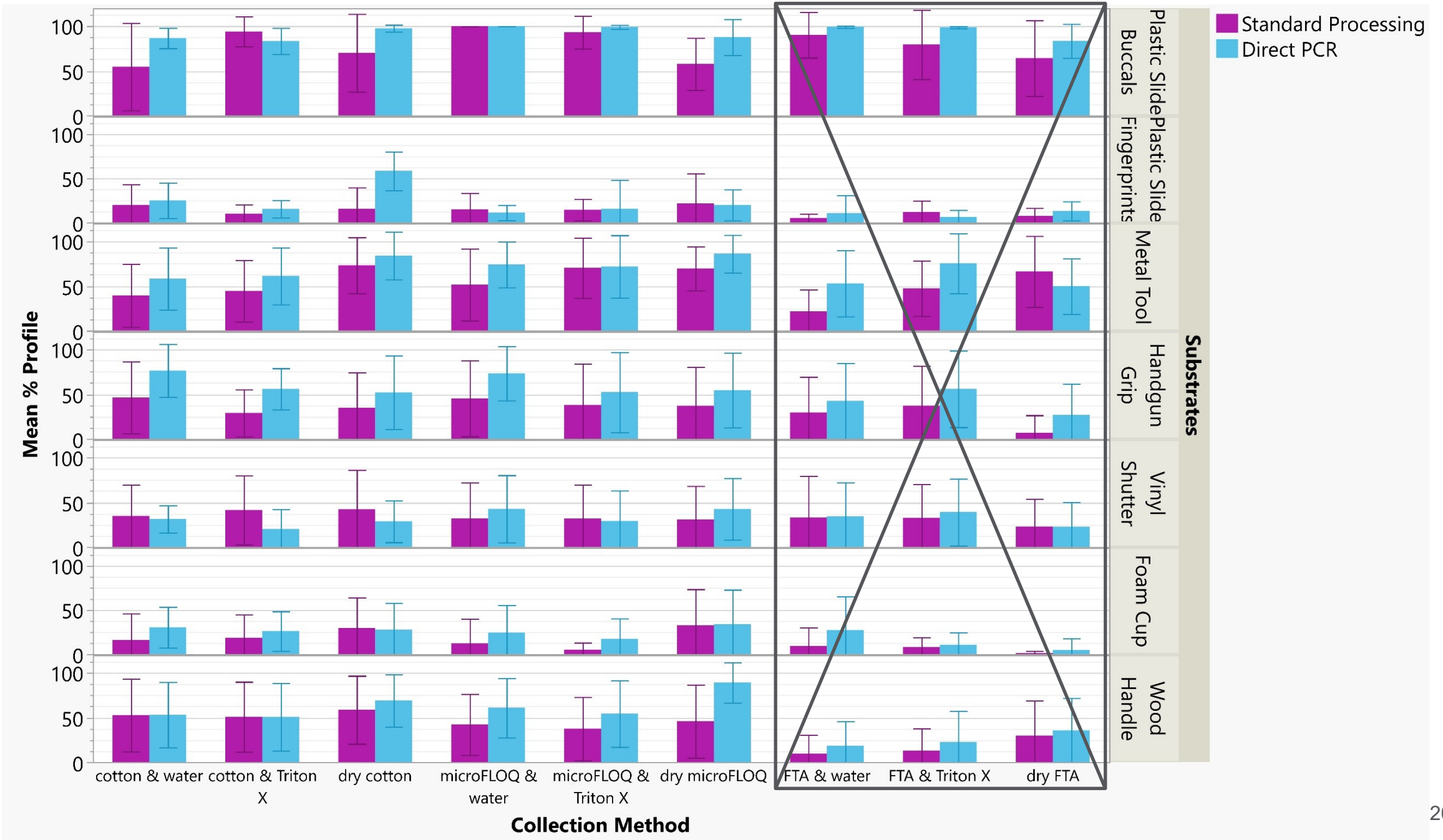
Processing methods

- Standard with DNA extraction and quantification
- Direct PCR

Eight replicates per substrate, donor, collection method, and processing method

- n = 3,376

Process	Method
Extraction	Qiagen Investigator® STAR™ Lyse&Prep
Concentration	Microcon® DNA Fast Flow
Quantification	Quantifiler® Trio
Amplification	GlobalFiler
CE	3500xL Genetic Analyzer
Data analysis	GeneMapper ID-X® v1.5 Analytical threshold 125 RFU Stochastic threshold 600 RFU
Evaluation metric	Percentage of profile obtained (% profile) CODIS eligibility


Standard Processing
Direct PCR
Mean % Profile
Substrates
Plastic Slide Buccals
Plastic Slide Fingerprints
Metal Tool
Handgun Grip
Vinyl Shutter
Foam Cup
Wood Handle
0
50
100
cotton & water
cotton & Triton X
dry cotton
microFLOQ & water
microFLOQ & Triton X
dry microFLOQ
FTA & water
FTA & Triton X
dry FTA
Collection Method

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PHASE I RESULTS

Processing Method	CODIS Eligibility Success Rates (%)						
	Plastic Slide Buccals	Polyester	Metal Tool	Handgun Grip	Wood Handle	Foam Cup	Plastic Slide Fingerprints
Standard	82	63	56	33	37	13	7
Direct PCR	**97**	**88**	**69**	**54**	**50**	**19**	**14**

Processing Method	CODIS Eligibility Success Rates (%)				
	Vinyl Shutter	Denim	100% Wool	Concrete Bricks	Cartridge Casings
Standard	**35**	**100**	**100**	**44**	**6**
Direct PCR	25	0	0	0	0

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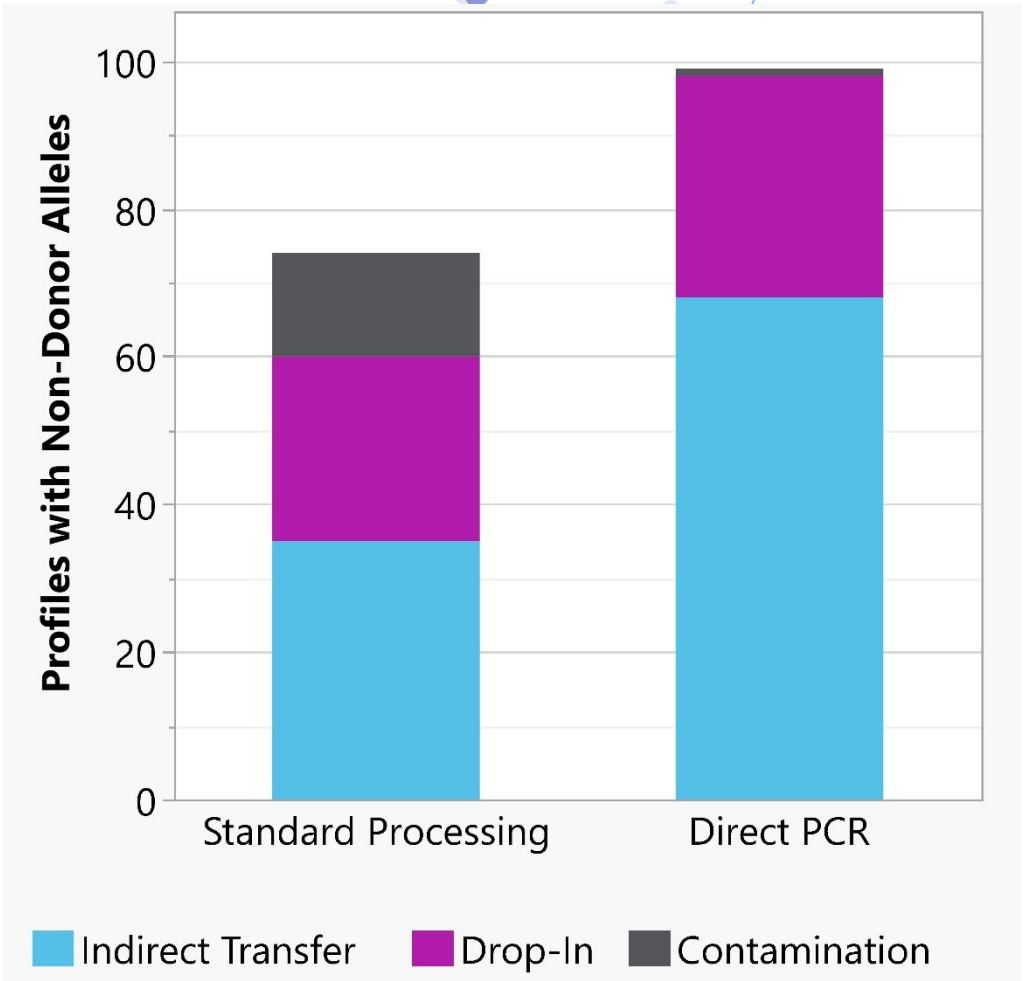
PHASE I RESULTS

Contamination Events

- Foreign alleles were observed in 5% of samples.
- 60% of contamination events were attributed to indirect DNA transfer (alleles consistent with a cohabitating member of one donor's household).
- These events highlight the sensitivity of the direct PCR method. Practitioners should be aware that mixture deconvolution may be needed.



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PHASE II OVERVIEW

Phase II Brief Summary

- Direct PCR with PowerPlex Fusion 6C; n = 2,336
- GF vs PPF6C comparison, 6-month time study, post-direct PCR re-sampling evaluation

GF vs PPF6C Comparison

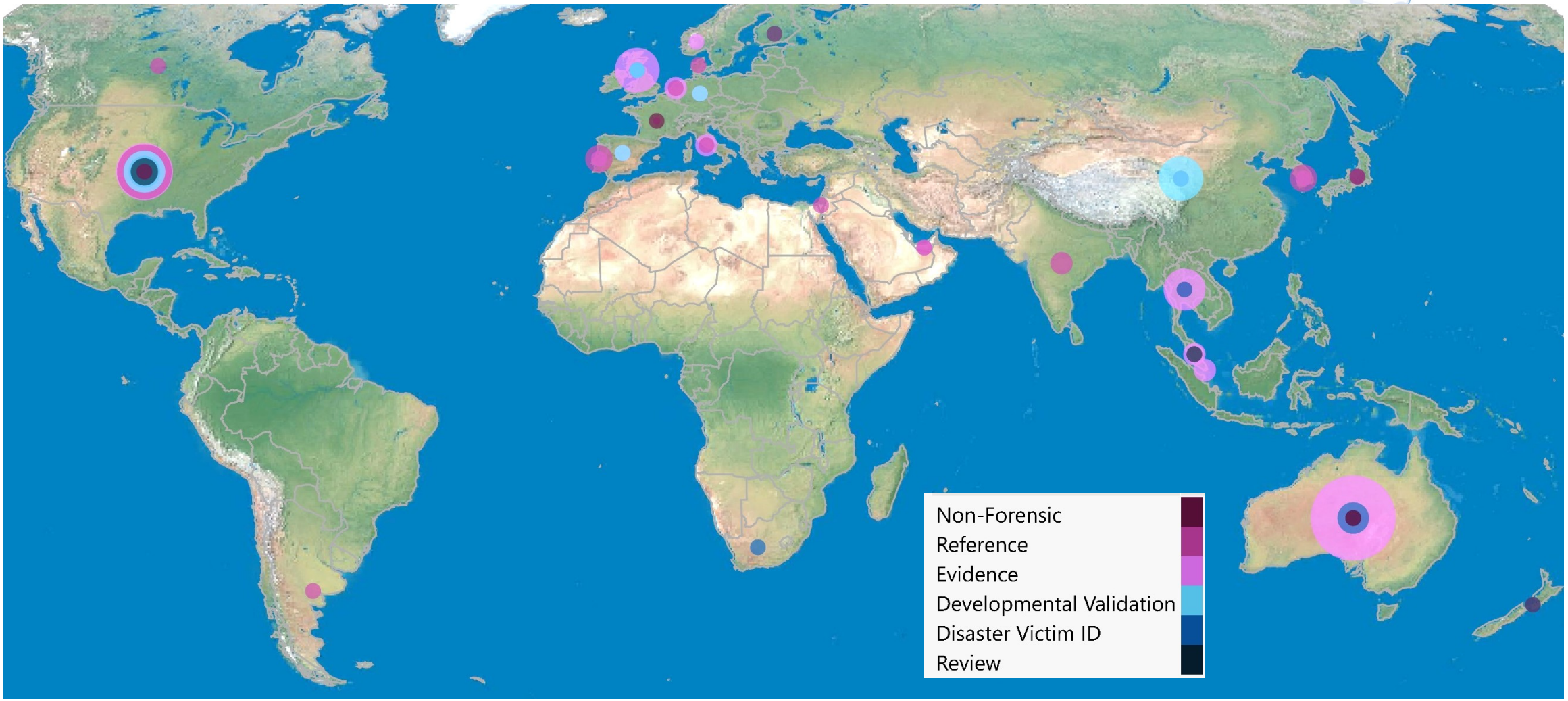
6-month Time Study

DIRECT PCR

Use In Casework

DIRECT PCR PUBLICATIONS

BY COUNTRY

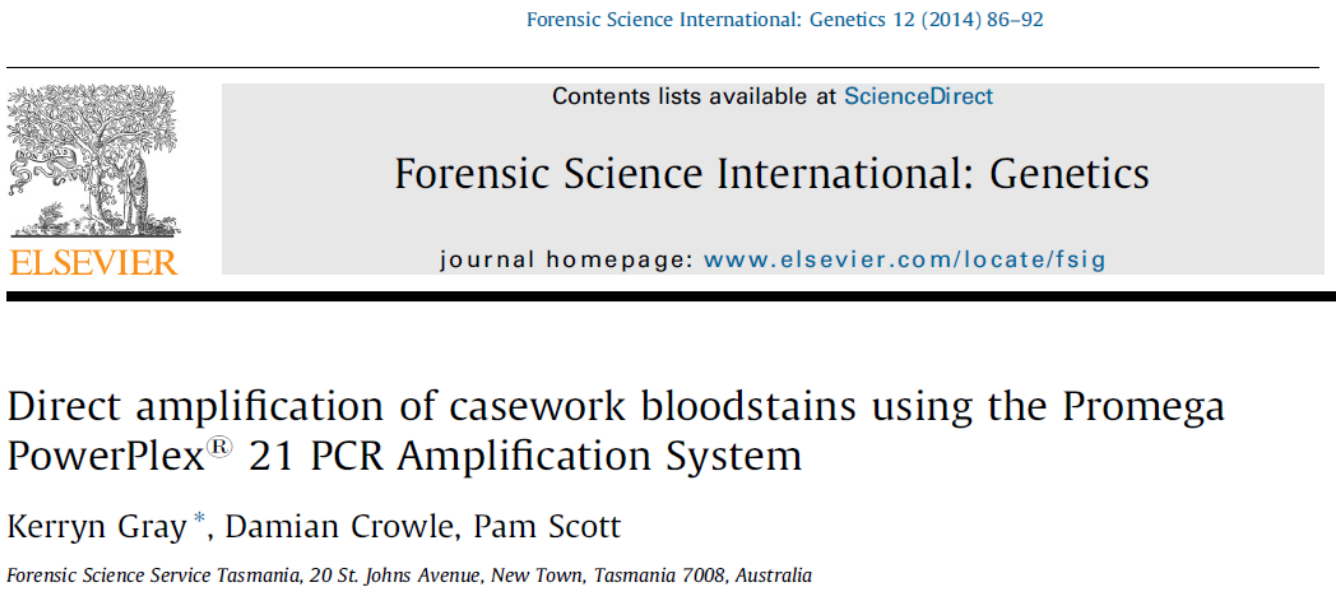


FORENSIC APPLICATIONS

USE IN CASEWORK

Direct PCR of casework bloodstains validated by Forensic Science Service Tasmania (FSST) in 2014

- Direct PCR of 1.0 mm bloodstain cuttings/punches with PowerPlex 21 (13 µL rxns)
- 340 real casework bloodstains processed with direct PCR
  - 90% produced acceptable profiles; 10% required subsequent organic extraction and qPCR
- Additional onus on examiners to assess the suitability of a stain for direct PCR and sample accordingly


Forensic Science International: Genetics 12 (2014) 86–92

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

ELSEVIER

Direct amplification of casework bloodstains using the Promega PowerPlex® 21 PCR Amplification System

Kerryn Gray *, Damian Crowle, Pam Scott

Forensic Science Service Tasmania, 20 St. Johns Avenue, New Town, Tasmania 7008, Australia

DIRECT PCR

Summary & Final Thoughts

DIRECT PCR ADVANTAGES

Faster Processing Times

- Complete profiles generated in less than 3 h
- Lab personnel save an estimated 3–4 h hands-on time

Reduced Costs

- Save 25% in reagent costs

Effective & Sensitive

- Direct PCR evidentiary type publications: blood (28%); saliva (20%), touch DNA (41%), and semen (9%)
- More work needed for semen and sexual assault mixture samples

DIRECT PCR LIMITATIONS

No DNA quantification

- Direct PCR reactions may contain variable/excessive quantities of template DNA
- May need to dilute the amplification product prior to CE or re-inject the sample for a shorter length of time

Interference from PCR inhibitors/challenging substrates

- Indigo dye, fired cartridge casings, etc.

Sensitivity

- May result in more complex mixtures that require mixture deconvolution

Sample Consumption

- No DNA extraction means that no extract will be available for re-analysis

FINAL THOUGHTS

FBI QAS Standard 9.4

The laboratory shall quantify or otherwise calculate the amount of human DNA in forensic samples prior to nuclear DNA amplification. Quantification of human DNA for casework reference samples shall not be required if a laboratory has a validated system demonstrated to reliably yield successful DNA amplification and typing without prior quantification.

Reassess the quant requirement?

- Direct PCR touch DNA
  - Overloaded: 0.6%
- Standard processing touch DNA
  - Consumed after quant: 99%
  - <1 pg/µl at quant but CODIS eligible: 0.5%
  - DNA extract for re-amp: 0.7%
- Direct PCR bloodstains [Gray et al. 2014]
  - Overloaded: 1%
  - Reprocessed: 10%

FINAL THOUGHTS

The purpose of Quality Assurance is to foster an environment of continued improvement.

Can concerns be addressed by establishing eligibility recommendations?

- Limit to specific substrate types
- Ensure there is enough sample for re-testing

How do we move forward?

- Critically consider and evaluate the existing research.
- Publish! Publish! Publish!
- Survey labs about their direct PCR use

ACKNOWLEDGEMENTS

Thank you to…

- Anna Salmonsen – GW Graduate fellow responsible for the laboratory work on NIJ Award # 2019-DU-BX-0009
- Jon Davoren – project PI
- Bode's Research Team

QUESTIONS?

Abby.Bathrick@bodetech.com

BODE TECHNOLOGY