



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

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

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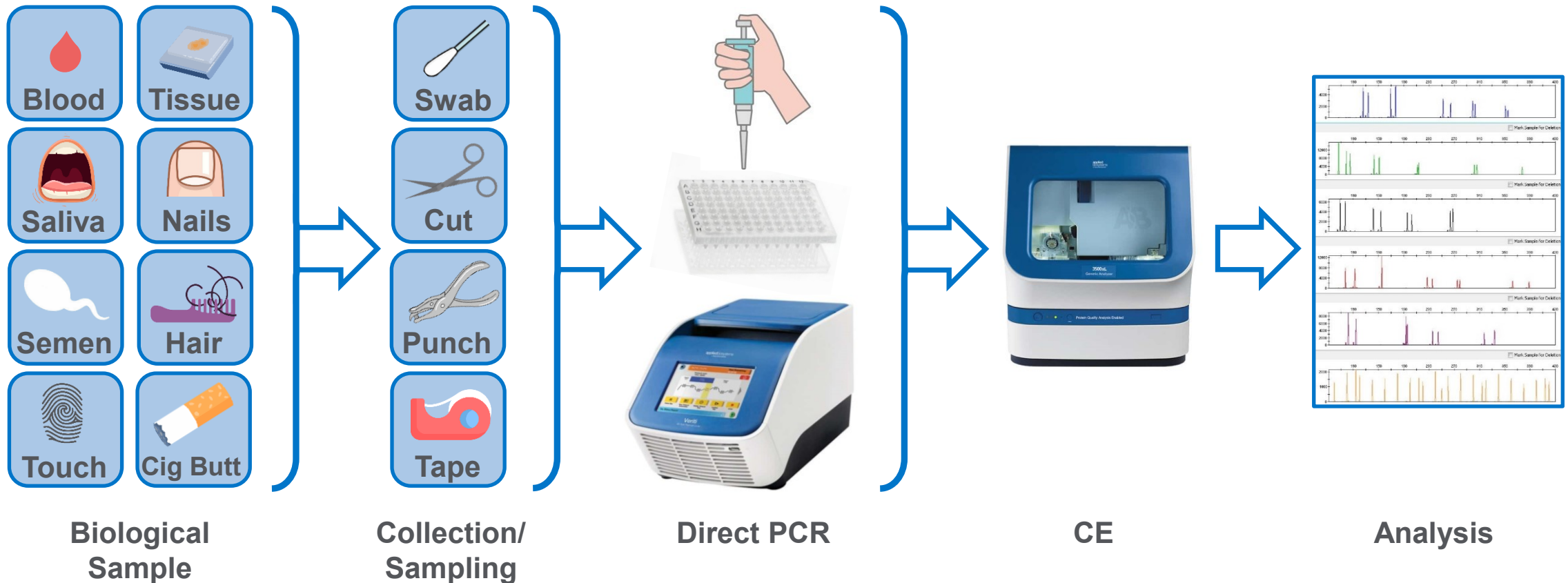
DIRECT PCR

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



Journal of Microbiological Methods

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



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

© 1990 Oxford University Press

Direct amplification of DNA from
of *Bacillus subtilis* and *Escherichia coli*
polymerase chain reaction

Per E.J. Saris¹, Lars G. Paulin¹, Mathias Uhlén²

[Show more](#) ▾

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

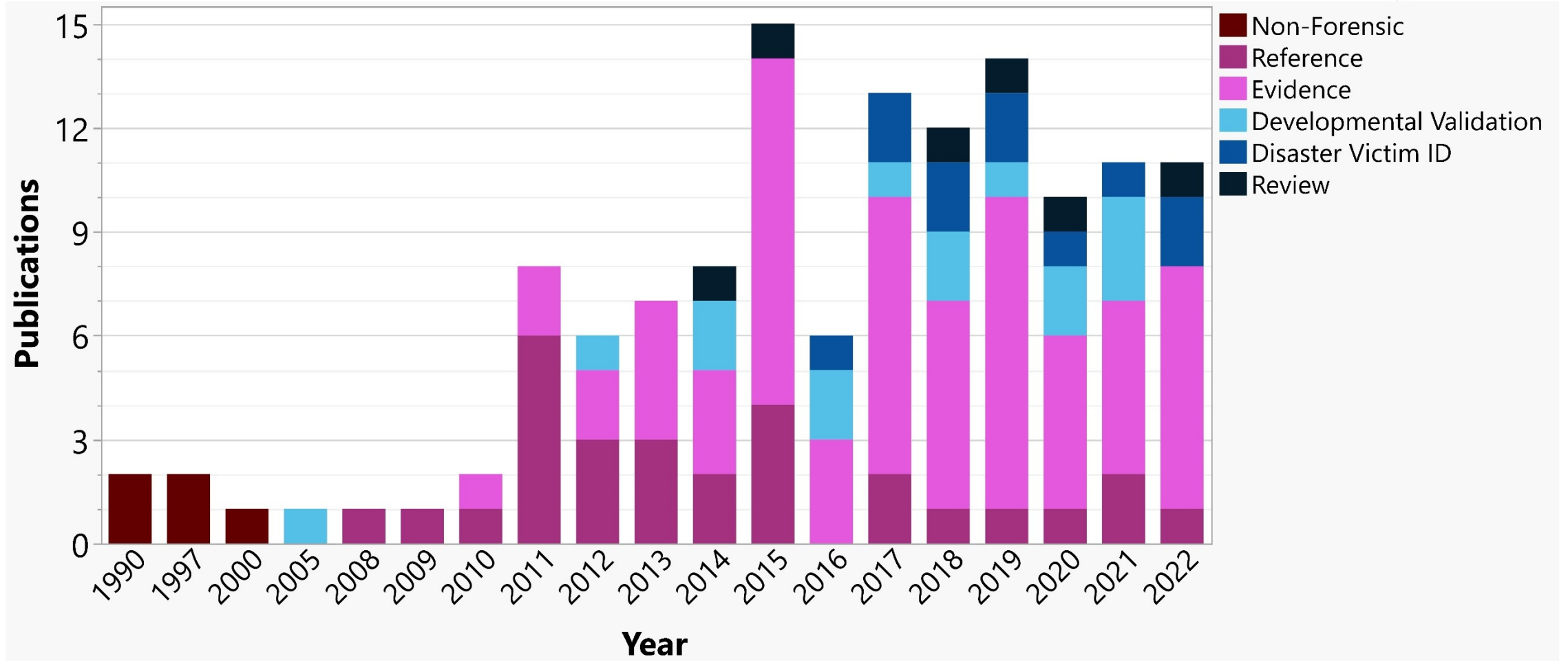
HIROSHI NAKAO AND TANJA POPOVIC*

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

TECHNICAL NOTE

Su Jeong Park,¹ Ph.D.; Jong Yeol Kim,² M.S.; Young Geun Yang,² Ph.D.; and Seung Hwan Lee,¹ Ph.D.
Forensic Science International: Genetics Supplement Series 3 (2011) e103–e104

J Forensic Sci
doi:
Available online at:

Forensic Science International: Genetics Supplement Series 3 (2011) e103–e104

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series



Direct STR
and Blood-
DNA Purific



For

Contents lists available at ScienceDirect



ELSEVIER

Forensic Science International

JOURNAL OF
**FORENSIC
SCIENCES**



J Forensic Sci, July 2013, Vol. 58, No. 4
doi: 10.1111/1556-4029.12166
Available online at: onlinelibrary.wiley.com

Research article
Direct amplifi
Dennis Y. Wang*,
Life Technologies, 850 Lincoln C



Forensic Science International

Cont

Direct PCR by
A. Barbaro*, P. C
Dept. Forensic Genetics, SIMI

TECHNICAL NOTE

CRIMINALISTICS

journal homepage: www.elsevier.com

Jeong Eun Sim,^{1,‡} M.S.; Su Jeong Park,^{1,‡} Ph.D.; Han Chul Lee,¹ Ph.D.; Se-Yong Kim,¹ M.S.;
Jong Yeol Kim,² M.S.; and Seung Hwan Lee,¹ Ph.D.

Amplification of non-FTA samples with AmpF
Direct PCR Amplification Kit

P. Brito^{a,b,*}, V. Lopes^{a,b}, V. Bogas^a, F. Balsa^{a,b}, L. Andrade^a
A.M. Bento^{a,b}, P. Cunha^a, M. Carvalho^{a,b}, F. Corte-Real^{b,c,d}, M.J. Anjos^{a,b}

^a Forensic Genetic Service, Centre Branch, National Institute of Legal Medicine, I.P., Coimbra, Portugal

^b CENCIFOR, Forensic Science Center, Portugal

^c National Institute of Legal Medicine I.P., Coimbra, Portugal

^d Faculty of Medicine, University of Coimbra, Portugal

High-Throughput STR Analysis for DNA
Database Using Direct PCR*,[†]

FORENSIC APPLICATIONS

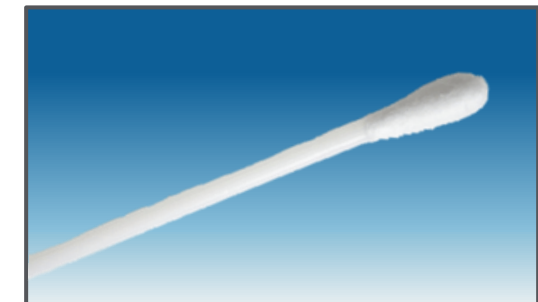
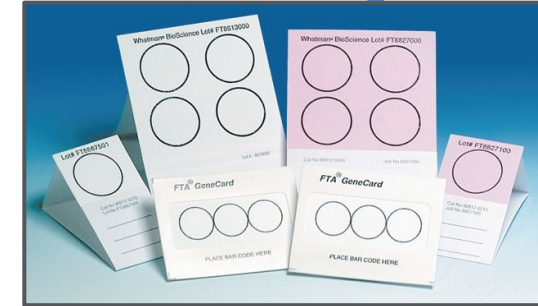
REFERENCES & DATABASING



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

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 & AmpF ℓ STR 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

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

ORIGINAL Forensic Science International: Genetics 46 (2020) 102256

A complete sample: Yuvaneshwari Copan and Seema Centre for Forensic Science

Forensic Science International: Genetics

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

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

Allison J. August Research paper

Received: 11 August 2019 Successful STR visualisation

Belinda Martin, David Armitt*,
 * College of Science & Eng
 † Forensic Science SA, GP
 ‡ Defence Science and Tech

Forensic Science International: Genetics

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

Generation of DNA profiles from fabrics without DNA extraction

Adrian Linacre*, Vera Pekarek, Yuvaneshwari Chandramouleeswaran, Shanan S. Tobe

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

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

TECHNICAL REPORT

3188 *Electrophoresis* 2014, 35, 3188–3192

DN Jian Tie, Seisaku Uchiyama
 Ren Department of Legal Medicine, Tokai University School of Medicine, Tokai, Japan

Science & Justice 58 (2018) 303–307

Received Feb 2018
 Revised May 2018
 Accepted Jun 2018

Forensic Science International: Genetics 57 (2022) 102653

Contents lists available at ScienceDirect

Science & Justice

Forensic Science International: Genetics

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

A novel forensic application of DNA amplification

Stephen G. Illingworth
 Department of Chemistry

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

Journal of Forensic and Legal Medicine 39 (2016) 50–60

Forensic Science International: Genetics Supplement Series 6 (2017) e208–e210

Forensic Science International 300 (2019) 43–50

Forensic Science International: Reports 4 (2021) 100243

Original communication
Direct Y-STR amplification of bo...
found crime scene substrates
Amanda Dargay, Reena Roy*
The Pennsylvania State University, Forensic Science Program, Eberly Coll

Direct amplification of biological evic...
Qiagen Investigator 24plex GO! Kit
Mary Habib^a, Angelita Pierre-Noel^a, Franz Fogt¹
^a John Jay College of Criminal Justice, New York, NY, United States
^b University of Pennsylvania Medical System, Dept. of Pathology and Laboratory M

A practical study on direct PCR amplifica...
PCR Amplification Kit on human bloodsta...
microFLOQ™ Direct swabs
Kevin Wai Yin Chong*, Yongxun Wong¹, Boon Kiat Ng,
Afiqah Razanah Rosli, Christopher Kiu Choong Syn
DNA Profiling Laboratory, Biology Division, Applied Sciences Group, Health Sciences Authority,

Comparison of six commercially available STR kits for their application to...
touch DNA using direct PCR
Belinda Martin^{a,b,*}, Duncan Taylor^{a,b,c}, Adrian Linacre^{a,b}
^a Biological Sciences Building, Bedford Park South Australia, Australia 5042
^b College of Science and Engineering, Flinders University, Bedford Park South Australia, Australia 5042
^c Forensic Science South Australia, 21 Diwett Pl, Adelaide SA 5000, Australia

FORENSIC APPLICATIONS

EVIDENTIARY SAMPLES

Evidentiary Sample Type Evaluations

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

Forensic Sci Med Pathol
DOI 10.1007/s12024-014-9626-8

TECHNICAL REPORT
Forensic Science International: Genetics Supplement Series 4 (2013) e224–e225

DNA
Renée O
Forensic Science International: Genetics Supplement Series
journal homepage: www.elsevier.com/locate/FSIGSS

The end of
Olivia Handt
School of Biological Sciences
Forensic Science International: Genetics Supplement Series 4 (2013) e224–e225

Int J Legal Med (2017) 131:87–94
DOI 10.1007/s00414-016-1461-x

ORIGINAL ARTICLE
A proof of principal study on the use of direct Y-STR and sex-typing protocols
International Journal of Legal Medicine (2022) 136:1237–1245
https://doi.org/10.1007/s00414-022-02858-2

ORIGINAL ARTICLE
Direct STR of different body fluids
Thitika Kitpipit¹
Forensic Science International: Genetics Supplement Series 4 (2013) e224–e225

Original communication
Direct Y-STR amplification of body fluids found on crime scene substrates
Amanda Dargay, Reena Roy^{*}
^{*} The Pennsylvania State University, Forensic Science Program, Eberly College of Science, University Park, PA 16802.

Received: 21 January
© The Author(s), and
A protocol for direct and rapid multiplex PCR amplification of relevant samples
Saskia Verheij^{b,1}, Joyce Hartevelde^{a,1}, Titia Sijen^{a,*}
^a Netherlands Forensic Institute, Laan van Yperburg 6, The Hague 2497 GB, The Netherlands
^b National crime squad, Hoofdstraat 54, 3972 LB Driebergen, The Netherlands

Forensic Science International: Genetics Supplement Series 4 (2013) e224–e225

Forensic Science International: Genetics Supplement Series
journal homepage: www.elsevier.com/locate/FSIGSS

Genetic Reports
Forensic Science International: Genetics 46 (2020) 102256

Jennifer Te
Adrian Linacre^a
School of Biological Sciences
Forensic Science International: Genetics
journal homepage: www.elsevier.com/locate/fsigen

Research paper
Successful STR amplification of post-blast IED samples by fluorescent visualisation and direct PCR
Belinda Martin^{a,*}, Piyamas Kanokwongnuwut^a, Duncan Taylor^{a,b}, K. Paul Kirkbride^a, David Armit^c, Adrian Linacre^a
^a College of Science & Engineering, Flinders University, Adelaide, Australia
^b Forensic Science SA, GPO Box 2790, Adelaide, SA, Australia
^c Defence Science and Technology Group (DST), Adelaide, Australia

DIRECT PCR

Current Research

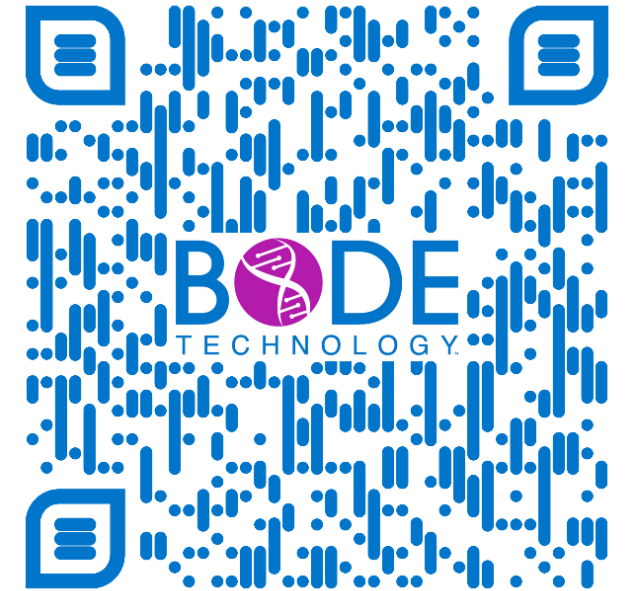


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OVERVIEW

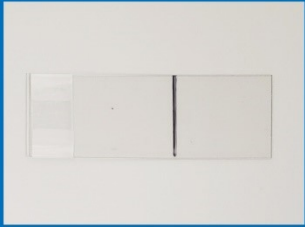
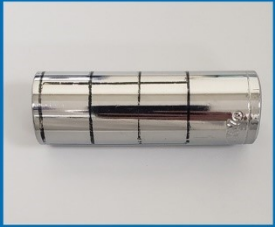





Direct PCR & Touch DNA Research at Bode

- 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



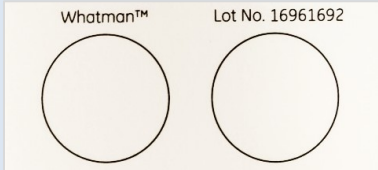
Plastic Slide	Metal Tool	Handgun Grip	Vinyl Shutter	Wool & Polyester
				
				
Cartridge Casing	Foam Cup	Concrete Brick	Wood Handle	Denim

- 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 \geq 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 ₂ O
			0.1% Triton X
			None - dry
Copan microFLOQ [®] swab 	1.2-2.0	Swabbing	Sterile H ₂ O
			0.1% Triton X
			None - dry
Non-indicating FTA paper 	1.2	Rubbing/ scraping	Sterile H ₂ O
			0.1% Triton X
			None - dry
Fabric	2.0	Cutting	None - dry

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PHASE I SAMPLE PROCESSING

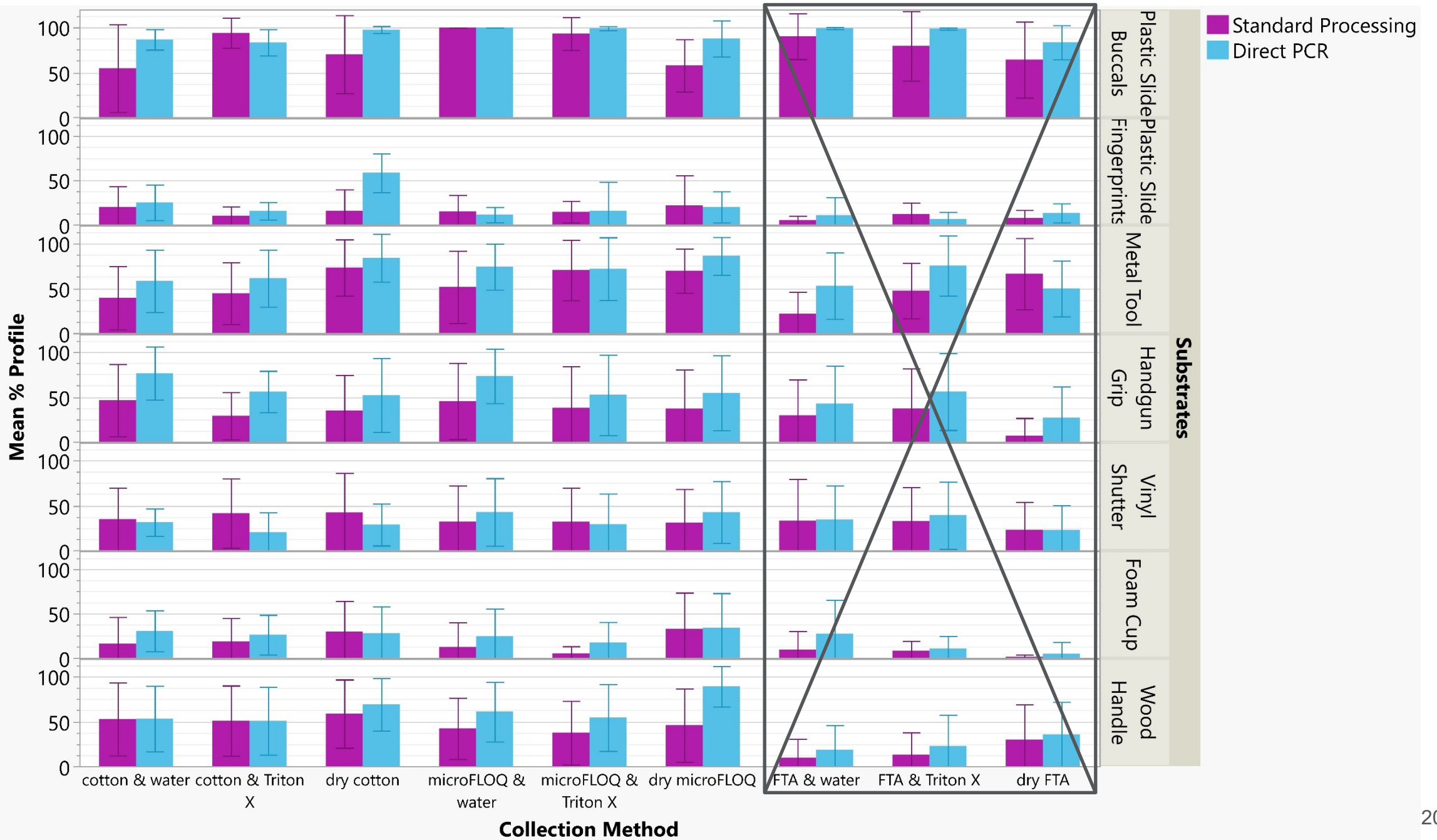
Processing methods

- 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



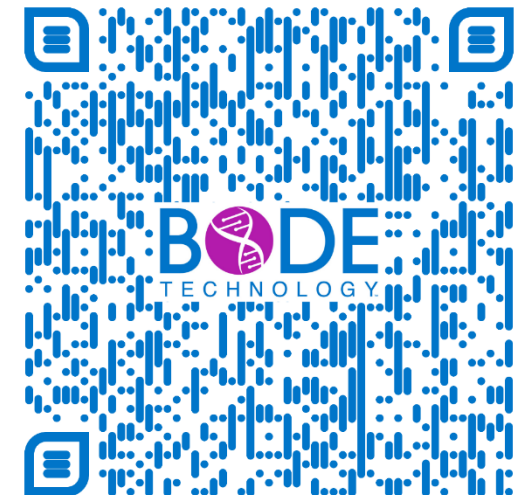
NIJ AWARD # 2019-DU-BX-0009

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

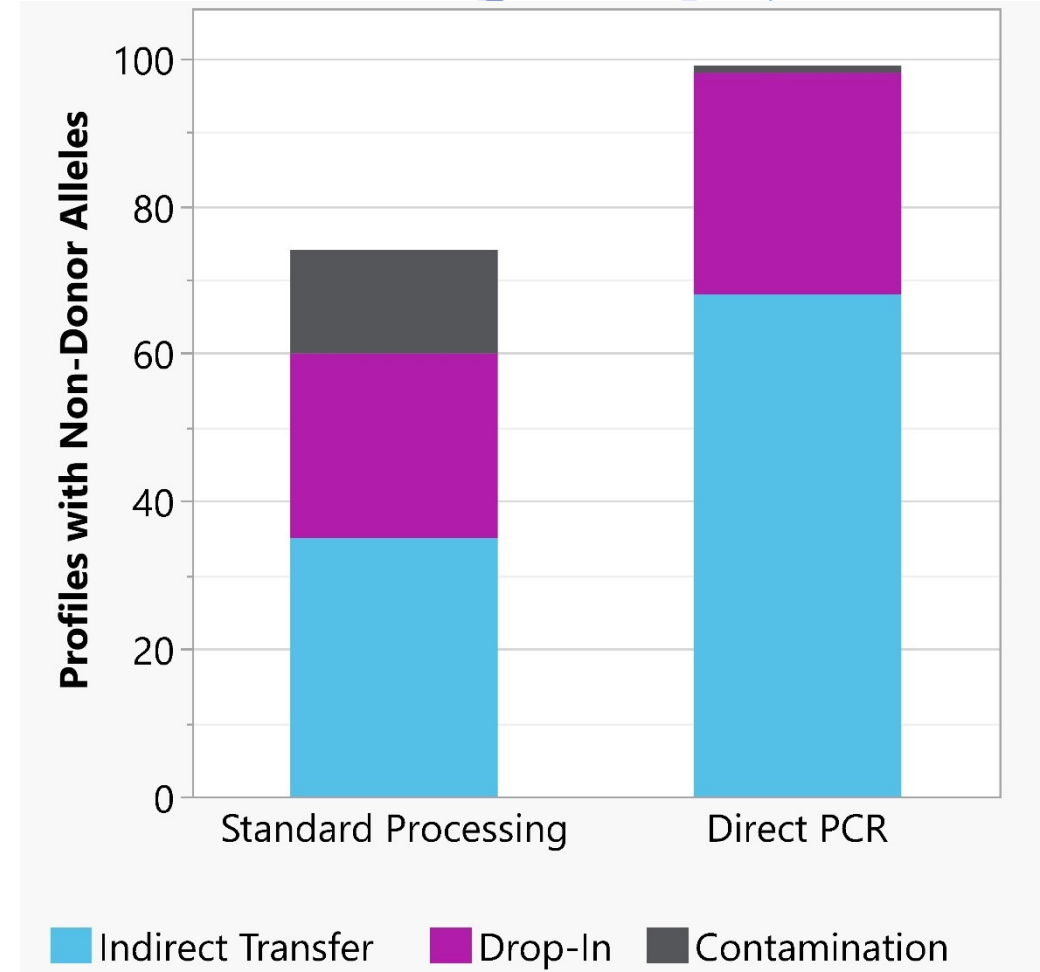


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PHASE I RESULTS

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.



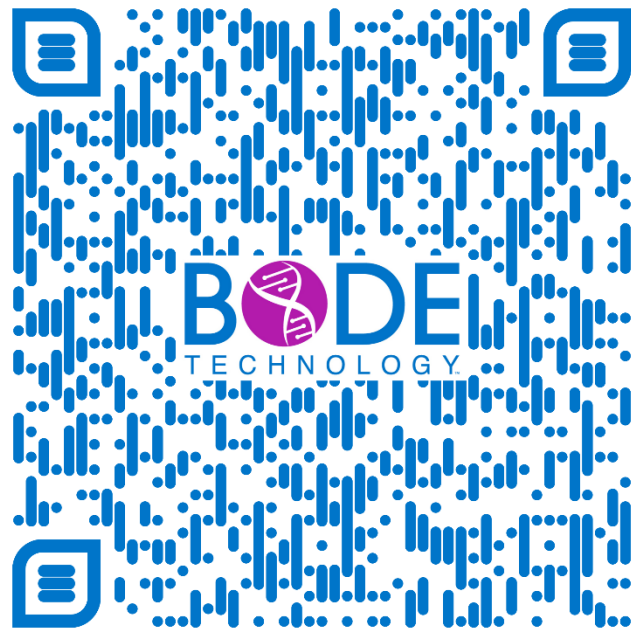
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PHASE II OVERVIEW

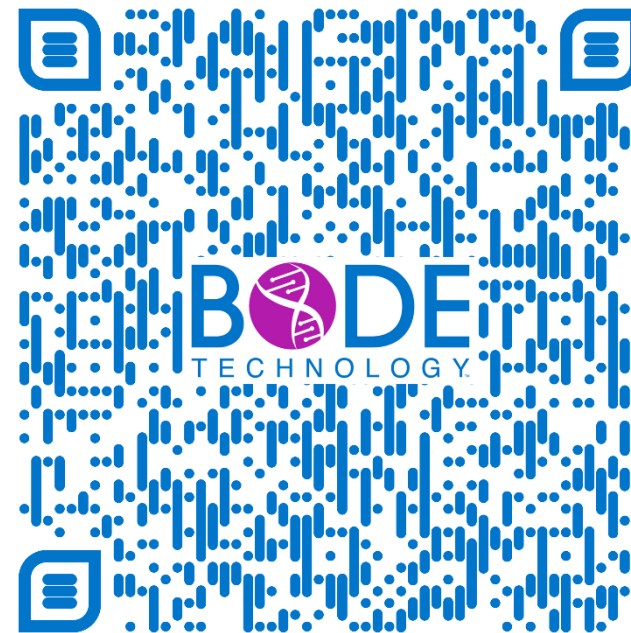


Phase II Brief Summary

- 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

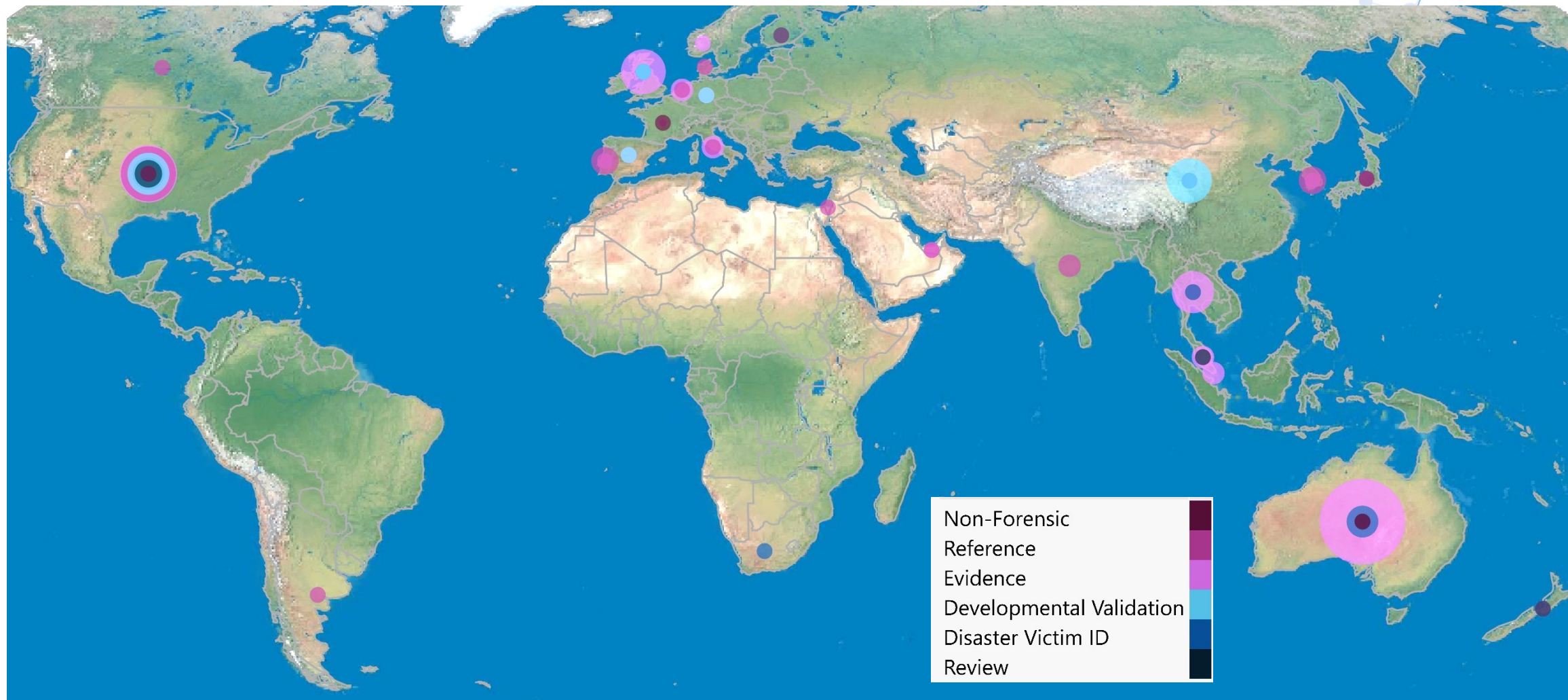
DIRECT PCR

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 μ L 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



ELSEVIER

Contents lists available at ScienceDirect

Forensic Science International: Genetics

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

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/ μ l 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 Salmonsens – 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