Direct PCR Artifacts Identified in Touch DNA from Fired Cartridge Casings

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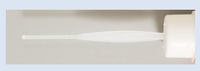
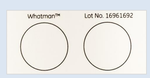
Introduction

- Direct polymerase chain reaction (PCR) is a DNA processing method in which a sample is added directly to an amplification reaction without prior purification or quantification.
- Reduces costs, labor, processing time, and DNA loss
- Downstream processing issues can arise due to the presence of contaminants and inhibitors
- The objective of this poster is to report PCR artifacts observed following direct PCR of swabs collected from brass cartridge casings that were handled and fired.

Methods

Collection Substrate	Diameter (mm^2)	Collection Method	Moistening Agent	Method Abbr.
Puritan® cotton swab 	3.0	Swabbing	Sterile H_2O	CW
			0.1% Triton X	CX
			None - dry	CD
Copan microFLOQ® swab 	1.2-2.0	Swabbing	Sterile H_2O	NW
			0.1% Triton X	NX
			None - dry	ND
Non-indicating FTA paper 	1.2	Rubbing/ scraping	Sterile H_2O	FW
			0.1% Triton X	FX
			None - dry	FD

9 mm Luger brass rounds
Fired with Glock 19

- Three touch DNA donors
- Eight replicates per collection method
- GlobalFiler™ – 25 µl, 29 cycles
  - Collection methods used: all
  - n = 216
- PowerPlex® Fusion 6C – 25 µl, 29 cycles
  - Collection methods used: CW & NX
  - n = 48

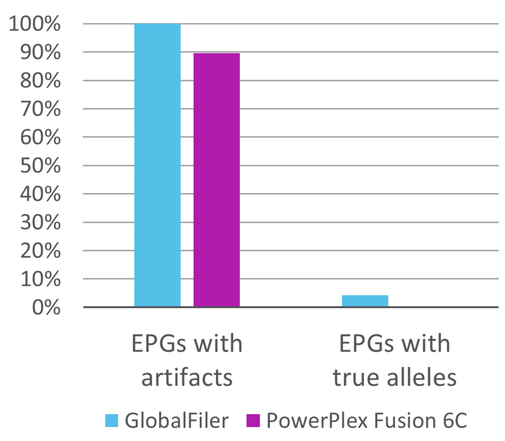
Process	Method	
Direct PCR	GlobalFiler	PowerPlex Fusion 6C
CE	3500xL Genetic Analyzer	
Data analysis	GeneMapper ID-X® v1.5	
Analytical threshold (AT)	125 RFU	100 RFU

Results

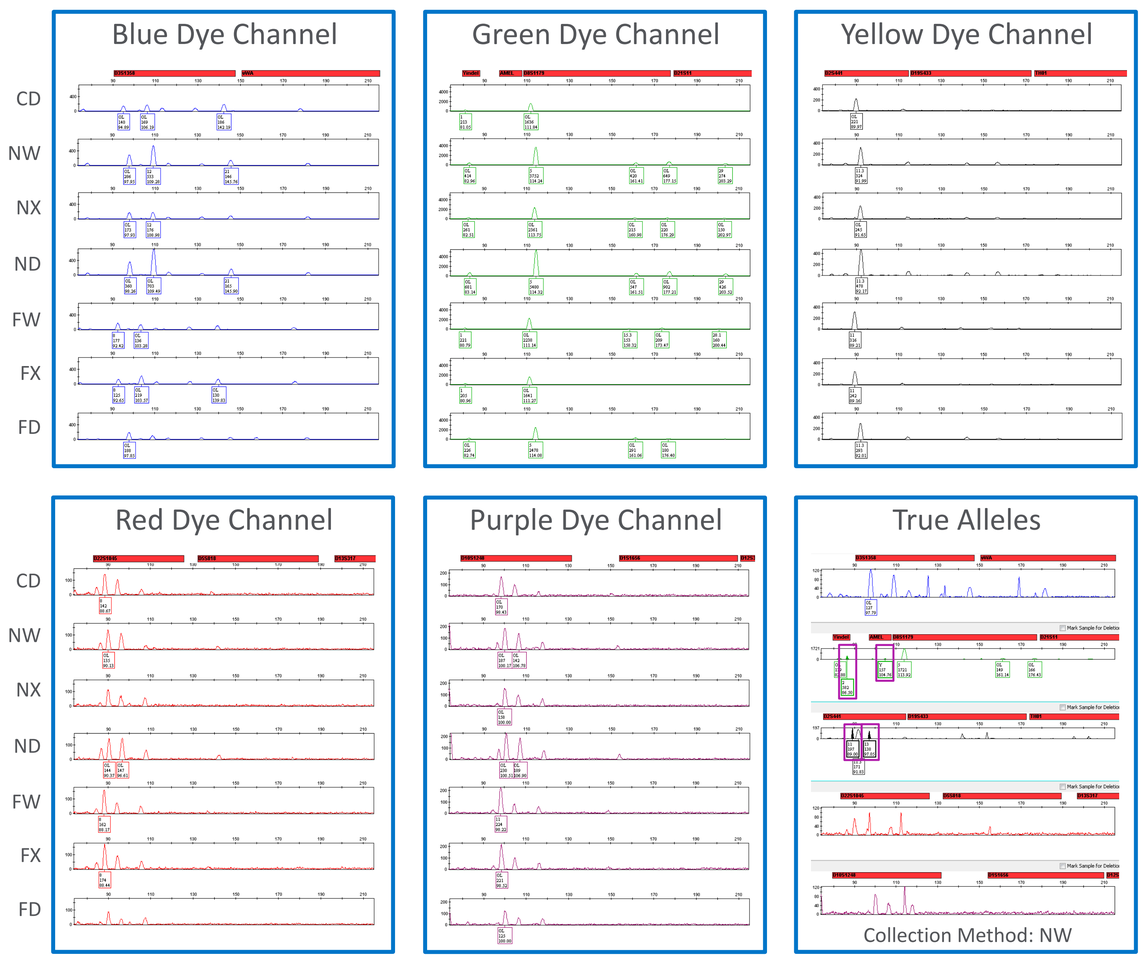
- Distinct peak morphology differentiated artifacts from true alleles.
- Peaks determined to be artifacts were tallied by size per amplification system.
- All artifacts were <205 bp and predominantly in the blue and green dye channels.

GlobalFiler													
				# artifacts above AT									
Dye	Locus	Size (bp)	Affected bin(s)	CW	CX	CD	NW	NX	ND	FW	FX	FD	Total
Blue	D3S1358	92-99	8, 9	0	1	3	11	11	10	4	8	5	53
Blue	D3S1358	103-110	12	0	0	2	10	7	12	2	1	0	34
Blue	D3S1358	140-146	21	0	0	3	1	0	3	0	1	0	8
Green	Y indel	80-84	1	2	8	9	15	15	15	9	9	9	91
Green	D8S1179	110-115	5	24	24	24	24	24	24	24	24	24	216
Green	D8S1179	158-162	15.3, 16	0	0	0	16	12	13	6	8	4	59
Green	D8S1179	173-178	20	0	0	0	15	15	13	4	0	1	48
Green	D21S11	200-204	28.1, 28.3, 29	0	0	0	6	5	12	2	0	0	25
Green	D21S11	237	N/A	0	0	0	0	0	1	0	0	0	1
Yellow	D2S441	89-93	11, 11.3	2	9	8	18	20	14	13	14	17	115
Red	D22S1045	88-90	8	0	2	4	3	0	1	2	1	0	13
Red	D22S1045	97	N/A	0	0	0	0	0	1	0	0	0	1
Purple	D10S1248	98-101	11	0	6	8	9	7	8	6	6	6	56
Purple	D10S1248	107	N/A	0	0	0	1	0	2	0	0	0	3
			Total	28	50	61	129	116	129	72	72	66	723

PowerPlex Fusion 6C						
				# artifacts above AT		
Dye	Locus	Size (bp)	Affected bin(s)	CW	NX	Total
Blue	D3S1358	92-96	8	24	17	41
Blue	D3S1358	103-107	11	16	17	33
Blue	D3S1358	113-114	N/A	14	0	14
Blue	D3S1358	130	16.2	14	0	14
Blue	D3S1358	140-144	19	19	8	27
Blue	D1S1656	178-179	14	3	0	3
Blue	D1S1656	200-201	N/A	11	0	11
Green	D16S539	117-118	13.3	0	8	8
Green	D18S51	148-149	11	4	0	4
			Total	105	50	155

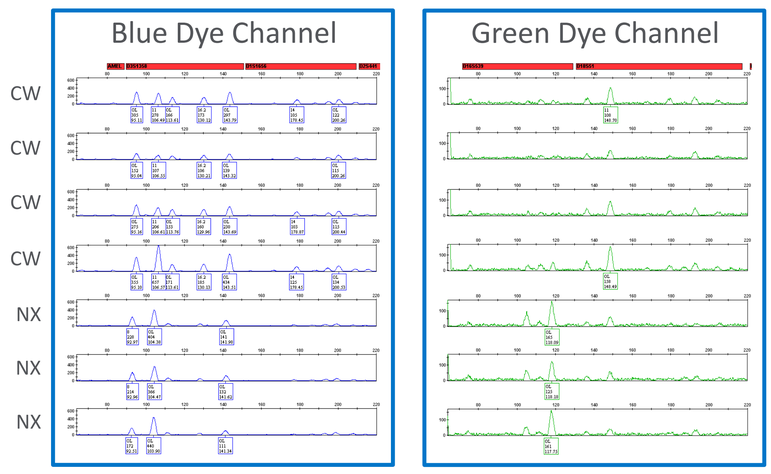


GlobalFiler



- All controls performed as expected. No artifacts were detected in amp positives or negatives.
- ILS performed as expected. No artifacts were detected, peaks were balanced, and mean peak height was ~1600 RFU.
- No CODIS-eligible profiles were obtained.
  - One true allele was observed in five samples.
  - Two true alleles were observed in three samples.
  - Four true alleles were observed in one sample (shown above).

PowerPlex Fusion 6C



- All controls performed as expected. No artifacts were detected in amp positives or negatives.
- ILS performed as expected: no artifacts, balanced peaks, mean peak height ~400 RFU
- No artifacts in yellow, red, or purple dye channels
- No true alleles

Conclusions

- Direct PCR is not optimal for swabs collected from fired cartridge casings when using unmodified GF or PPF6C amplification reactions. Higher quality DNA typing results can be obtained using standard processing and new/optimized collection methods[1].
- If optimizing GF or PPF6C reactions for direct PCR of cartridge casing samples, artifacts will appear as slightly broadened peaks that may mask true alleles if they fall into bins.
- In GF, more artifacts were observed in samples collected with microFLOQ swabs.
- In PPF6C, more artifacts were observed in samples collected with cotton swabs. Additionally, PPF6C artifacts at 113-114, 130, 148-149, 178-179, and 200-201 only appeared in samples collected with CW, and artifacts at 117-118 only appeared in samples collected with NX.

[1]Bille TW, Fahrig G, Weitz SM, Peiffer GA. An improved process for the collection and DNA analysis of fired cartridge cases. Forensic Sci Int Genet. 2020;46:102238.

This project was supported by Award No. 2019-DU-BX-0009, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this exhibition are those of the authors and do not necessarily reflect those of the Department of Justice.

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