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Investigation into Mixture found in FTA Evidence Sample [redacted] (barcode [redacted])

Maria Aguilera, Scientist, Allan McNevin, Senior Scientist,
DNA Analysis, Forensic and Scientific Services, Queensland Health.

Abstract

On the 24th of May 2008, during the Genescan analysis of capillary electrophoresis batch CEPRF20080521_01 (to become batch GEN9REF20080526_01), a mixture was noted in FTA sample barcode [redacted] Initially FTA sample [redacted] was processed through routine FTA processing procedures (as outlined above) on batch FTA20080207_01 (to become GEN9REF20080225_03), and yielded no DNA profile. An investigation under OQI # 19767 commenced to determine the cause for this mixture. [redacted]

Background

Within DNA Analysis processing of person (Reference) samples is performed using FTA [redacted] are transferred onto FTA™ paper and provided to the laboratory, or whole blood is provided and this transferred to the FTA™ paper within the laboratory.

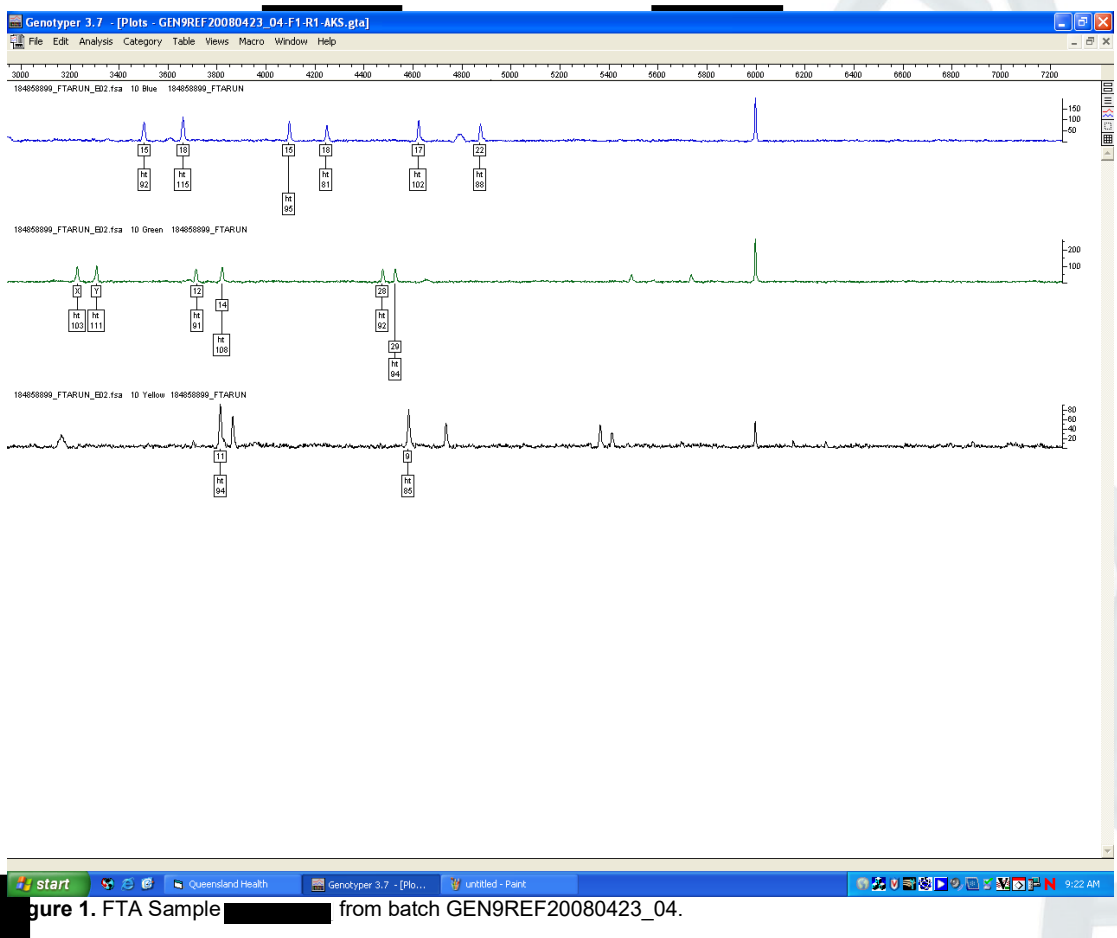
These samples are processed through standard laboratory procedures. Briefly, punches of FTA paper were transferred to a 96-well half-skirt PCR plates using the BSD Duet 600 (BSD Robotics, Australia) semi automated dried sample punch instrument. The dried punches were washed with TE buffer (blood punches were washed in weak NaOH solution followed by TE buffer), and dried on a hot block. The samples are then processed through to STR PCR amplification. This was performed by the addition of TE and PCR mastermix (Applied Biosystems AMPFℓSTR® Profiler Plus® PCR Amplification kit), the plate was sealed and amplified on a GeneAmp® PCR System 9700 thermalcycler. After amplification stage, fragment analysis was performed on a portion of the PCR product by capillary electrophoresis on the Applied Biosystems Prism® 3130*xl* Genetic Analyser, and the data analysed using a combination of Genescan (version 3.7.2) with Genotyper (version 3.7.1) software.

If an unacceptable profile was obtained further processing would be required, depending on the sample and result, the sample may have been re-processed through the procedure outlined above with the same, more or less punches of the FTA paper. If these results were still unacceptable further processing was performed. This involved further punches (with a larger manual hole punch) being placed into individually labelled DNA free 1.5mL tubes. The punches were then transferred to a Slicprep™ 96 device (Promega) via the use of the *utomate.it* STORstar system (Process Analysis & Automation Ltd. Hampshire. UK). There ere then processed through automated DNA extraction on a PerkinElmer MultiPROBE® II LUS HT EX with Gripper™ Integration platform with Promega DNA IQ™ DNA extraction kit. ach extraction batch includes a positive and negative extraction control and a negative unching control for quality purposes. All samples on the one batch are processed under the ame conditions as each other according standard laboratory procedures (QIS document 4897).

fter extraction, between and after the following processes, the DNA extracts were stored rozen at -20°C. Following extraction, the DNA extracts were then quantified using Applied Biosystems Quantifiler® Human DNA Quantification Kit on an Applied Biosystems Prism 7000 Sequence Detection System real-time PCR instrument. The DNA extract was then amplified using Applied Biosystems AMPFℓSTR® Profiler Plus® amplification kit on a Perkin Elmer GeneAmp 9700. The PCR product was then prepared for capillary electrophoresis and run through an Applied Biosystems Prism 3130*xl* Genetic Analyser and analysed using Genescan (version 3.7.2) and Genotyper (version 3.7.1) software.

Investigation [redacted]

On the 24th of May 2008 during the Genescan analysis of capillary electrophoresis batch CEPRF20080521_01 (to become batch GEN9REF20080526_01), a mixture was noted in FTA sample barcode [redacted]. Initially FTA sample [redacted] processed through routine FTA processing procedures (as outlined above) on batch FTA20080207_01 (to become GEN9REF20080225_03), and yielded no DNA profile. The sample was reprocessed with extra punches on batch FTARUN20080318_02 (to become batch GEN9REF20080423_04). This resulted in a partial DNA profile shown in Figure 1 below.



[redacted] **gure 1.** FTA Sample [redacted] from batch GEN9REF20080423_04.

[redacted] he FTA sample was further processed through automated extraction procedures outlined bove on batch FTAEXT20080515_01 (to become batch RFIQEXT20080515_01). After xtraction, the sample was processed through routine quantification and amplification ocedures as outlined above. The DNA extract yielded a quantification value of 0.0558ng/µL, sulting in 20µL of the DNA extract being added to the amplification reaction. This amplified oduct was analysed on capillary electrophoresis batch CEPRF20080521_01 (to become atch GEN9REF20080526_01). This yielded a mixed DNA profile shown in Figure 2 below.

[redacted]

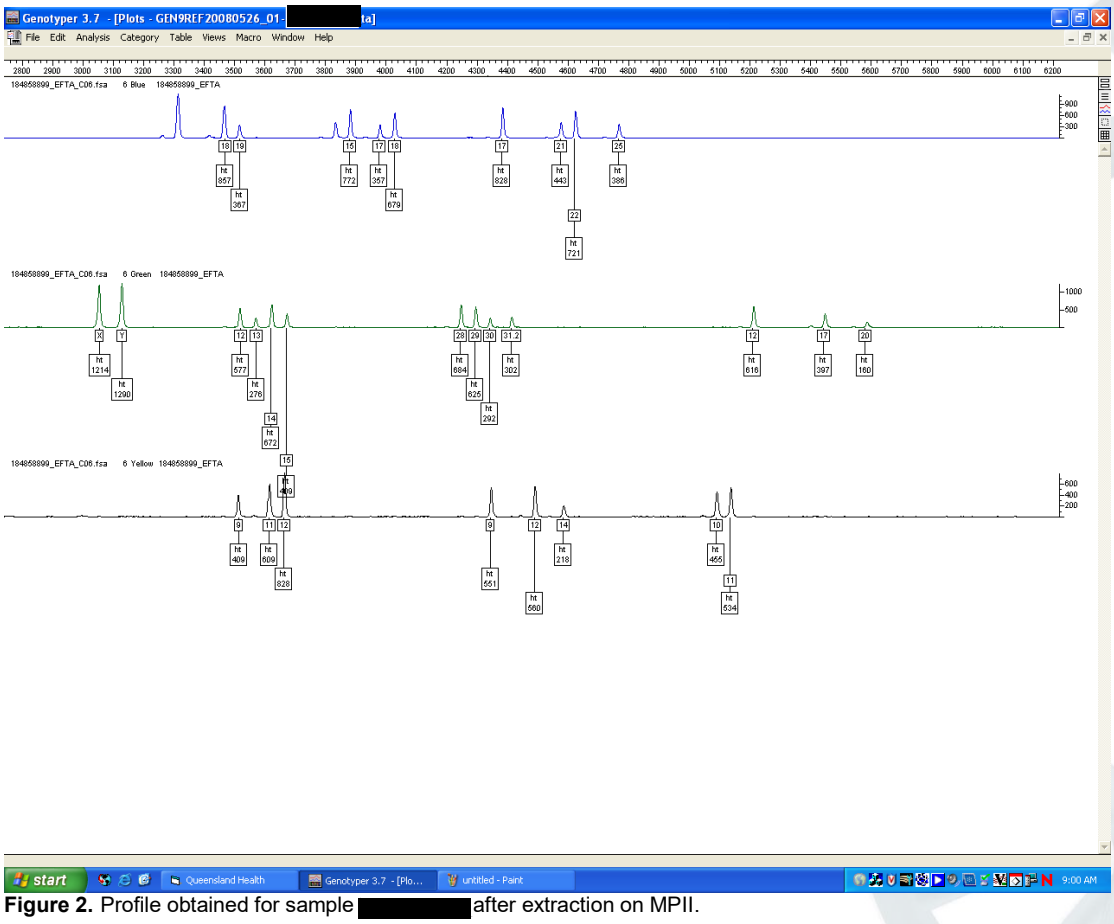


Figure 2. Profile obtained for sample [redacted] after extraction on MPII.

The sample [redacted] was reprepared and re-run on t[redacted]tic Analyser and re-analysed [redacted]enescan software. The mixed DNA profile was shown to be reproducible.

The DNA extract was re-amplified on amplification batch 9AMPR200820080527_01 (to become batch GEN9REF20080602_02). The mixed DNA profile was shown to be reproducible as shown in Figure 3 below.

[redacted]

[redacted]

[redacted]

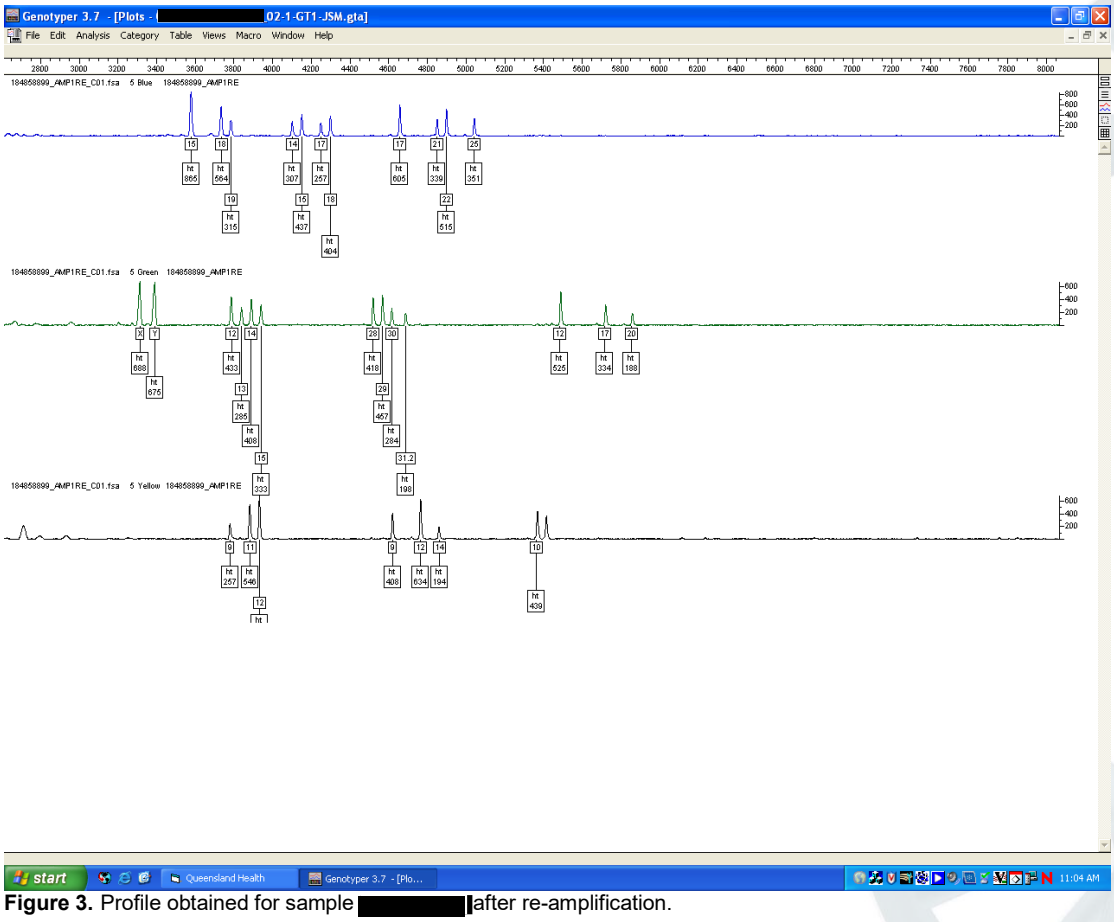


Figure 3. Profile obtained for sample [redacted] after re-amplification.

This demonstrates that the DNA extract of sample [redacted] was contaminated with a second DNA [redacted] e-extraction for the FTA sample [redacted] performed under barcode [redacted] and this yielded a full 9-loci single source DNA profile shown in Figure 4 below.

[redacted]

[redacted]

[redacted]

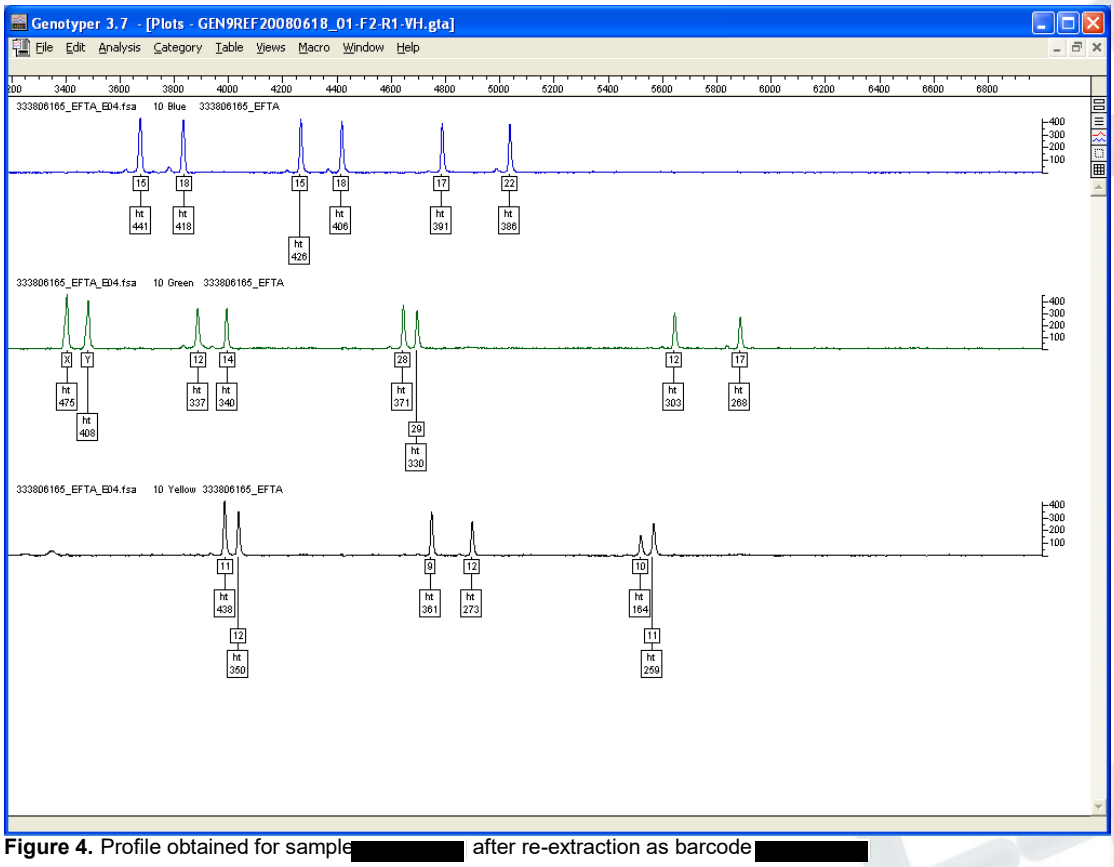


Figure 4. Profile obtained for sample [redacted] after re-extraction as barcode [redacted]

This profile was consistent with [redacted]nal partial profile shown in Figure 1 above.

The mixed DNA profile from barcode [redacted] w[redacted]parated into the known DNA profile and th[redacted]ating profile contributing to the m[redacted]s then searched against a[redacted]btained from samples on extraction batch RFIQEXT20080515_01. A match was found with sample [redacted]. This is shown in Table 1 below.

Table 1. Summary or results for FTA sample 184858899.

Sample	Processing	Amel	D3	D8	D5	vWA	D21	D13	FGA	D7	D18
[redacted]	FTA	X, [redacted]	[redacted] 18	12, 14	11, NR	15, 18	28, 29	9, NR	17, 22	NSD	NSD
	DNA IQ extraction	X, Y	15, 18, 19	12, 13, 14, 15	9, 11, 12	14, 15, 17, 18	28, 29, 30, 31.2	9, 12, 14	17, 21, 22, 25	10, 11	12, 17, 20
	DNA IQ extraction	X,Y	15,18	12,14	11,12	15,18	28,29	9,12	17,22	10,11	12,17
		X, Y	15,19	13, 15	9, 12	14, 17	30, 31.2	12, 14	21, 25	10, 11	12, 17, 20
	DNA IQ extraction	X,Y	15,19	13,15	9,12	14,17	30,31.2	12,14	21,25	10,11	12,20

NR = no reportable allele, NSD = No Sizing Data (no alleles detected), *333806165 was a re-extraction of 184858899

A representation of the plate layout for extraction batch RFIQEXT20080515_01 is shown in Figure 5 below, with the relative positions of samples [redacted] indicated in yellow and green respectively.

	1	2	3	4	5	6	7	8	9	10	11	12
A	POS	sample	sample	[redacted]	sample	sample	[redacted]	[redacted]	[redacted]	sample	sample	sample
B	NEG	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
C	NEG	sample	sample	[redacted]	sample	sample	sample	[redacted]	sample	sample	sample	empty
D	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	empty
E	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	empty
F	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	empty
G	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	empty
H	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	empty

Figure 5. Plate layout for extraction batch RFIQEXT20080515_01

The profile obtained from extraction batch RFIQEXT20080515_01 for sample [redacted] was consistent with all 16 alleles obtained from previous FTA processing of the same sample. Therefore the profile that was obtained from this sample was not from an exogenous source. The presence of a reproducible DNA profile from within the extract and with both samples [redacted]) being both processed alongside each other on initial quantification and amplification batches indicates that contamination has occurred at the point of the first Amplification set-up or earlier (e.g. quantification or extraction).

Conclusion

During the course of investigation information has been gained that when viewed alongside other previous and subsequent quality events (namely OQI's #19330, 19349, 19477, & 19768) a picture of potential systematic quality failure of the quality processes from the automated extraction processes is present. Further information is required to further elucidate and rectify if necessary any problems. A full process audit (Audit #8227 – DNA IQ) has been commissioned to thoroughly review all facets of the automated extraction process. This had been planned as a post implementation review but has been brought forward in view of events mentioned above. An extra-ordinary meeting of the DNA Analysis management meeting was held 14/07/2008 and the following actions were agreed upon:

- Processing of Reference samples only on Extraction platform A (initial investigations indicated events were likely related to platform A)
- Processing of Casework samples on Extraction platform B in a checkerboard pattern with extraction reagent blanks (layout shown in Figure 6 below).
- Urgent progression of audit mentioned above and investigation into findings
- A full information review of results from automated extractions with documented quality events and extractions without documented quality events to gain further information

[redacted]

	1	2	3	4	5	6	7	8	9	10	11	12
A	Neg	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank	sample
B	Pos	blank	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank
C	blank	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank	sample
D	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank
E	blank	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank	sample
F	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank
G	blank	sample	blank	sample	blank	sample	blank	[redacted]	blank	[redacted]ample	blank	sample
H	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank

Figure 6. Checkerboard arrangement of samples and extraction blanks on extraction batches during investigation period

Findings from [redacted] audit and investigations will be documented in the quality system against the audit and in a separate investigation report once complete. This adverse event has been documented as OQI#19767 in the quality system.

[redacted]