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Investigation into [redacted]e Extraction Control with a partial DNA profile [redacted]

Allan McNevin, Senior Scientist, Chiron Weber, Scientist
[redacted]
DNA Analysis, Forensic and Scientific Services, Queensland Health.

Abstract

During a review of extraction controls in response to quality concerns raised about the automated DNA IQ extraction process, it was noted that negative extraction control [redacted] (off-deck lysis batch CWIQLYS20080416_01 and extraction batch CWIQEXT20080417_01) was found to contain peaks above peak detection threshold but below reporting threshold at two loci. This appears to be a further example of well-to-well contamination during the automated DNA IQ extraction process.

Introduction

Within DNA Analysis, routine DNA extractions are performed using the PerkinElmer MultiPROBE® II PLUS HT EX with Gripper™ Integration platform in conjunction with The Promega DNA IQ™ kit. For each extraction process on the PerkinElmer MultiPROBE® II PLUS HT EX with Gripper™ Integration platform, each extraction batch includes a positive and negative extraction control for quality purposes. All samples on the one batch are processed under identical conditions according standard laboratory procedures (refer QIS document 2489[redacted]). Briefly, samples extracted using the Promega DNA IQ™ kit were processed through 2 distinct process, off-deck lysis and automated extraction. The off-deck lysis consisted of manual addition of extraction buffer to each sample followed by incubation and separation of the substrate from liquid components. The liquid component (lysate) was then manually transferred into an ABgene 2mL 96-deep well plate via the use of the *automate.it* STORstar system (Process Analysis & Automation Ltd. Hampshire. UK). The DNA from the lysates was then extracted on a PerkinElmer MultiPROBE® II PLUS HT EX with Gripper™ Integration platform using the Promega DNA IQ™ kit. After extraction DNA extracts were stored frozen (-20°C) in Nunc™ Bank-It tubes whilst waiting for further processing.

The DNA within each DNA extract was then quantified using the Applied Biosytems Quantifiler™ Human DNA Quantification kit. The PCR reaction was prepared on a dedicated (Pre-PCR) PerkinElmer MultiPROBE® II PLUS HT EX with Gripper™ Integration platform. The real-time PCR was [redacted]d out on an Applied Biosystems Prism® 7500 Sequence Detection System. Once the DNA quantification value had been obtained an appropriate amount of DNA template to be added to the STR amplification reaction was determined by mathematical calculation as programmed in the AUSLAB laboratory information management system. The DNA extracts were then amplified using the Applied Biosystems AMPF*l*STR® [redacted]us® PCR Amplification kit, prepared on a dedicated (Pre-PCR) PerkinElmer [redacted]BE® II PLUS HT EX with Gripper™ Integration platform, and amplified on a [redacted]® PCR System 9700 thermalcycler.

After amplification a portion of the amplified product was then submitted to fragment analysis. This was performed by capillary electrophoresis on an Applied Biosystems Prism® 3130*xl* Genetic Analyser, and the data analysed using a combination of Genescan (version 3.7.2) Genotyper (version 3.7.1) software. During preparation of quantification and amplification batches, sample storage was tracked using AUSLAB storage functionality, each sample was [redacted]zen (-20°C) whilst waiting for processing. For the preparation of PCR reactions, [redacted]bes were uncapped and recapped using a LifeTool™ RECAP 96M automated

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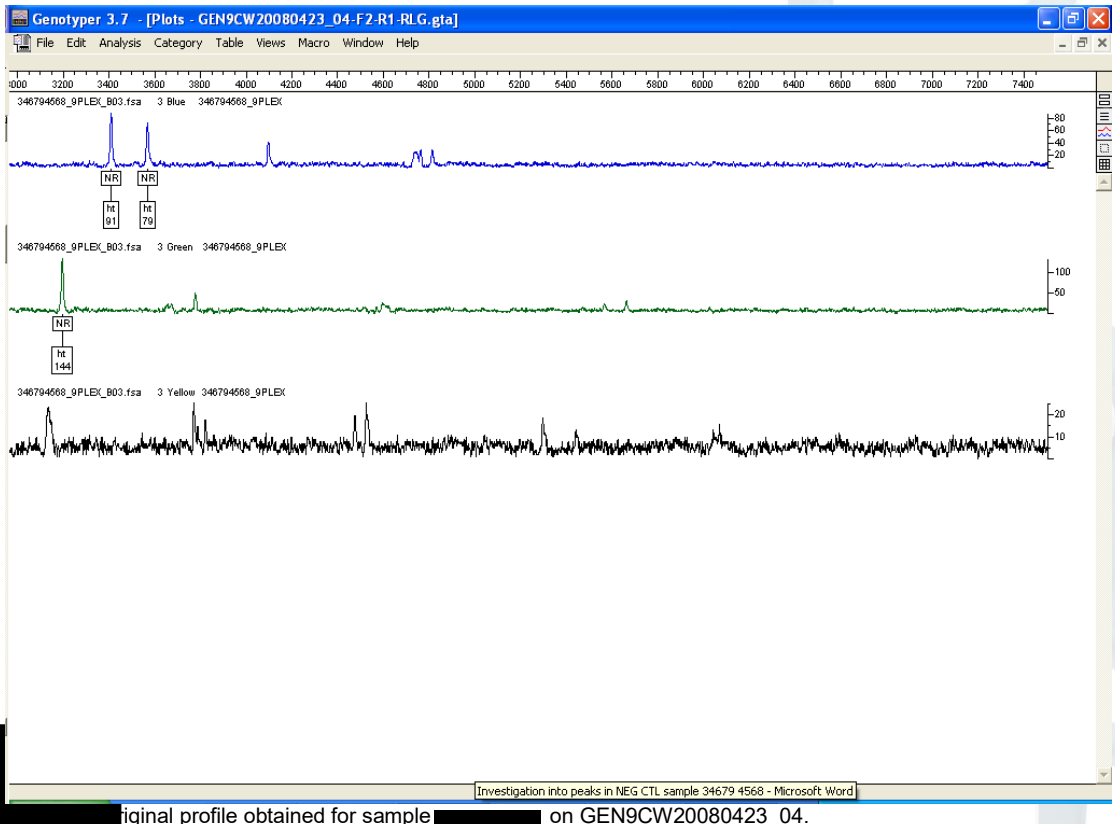
Queensland Government
Queensland Health

Investigation [redacted]

Negative extraction control sample [redacted] was extracted on batches CWIQLYS20080416_01 and CWIQEXT20080417_01 as outlined above. Negative extraction control sample [redacted] was one of two negative extraction controls present on these batches. The DNA extract was then quantified using the method described above, yielding a quantification value of 0.000934ng/µL. This value is less than the limit of reporting (0.00426ng/µL) as determined by previous in-house validation. The DNA extract of [redacted] was then amplified at maximum volume (20µL), a portion of the PCR product run through capillary electrophoresis and the results analysed using methods outlined above.

Results obtained from analysis using the Genotyper software (Genotyper batch GEN9CW20080423_04) showed 4 peaks above the 75 RFU peak detection threshold as shown in Figure 1 below.



[redacted]iginal profile obtained for sample [redacted] on GEN9CW20080423_04.

The results from this analysis were imported into AUSLAB, however no concern was raised to the Analytical senior scientist at either Genescan (allele sizing), Genotyper (allele designation) or result importing process. This displays a breakdown of two quality processes, whereby the Analytical 3130*xl* operator is to review all positive and negative extraction controls during the Genescan analysis of samples run on the instrument and Analytical staff [redacted] profiles into AUSLAB are to review all positive and negative extraction controls. [redacted]th of these processes the presence of peaks above detection threshold were not [redacted]

In July 2008, concern was raised as the quality of the automated DNA IQ extraction process, with a number of contamination events having occurred leading to evidence of well to well contamination during the automated DNA IQ extraction [redacted] process as outlined above. A review of all negative extraction controls from all batches performed

through this process was [redacted] It was noted that negative extraction control sample 346794568 showed the presence of [redacted] DNA profile in AUSLAB LIMS. The profile obtained from Genotyper batch GEN9CW2008042[redacted] analysed using a lowered peak detection [redacted] of 30RFU. The profile observed is shown in Figure 2 below.

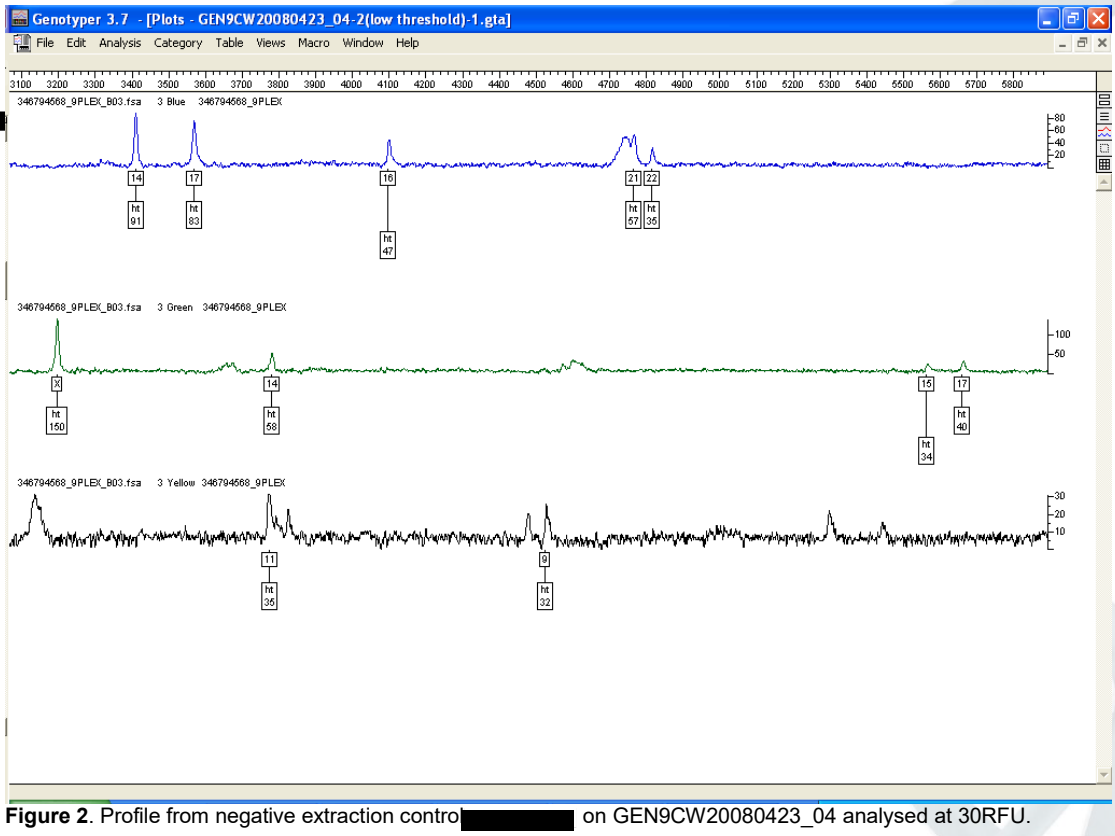


Figure 2. Profile from negative extraction contro[redacted] on GEN9CW20080423_04 analysed at 30RFU.

The DNA extract from [redacted] was then concentrated by centrifugal filtration with a Microcon YM-100 (Millipore) filter. The DNA extract was reduced in volume from 100µl to 37µl. This concentrated extract we re-quantified, amplified and analysed through capillary electrophoresis using methods described above. A quantification result of 0.0238ng/µL was obtained and two peaks were present above peak detection threshold. This profile is shown in [redacted] elow.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

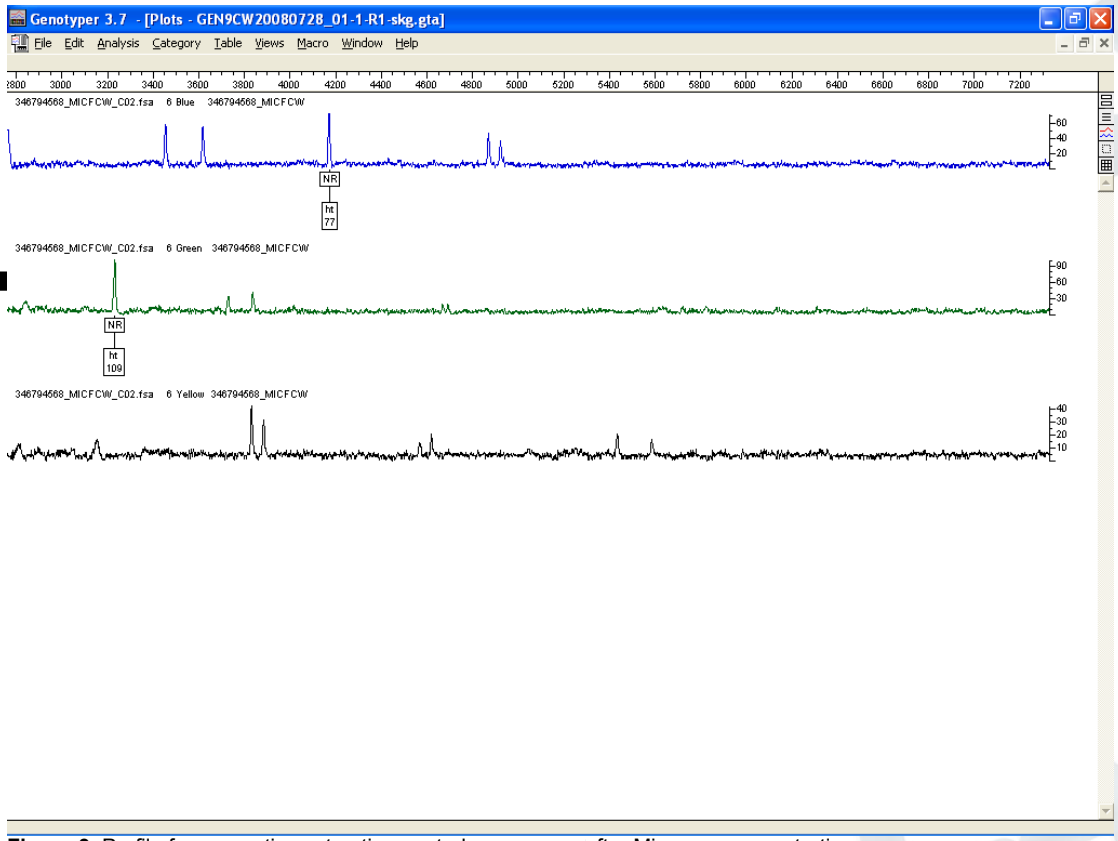


Figure 3. Profile from negative extraction control [redacted] after Microcon concentration

This profile was re-analysed at reduced peak detection thresholds (30RFU's). This is shown in Figure 4 below.

[redacted]

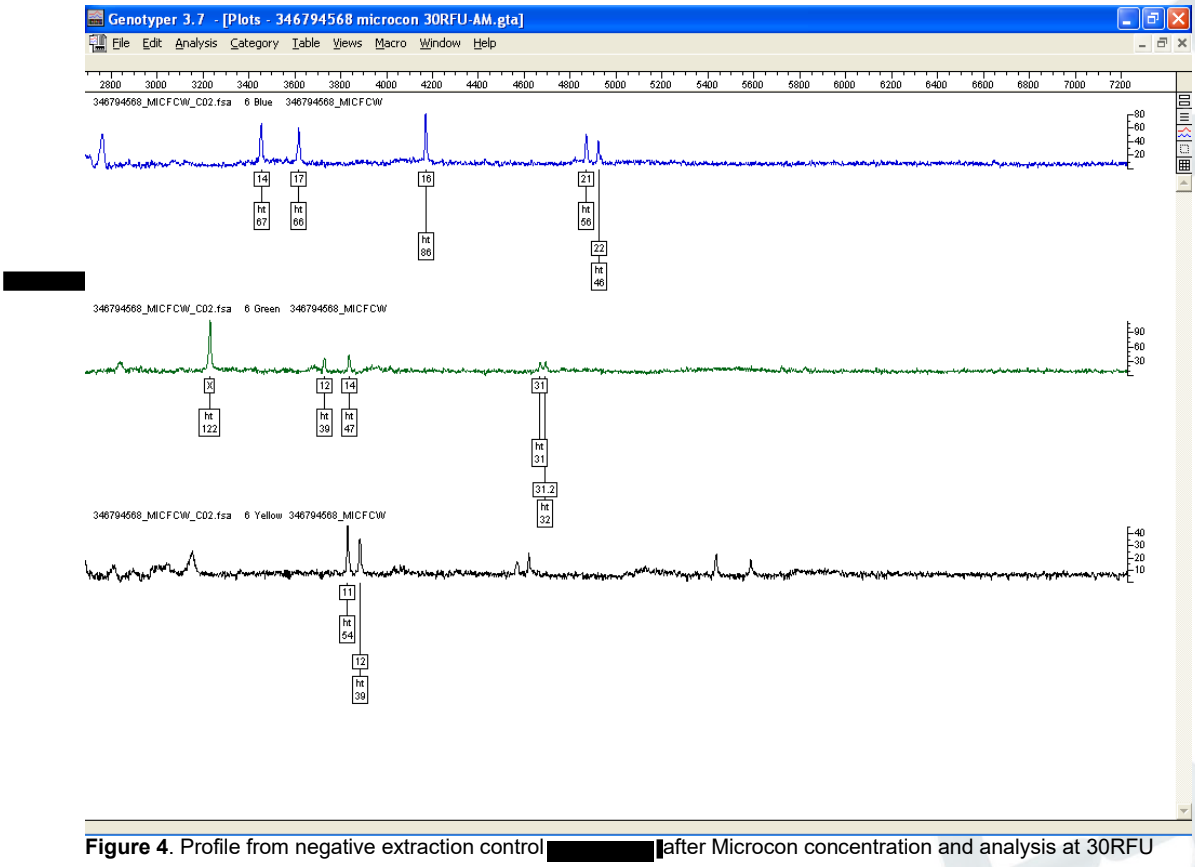


Figure 4. Profile from negative extraction control [redacted] after Microcon concentration and analysis at 30RFU

The results obtained from initial amplification and amplification after Microcon concentration are summarised in Table 1 below.

Table 1. Profiles obtained from 346794568 when analysed using 30RFU peak detection threshold

Sample	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
Initial extract	14,17	16	21,22	X,X	14	NSD	15,17	11	9	NSD
Concentrated extract	14,17	16,16	21,22	X,X	12,14	31,31.2	NSD	11,12	NSD	NSD
Combined profile	14,17	16,16	21,22	X,X	12,14	31,31.2	15,17	11,12	9,NR	NSD

NSD = No Sizing Data (i.e. no peaks detected) NR= not reportable (i.e. no allele designated)

[redacted] ined profile was then searched against all other profiles obtained from the same batch (CWIQEXT20080417_01). A match was found to sample 346802502, an [redacted] ental monitoring sample. When further analysis of the whole extraction batch was [redacted] at reduced peak detection thresholds, an additional four samples (346802405, 346802532, 346802482, and 346802446) were found to contain consistent alleles. These results are shown in Table 2 below.

Table 2. Profiles obtained from CWIQEXT20080417_01 matching to negative control 346794568

Sample ID	Quant	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
[redacted]	0.000934*	14,17	16,16	21,22	X,X	12,14	31,31.2	15,17	11,12	9,NR	NSD
	0.002810	14,NR	16,NR	21,NR	X,NR	12,14	NSD	NSD	11,12	8,NR	NSD
	0.000628	NSD	NSD	NSD	X,X	12,14	NSD	NSD	11,12	NSD	NSD
	0.006560	14,17	16,NR	21,22	X,X	12,14	31,NR	17,NR	11,12	NSD	NSD
	0.002600	14,17	16,NR	NSD	X,X	14,NR	NSD	NSD	NSD	NSD	NSD
	1.620000	14,17	16,16	21,22	X,X	12,14	31,31.2	15,17	11,12	8,9	10,13

NSD = No Sizing Data (i.e. no peaks detected) NR= not reportable (i.e. no allele designated), Qua[redacted] concentration in the extract as ng/µL, * = Quantification value before concentration, ** = quantification and profile after clean-up procedure (inhibition detected from original quantification)
[redacted]

After reviewing the quantif[redacted]es for the samples outlined in Table 2 above, it was determined that sample 346802502[redacted] most likely the source of the contamination as this sample had the highest quantification value (1.62n[redacted]). [redacted]346802502 was a blind positive control [redacted] of a buccal swab and was submitted alongside environmental monitoring samples collected for that month. The relative layout of samples on extraction batch CWIQEXT20080417_01 are shown in Figure 5 below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	[redacted] (0.000934)	[redacted] Enviro (0.002810)	[redacted] Enviro (0.0026)	[redacted] Enviro (1.62)	Proj sample	Proj sample	sample	sample	sample	sample	sample	sample
B	Pos	[redacted] Enviro (0.000628)	Enviro sample	Proj sample	Proj sample	sample	sample	sample	sample	sample	sample	sample
C	Neg	Enviro sample	Enviro sample	Proj sample	Proj sample	sample	sample	sample	sample	sample	sample	sample
D	Pos	Enviro sample	Enviro sample	Proj sample	Proj sample	sample	sample	sample	sample	sample	sample	sample
E	Enviro sample	Enviro sample	Enviro sample	Proj sample	Proj sample	sample	sample	sample	sample	sample	sample	sample
F	Enviro sample	Enviro sample	Enviro sample	Proj sample	Proj sample	sample	sample	sample	sample	sample	sample	sample
G	Enviro sample	[redacted] Enviro (0.006560)	Enviro sample	Proj sample	Proj sample	sample	sample	sample	sample	sample	sample	sample
H	Enviro sample	Enviro sample	Enviro sample	Proj sample	Proj sample	sample	sample	sample	sample	sample	sample	sample

Figure 5. Relative layout of extraction batch CWIQEXT20080417_01
Pos = Positive extraction control, Neg = Negative extraction control, Enviro = Environmental monitoring sample, Proj = Sample for 4N6 swab trial, Sample = routine casework sample

[redacted]

A review of the AUSLAB audit trails for each of the affected samples shows that although sample 346802502 was quantified and amplified on the same batch as some of the affected samples, not all were processed on the same batches (see Table 3 below) and therefore the contamination of these samples (includ[redacted]ng the negative extraction control) must have occurred during the extraction procedure (either during off-deck lysis, or during automated extraction). [redacted]

Table 3. Quantification and Amplification batch ID's for affected samples

Sample ID	Sample type	Initial Quantification		Initial Amplification	
		Batch	Position	Batch	Position
[redacted]	Negative Control	QUACW20080422_01	27	9AMPC20080422_01	18
	Environmental	QUACW20080423_01	96	Nuc Cleanup	
	Environmental	QUACW20080424_01	39	9AMPC20080424_03	29
	Environmental	QUACW20080424_01	44	9AMPC20080424_03	34
	Environmental	QUACW20080424_01	47	9AMPC20080424_03	46
	Environmental	QUACW20080424_01	58	9AMPC20080424_03	49

Conclusion

[redacted]sion, the cause of contamination could be positively identified as having occurred [redacted]e extraction process. Although the contamination could not be isolated to a single [redacted]e process, contamination most likely occurred during the automated extraction on [redacted]however the off-deck lysis component cannot be excluded. The off-deck lysis [redacted]nt is least likely as this is a manual process, during which only one tube is opened at a time and samples are processed sequentially, as negative extraction control [redacted] was the first sample processed and affected samples [redacted] were processed as samples 9, 10, 15, 17 & 25 respectively, it is

highly unlikely for sample [redacted] to have contaminated multiple samples in a reverse direction.

[redacted]

This event has been documented in the FSS quality system as OQI#20231. Appropriate specimen notes, UR notes and batch audit entries have been made in AUSLAB. This event will be discussed in the next available Analytical team meeting and has formed part of the investigations already underway into the automated DNA IQ extraction procedure (including Audit #8887). Particular attention will additionally be placed on the initial acceptance of the [redacted] control [redacted] co[redacted]sing through the quality process). The need for due care when reviewing control results will be highlighted.

[redacted]

[redacted]

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[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]