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Investigation into a partial DNA profile negative extraction control sample (barcode [redacted])

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Abstract

On the 9th May 2008, it was [redacted] on CWIQLYS20080430_01/CWIQEXT20080430_01 contained a partial profile result in the DNA extract which instead should have resulted in 'NSD' which is expected for any negative control. An investigation under OQI # 19477 commenced to determine the cause for the contamination of the negative control.

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 the same conditions as each other according to SOP 24897. The Promega DNA IQ™ method consists of 2 parts, off-deck lysis and automated extraction. The off-deck lysis consists of manual addition of extraction buffer to the samples and then incubated, then manually transferred into a Slicprep™ 96 device (Promega) via the use of the *automate.it* STORstar system (Process Analysis & Automation Ltd. Hampshire. UK). The samples are then submitted for automated extraction on PerkinElmer MultiPROBE® II PLUS HT EX with Gripper™ Integration platform using Promega DNA IQ™ kit.

These samples are then progressed to the quantification stage using the Applied Biosytems Quantifiler™ Human DNA Quantification kit and are prepared on a dedicated (Pre-PCR) PerkinElmer MultiPROBE® II PLUS HT EX with Gripper™ Integration platform. The real-time PCR is then performed on an Applied Biosystems Prism® 7500 Sequence Detection System. Once the DNA quantification has been performed and an appropriate amount of DNA template to be added to the STR amplification reaction is determined, these samples are then progressed through to the amplification stage. This process is carried out by amplification using the Applied Biosystems AMPF*l*STR® Profiler Plus® PCR Amplification kit prepared by the dedicated (Pre-PCR) PerkinElmer MultiPROBE® II PLUS HT EX with Gripper™ Integration platform and amplified on a GeneAmp® PCR System 9700 thermalcycler, ultimately yielding DNA profiles.

After this amplification stage, fragment analysis is performed by capillary electrophoresis on an Applied Biosystems Prism® 3130*xl* Genetic Analyser, and the data analysed using the combination of Genescan (version 3.7.2) with Genotyper (version 3.7.1) software. During preparation of quantification and amplification batches, samples are storage is tracked using AUSLAB storage functionality, samples are stored frozen (-20°C) and at 4°C whilst waiting for processing. Sample tubes are uncapped and recapped using a LifeTool™ RECAP 96M [redacted]mated capper.

[redacted] extracts stored in Nunc™ Bank-It tubes and stored frozen (-20°C).

[redacted]stigation

[redacted]tive extraction control sample (barcode [redacted]) was extracted, quantified, amplified [redacted]analysed using methods described above. This resulted in a partial profile (an alleles at Amelogenin below casework reporting threshold). The DNA extract was re-amplified and analysed confirming this result. The sample was re-analysed at a reduced peak height

detection thresholds (30RFU) in order to obtain a possible profile. This profile is shown in Figure 1 below.

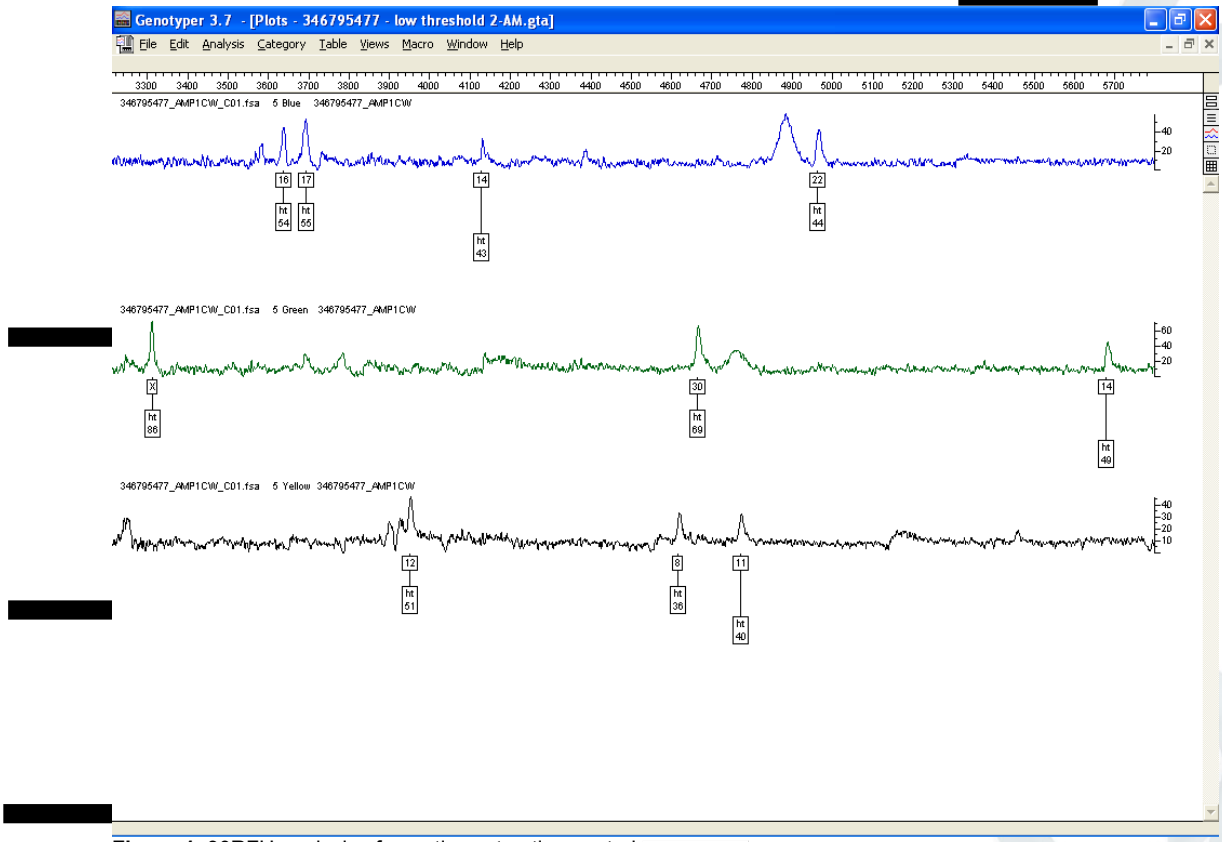

Figure 1. 30RFU analysis of negative extraction control [redacted].

The DNA extract [redacted] was then concentrated via centrifugal membrane filtration using a Microcon Y-100 (Millipore) filter to a final volume of approximately 29µL in order to obtain more detectable alleles. This resulted in the visualisation of more alleles below casework reporting threshold after quantification, amplification and analysis. These were further analysed using a 30RFU peak height detection threshold. The profile visualised is shown in Figure 2 below.

[redacted]

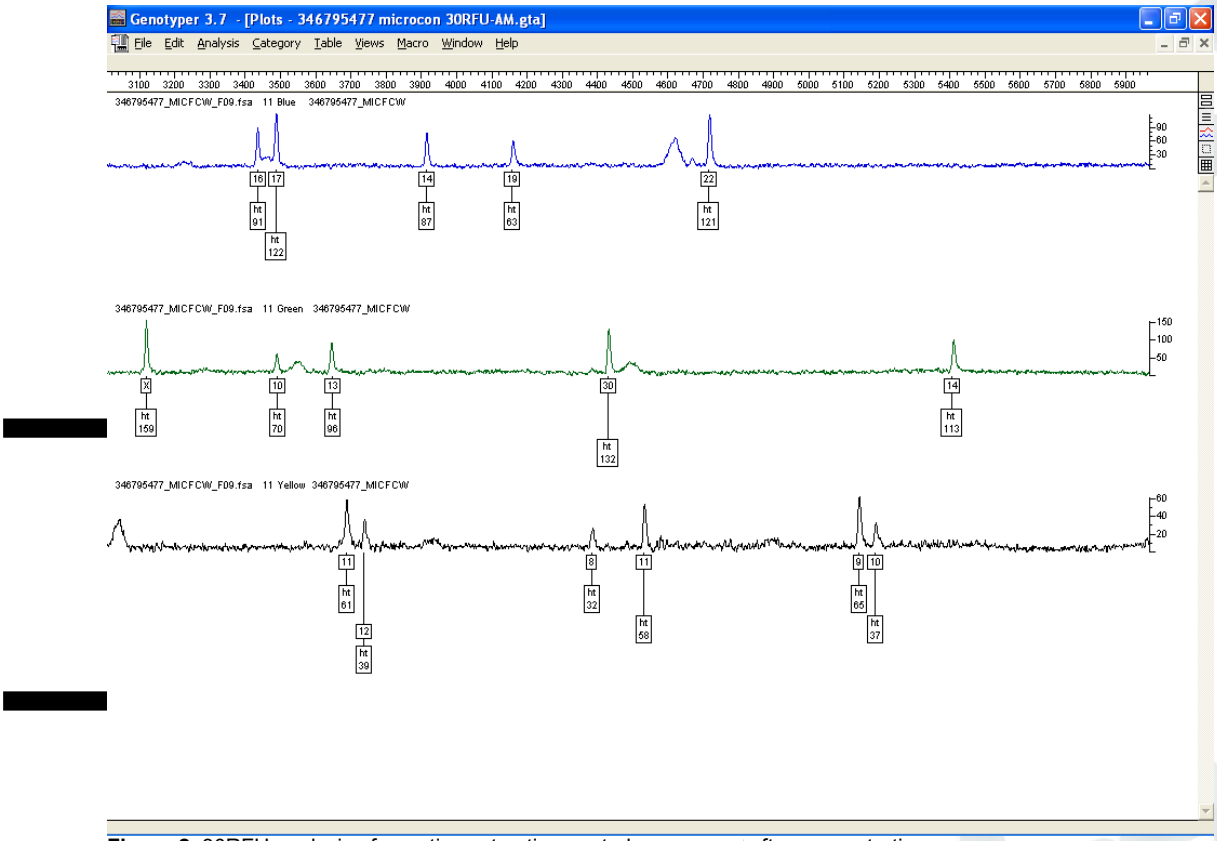

Figure 2. 30RFU analysis of negative extraction control [redacted] after concentration.

[redacted]The [redacted]s a[redacted]marised in Table 1 below.

Table 1. Summary of Results for [redacted] when analysed at 30RFU threshold

Result	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
9PLEX	16,17	14,NR	22,NR	X,X	10,NR	30,NR	14,NR	11,NR	NSD	NSD
AMP1CW	16,17	14,NR	22,NR	X,X	NSD	30,NR	14,NR	12,NR	8,11	NSD
MICFCW	16,17	14,19	22,22	X,X	10,13	30,30	14,14	11,12	8,11	9,10

9PLEX = Casework processing (original result), AMP1CW = re-amplification , MICFCW = Microcon concentration
NR = non reportable alleles, NSD = no peaks detected

A search was conducted against profiles obtained from all samples from the same extraction batch (CWIQEXT20080430_01). A number of matches were found. During the course of investigation, a further match was found to another sample from the same extraction batch that had undergone a cleanup procedure using the Macherey-Nagel NucleoSpin Tissue Kit. These matches are shown in Table 2 below.

Table 2. Summary of extracts matching to 346795477

Sample ID	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
[redacted]	16,17	14,19	22,22	X,X	10,13	30,30	14,14	11,12	8,11	9,10
	16,17	14,19	22,22	X,X	10,13	30,30	14,14	11,12	8,11	9,NR
	16,17	14,19	22,22	X,X	10,13	30,30	14,14	11,12	8,11	NR,NR
	16,NR	NSD	22,NR	X,NR	10,NR	30,NR	14,NR	NSD	NSD	NSD
	16,17	14,19	22,22	X,X	10,13	30,30	14,14	11,12	8,11	9,10
	16,17	14,19	22,22	X,X	10,13	30,30	14,14	11,12	8,11	9,10
	16,17	14,19	22,22	X,X	10[redacted]	30,30	14,14	11,12	8,11	NR,NR
	15,16,17	14,17,19	NR,22,24	X,X	10,13,14,NR	28,30	14,NR,17,NR	11,12,14	8,11,12	NR,10,11
	16,17	14,19	22,22	X,X	10,13	30,30	14,14	11,12	8,11	9,10

NR = non reportable alleles, NSD = no peaks detected, * Profile after NucleoSpin cleanup

A further search against the staff elimination database showed no matching profiles. Each of the matching samples were quantified and amplified on the same batches (QUACW20080501_01 & 9AMPC20080502_01 res[redacted]ples [redacted] all were from the same case and the profile obtained was consistent with profiles from this case. The profile obtained from sample 288908564 after cleanup was not consistent with other profiles from the same case. Sample 333810182 has not been assessed at the time of writing. This information suggests that one or more of samples [redacted], & [redacted] was the source of contamination, or one of these samples had also contaminated other samples from the same case. The layout of the abovementioned samples as extracted on extraction batch CWIQEXT20080430_01 is shown below in Figure 3.

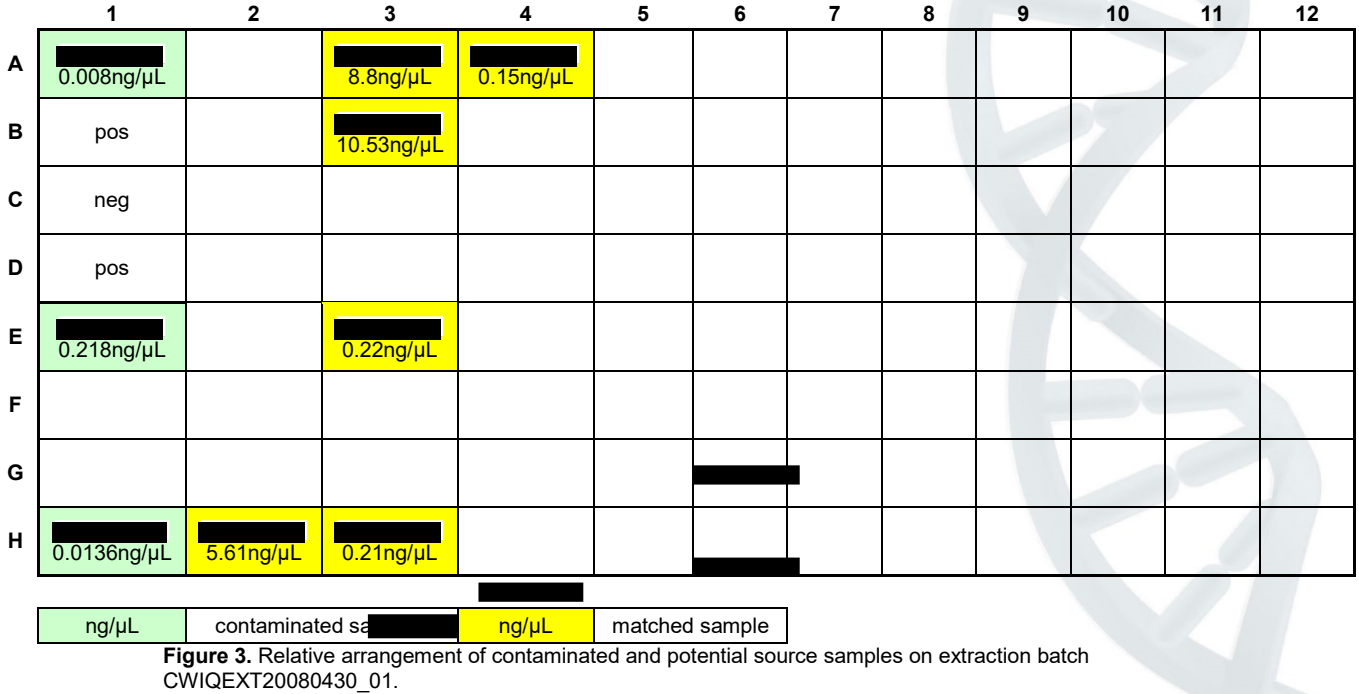

Figure 3. Relative arrangement of contaminated and potential source samples on extraction batch CWIQEXT20080430_01.
* Result after NucleoSpin cleanup

The quantification value for each sample has also been shown in Figure 3. Given the relative quantification values obtained, it is most likely that the samples with the highest concentration of DNA would be the source as relatively small volumes of DNA extract would be required to yield visible amplified DNA.

Conclusion

During the course of the investigation two further examples of potential well-to-well contamination have been identified and taken in conjunction with two previously documented events, these events build a picture of potential systematic problems. This event has been [redacted]mented in the FSS quality system as OQI#19477. The other documented events have [redacted] entered into the quality system as OQI#19330, OQI#19349, OQI#19767, & OQI#19768.

[redacted] process audit (Audit #8227 – DNA IQ) has been commissioned to thoroughly review all [redacted]s of the automated extraction process. This had been planned as a post implementation [redacted]w but has been brought forward in view of [redacted] mentioned above. An extra-ordinary [redacted]ng 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 Extra[redacted] in a [redacted]d pattern [redacted] 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]

[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	[redacted]	blank	sample	blank	sample	blank	sample	blank	sample	blank
C	blank	[redacted]	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	[redacted]	blank	sample	blank	sample	blank	sample	blank	sample	blank
G	blank	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank	sample
H	sample	blank	sample	blank	sample[redacted]	blank	sample	blank	sample	blank	sample	blank

[redacted]**Figu**[redacted]ker[redacted]gement of samples and extraction blanks on extraction batches during investigation period

[redacted]

Findings from the abovementioned audit and investigations will be documented in the quality system against the audit and in a separate investigation report once complete.

[redacted]

[redacted]