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Investigation into a partial DNA profile negative extraction control sample (barcode 346795477)

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Abstract

On the 9th May 2008, it was noted that negative extraction control sample 346795477 which was extracted on CWIQLYS20080430_01/CWIIQEXT20080430_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 Biosystems 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/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® 3130xl 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 automated capper.

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

Investigation

Negative extraction control sample (barcode 346795477) was extracted, quantified, amplified and 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

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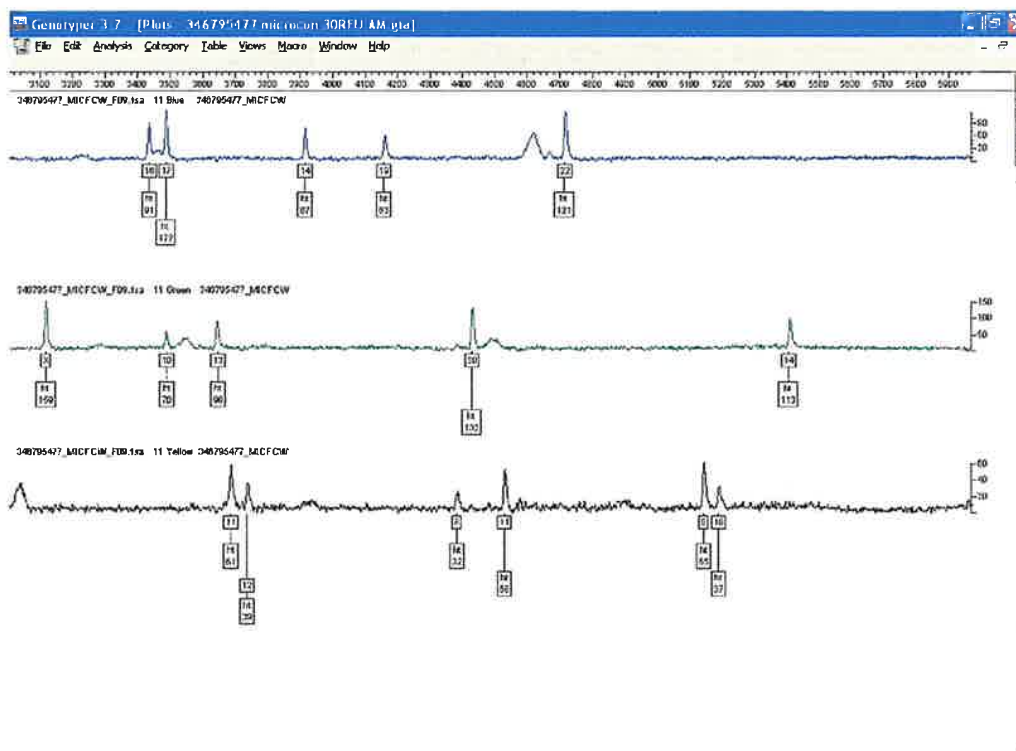


Figure 2. 30RFU analysis of negative extraction control 346795477 after concentration.

These results are summarised in Table 1 below.

Table 1. Summary of Results for 346795477 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
346795477	16,17	14,19	22,22	X,X	10,13	30,30	14,14	11,12	8,11	9,10
320124349	16,17	14,19	22,22	X,X	10,13	30,30	14,14	11,12	8,11	9,NR
320124335	16,17	14,19	22,22	X,X	10,13	30,30	14,14	11,12	8,11	NR,NR
320124326	16,NR	NSD	22,NR	X,NR	10,NR	30,NR	14,NR	NSD	NSD	NSD
320124371	16,17	14,19	22,22	X,X	10,13	30,30	14,14	11,12	8,11	9,10
320124362	16,17	14,19	22,22	X,X	10,13	30,30	14,14	11,12	8,11	9,10
334116189	16,17	14,19	22,22	X,X	10,13	30,30	14,14	11,12	8,11	NR,NR
333810182	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
288908564*	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

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- 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 4 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

	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	sample	blank	sample	blank	sample
H	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank

Figure 4. Checkerboard arrangement of samples and extraction blanks on extraction batches during investigation period

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.

? what about lab clean down & environmental monitoring.?