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Investigation into Negative Extraction Control with a partial DNA profile (barcode 346794568)

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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 346794568 (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 24897) 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 then carried 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® Profiler Plus® PCR Amplification kit, prepared on a dedicated (Pre-PCR) PerkinElmer MultiPROBE® II PLUS HT EX with Gripper™ Integration platform, and amplified on a GeneAmp® 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 stored frozen (-20°C) whilst waiting for processing. For the preparation of PCR reactions, sample tubes were uncapped and recapped using a LifeTool™ RECAP 96M automated capper.

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through this process was conducted. It was noted that negative extraction control sample 346794568 showed the presence of a partial DNA profile in AUSLAB LIMS. The profile obtained from Genotyper batch GEN9CW20080423_04 was re-analysed using a lowered peak detection threshold of 30RFU. The profile observed is shown in Figure 2 below.

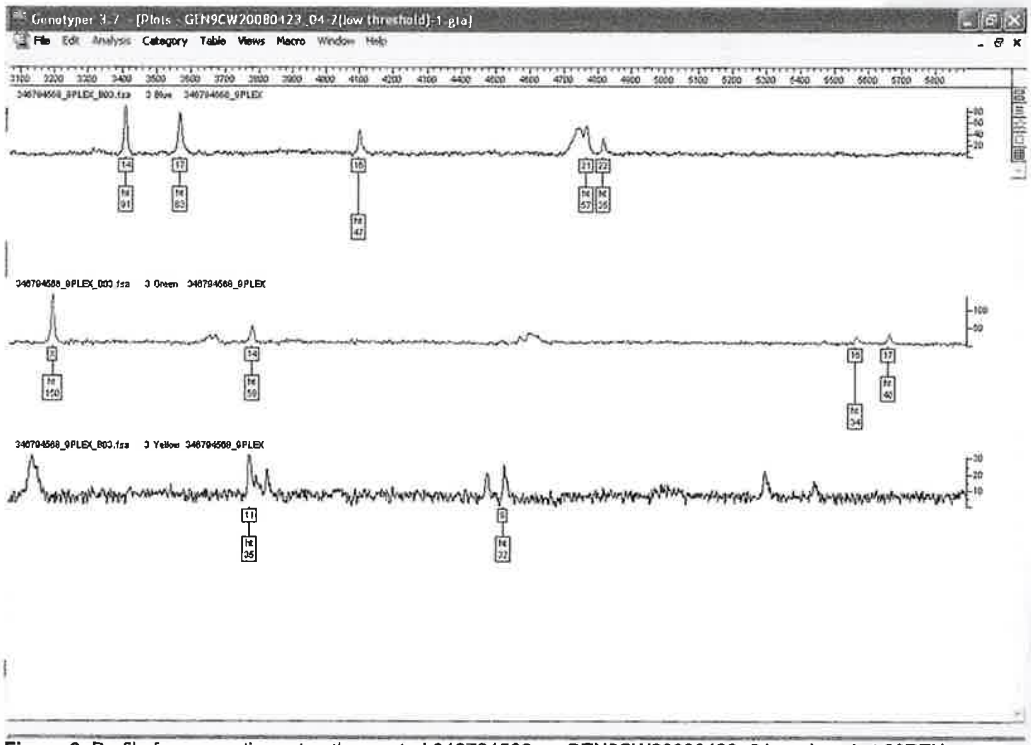

Figure 2. Profile from negative extraction control 346794568 on GEN9CW20080423_04 analysed at 30RFU.

The DNA extract from 346794568 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 Figure 3 below.

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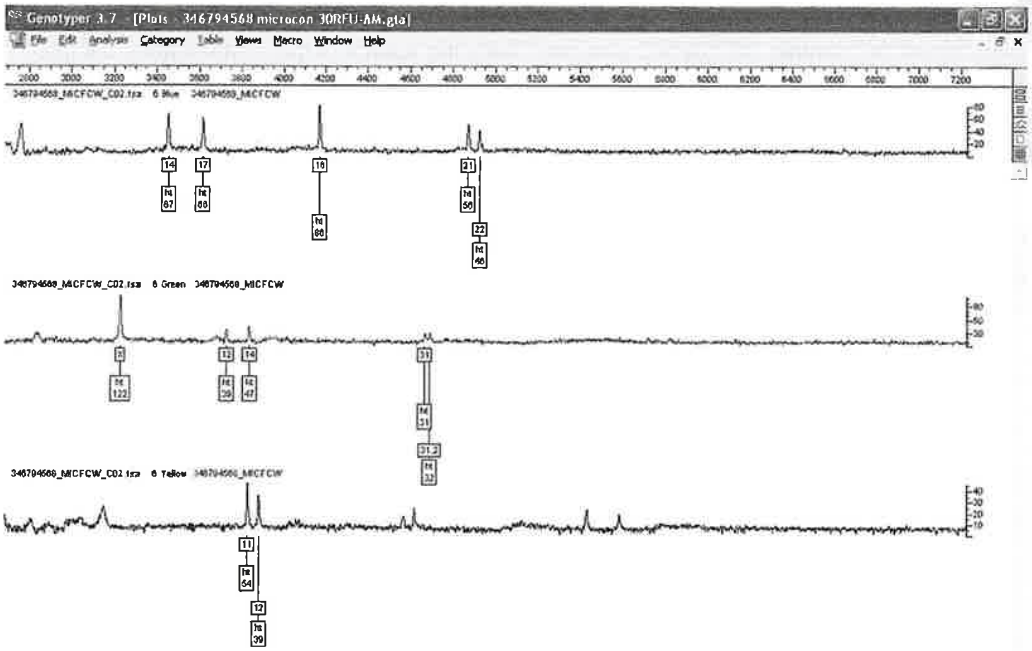

Figure 4. Profile from negative extraction control 346794568 after Microcon concentration and analysis at 30RFU

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)

The combined profile was then searched against all other profiles obtained from the same extraction batch (CWIQEXT20080417_01). A match was found to sample 346802502, an environmental monitoring sample. When further analysis of the whole extraction batch was conducted at reduced peak detection thresholds, an additional four samples (346802405, 346802352, 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
346794568	0.000934*	14,17	16,16	21,22	X,X	12,14	31,31.2	15,17	11,12	9,NR	NSD
346802405**	0.002810	14,NR	16,NR	21,NR	X,NR	12,14	NSD	NSD	11,12	8,NR	NSD
346802352	0.000628	NSD	NSD	NSD	X,X	12,14	NSD	NSD	11,12	NSD	NSD
346802482	0.006560	14,17	16,NR	21,22	X,X	12,14	31,NR	17,NR	11,12	NSD	NSD
346802446	0.002600	14,17	16,NR	NSD	X,X	14,NR	NSD	NSD	NSD	NSD	NSD
346802502	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), Quant = DNA concentration in the extract as ng/µL, * = Quantification value before concentration, ** = quantification and profile after clean-up procedure (inhibition detected from original quantification)

highly unlikely for sample 346802502 to have contaminated multiple samples in a reverse direction.

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 #8227). Particular attention will additionally be placed on the initial acceptance of the Negative control (i.e. the control passing through the quality process). The need for due care when reviewing control results will be highlighted.