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Investigation into Positive Control with extra peaks (barcode [redacted])

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Abstract

On the 17 April 2008, during the Genotyper analysis of batch GEN9CW20080411_01, it was noted that extra peaks were found in a positive extraction control sample [redacted] (Figure 1). An investigation ensued and the extra peaks were determined to be a combination of stutter peaks and a raised level of background florescence, possibly due to poor spectral calibration, array over use-age or low quality polymer.

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 SOP 24897.

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® 7000 or 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. These 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, sample 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.

Investigation

Initially, positive extraction control sample [redacted] was extracted on a PerkinElmer MultiPROBE® II PLUS HT EX with Gripper™ Integration platform using Promega DNA IQ™ kit (batch CWIQEXT20080402_01). The DNA extract was then quantified using Applied Biosystems Quantifiler® Human DNA Quantification Kit on an Applied Biosystems Prism 7500 Sequence Detection System real-time PCR instrument. The DNA extract [redacted] was quantified and produced a quant value of 1.11ng/µL. 1µL of the DNA extract was then amplified using Applied Biosystems AMPF*l*STR® Profiler Plus® amplification kit on an ABI GeneAmp 9700. The PCR product was then prepared and run through an Applied Biosystems 3130*xl* Prism Genetic Analyser (3130*xl)* as batch 3100CW20080409_01, and analysed using Genescan (version 3.7.2). This batch progressed to genotyper batch GEN9CW20080411_01 and analysed with Genotyper (version 3.7.1) software. A mixture was noted during analysis of positive extraction control sample [redacted] (Figure 1).

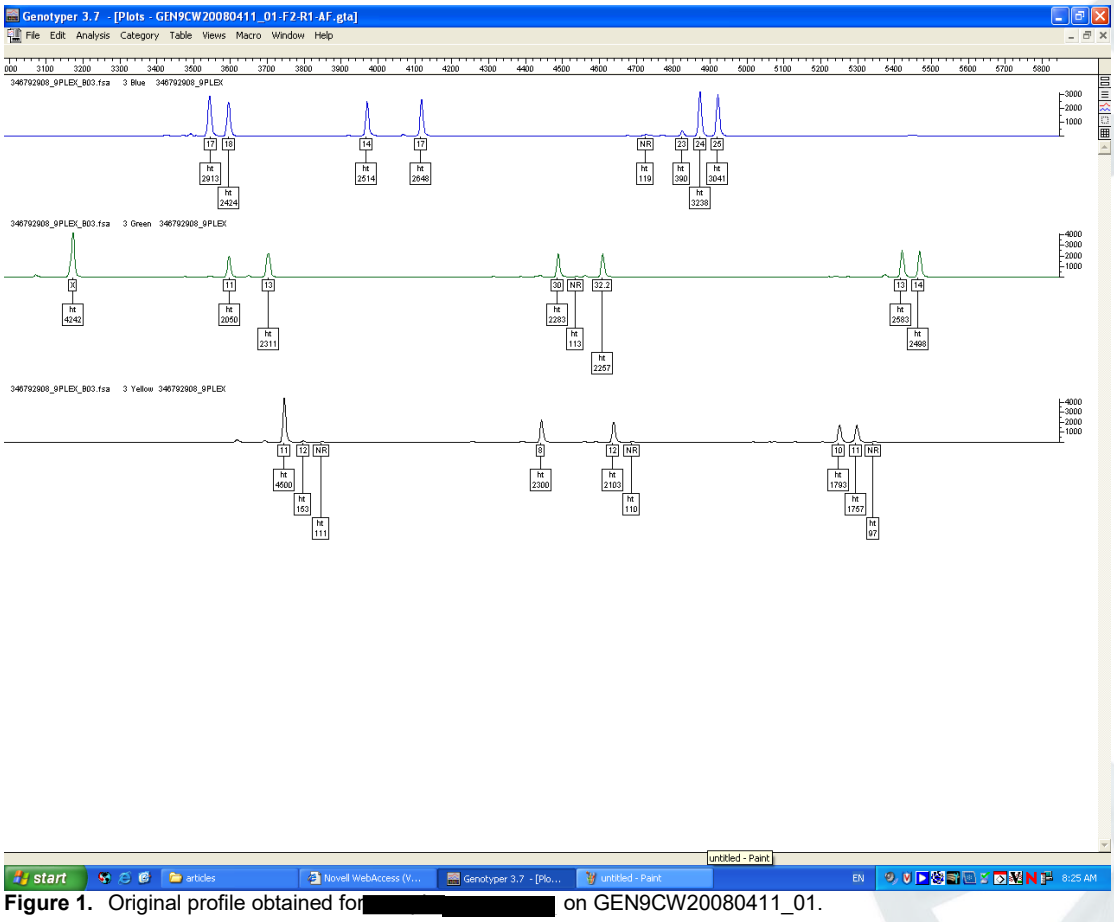

Figure 1. Original profile obtained for [redacted] on GEN9CW20080411_01.

The sam[redacted] prepared and run on batch CERRCW20080417_01 and analysed at lower thresholds (30 RFU's). The presence of extra peaks consistent with initial results were observed (Figure 2).

[redacted]

[redacted]

[redacted]

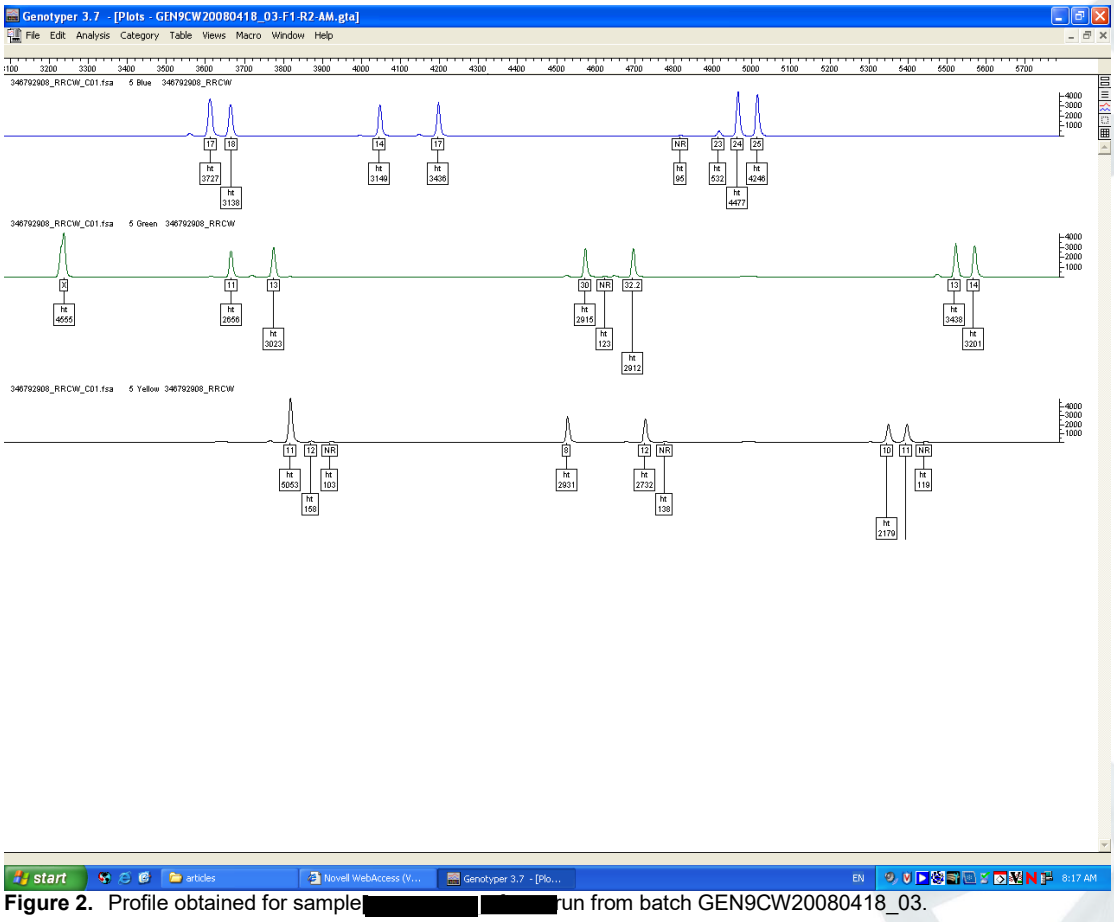

Figure 2. Profile obtained for sample [redacted] [redacted] run from batch GEN9CW20080418_03.

Sample [redacted] was re-amplified on batch 9AMPC20080514_03 (progressing to batch GEN9CW20080516_01) and analysed demonstrating a single extra peak at the D8 locus in the stutter position of the longer allele (Figure 3).

[redacted]

[redacted]

[redacted]

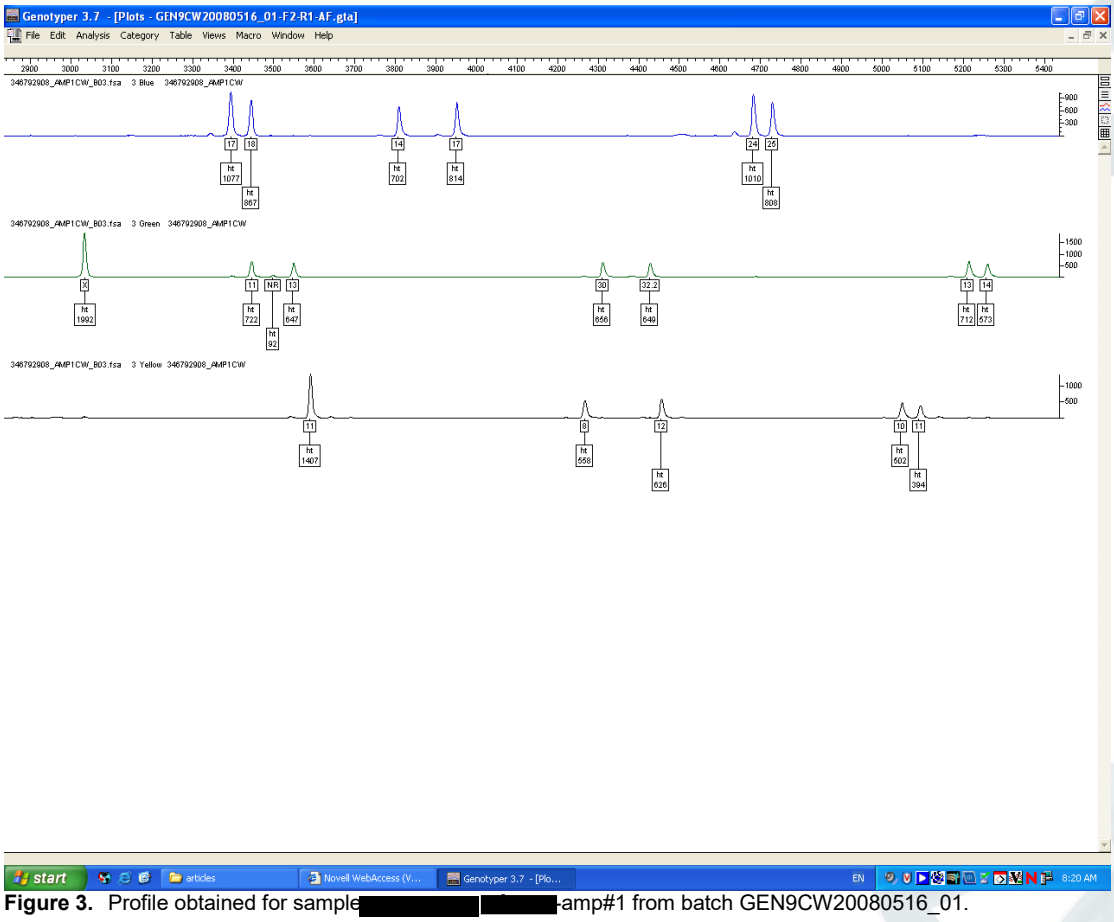

Figure 3. Profile obtained for sample [redacted] [redacted]-amp#1 from batch GEN9CW20080516_01.

The sam[redacted]ther amplified and analysed on batch GEN9CW20080625_02 (Figure 4), no evidence of a mixture was seen.

[redacted]

[redacted]

[redacted]

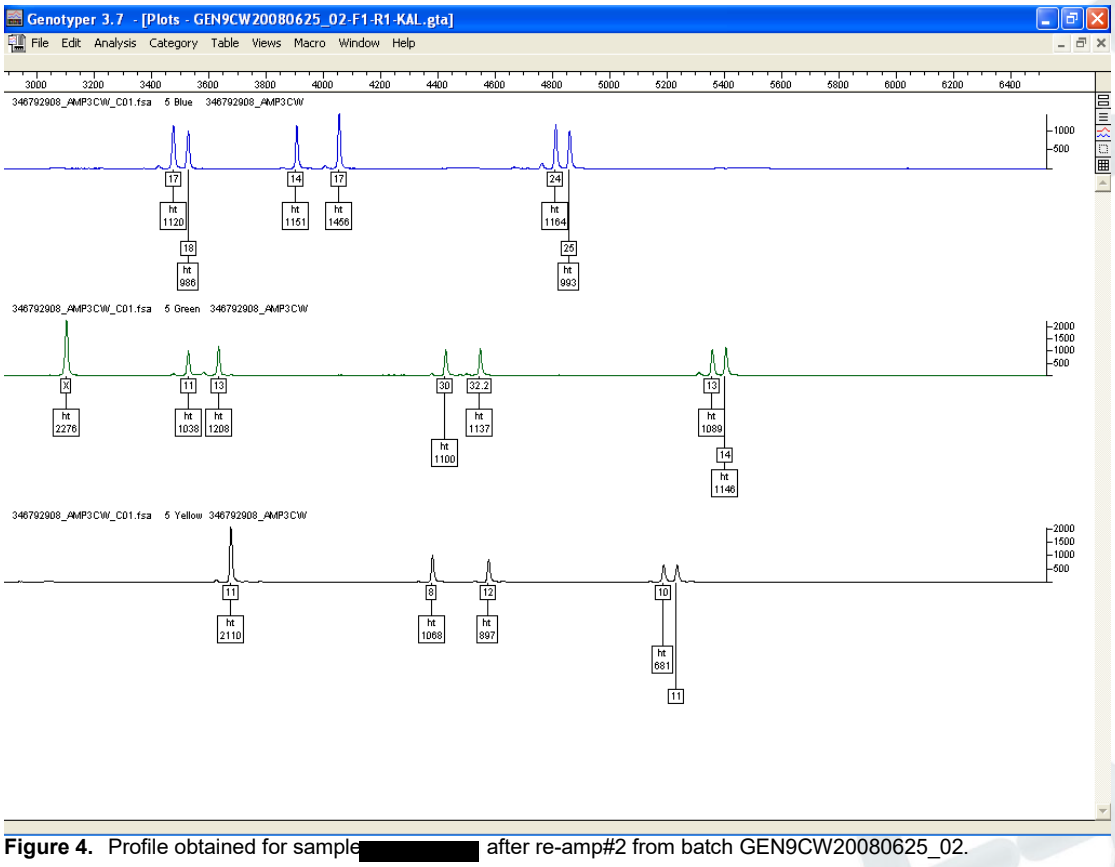

Figure 4. Profile obtained for sample [redacted] after re-amp#2 from batch GEN9CW20080625_02.

Conclusion

During the time span of the initial analysis of sample [redacted] the laboratory was experiencing some analysis difficulties due to poor quality of polymer from the supplier. This difficulty was experienced at other Forensic sites interstate. This resulted in poor spectral differentiation and the presence of some shadow peaks approximately 8-12 base-pairs smaller than the true peak. Re-amplification of the sample failed to show reproducible evidence of a mixture. AUSLAB specimen notes were made for sample [redacted] during the investigation. Due to the investigation findings, no OQI was required.

[redacted]