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Investigation into Mixture found in FTA Evidence Sample (barcode 184858899)

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Abstract

On the 24th of May 2008, during the Genescan analysis of capillary electrophoresis batch CEPRF20080521_01 (to become batch GEN9REF20080526_01), a mixture was noted in FTA sample barcode 184858899. Initially FTA sample 184858899 was processed through routine FTA processing procedures (as outlined above) on batch FTA20080207_01 (to become GEN9REF20080225_03), and yielded no DNA profile. An investigation under OQI # 19767 commenced to determine the cause for this mixture.

Background

Within DNA Analysis processing of person (Reference) samples is performed using FTA paper. Buccal cells or blood are transferred onto FTA™ paper and provided to the laboratory, or whole blood is provided and this transferred to the FTA™ paper within the laboratory.

These samples are processed through standard laboratory procedures. Briefly, punches of FTA paper were transferred to a 96-well half-skirt PCR plates using the BSD Duet 600 (BSD Robotics, Australia) semi automated dried sample punch instrument. The dried punches were washed with TE buffer (blood punches were washed in weak NaOH solution followed by TE buffer), and dried on a hot block. The samples are then processed through to STR PCR amplification. This was performed by the addition of TE and PCR mastermix (Applied Biosystems AMPFℓSTR® Profiler Plus® PCR Amplification kit), the plate was sealed and amplified on a GeneAmp® PCR System 9700 thermalcycler. After amplification stage, fragment analysis was performed on a portion of the PCR product by capillary electrophoresis on the Applied Biosystems Prism® 3130*x*l Genetic Analyser, and the data analysed using a combination of Genescan (version 3.7.2) with Genotyper (version 3.7.1) software.

If an unacceptable profile was obtained further processing would be required, depending on the sample and result, the sample may have been re-processed through the procedure outlined above with the same, more or less punches of the FTA paper. If these results were still unacceptable further processing was performed. This involved further punches (with a larger manual hole punch) being placed into individually labelled DNA free 1.5mL tubes. The punches were then transferred to a Slicprep™ 96 device (Promega) via the use of the *automate.it* STORstar system (Process Analysis & Automation Ltd. Hampshire. UK). There were then processed through automated DNA extraction on a PerkinElmer MultiPROBE® II PLUS HT EX with Gripper™ Integration platform with Promega DNA IQ™ DNA extraction kit. Each extraction batch includes a positive and negative extraction control and a negative punching control for quality purposes. All samples on the one batch are processed under the same conditions as each other according standard laboratory procedures (QIS document 24897).

After extraction, between and after the following processes, the DNA extracts were stored frozen at -20°C. Following extraction, the DNA extracts were then quantified using Applied Biosystems Quantifiler® Human DNA Quantification Kit on an Applied Biosystems Prism 7000 Sequence Detection System real-time PCR instrument. The DNA extract was then amplified using Applied Biosystems AMPFℓSTR® Profiler Plus® amplification kit on a Perkin Elmer GeneAmp 9700. The PCR product was then prepared for capillary electrophoresis and run through an Applied Biosystems Prism 3130*x*l Genetic Analyser and analysed using Genescan (version 3.7.2) and Genotyper (version 3.7.1) software.

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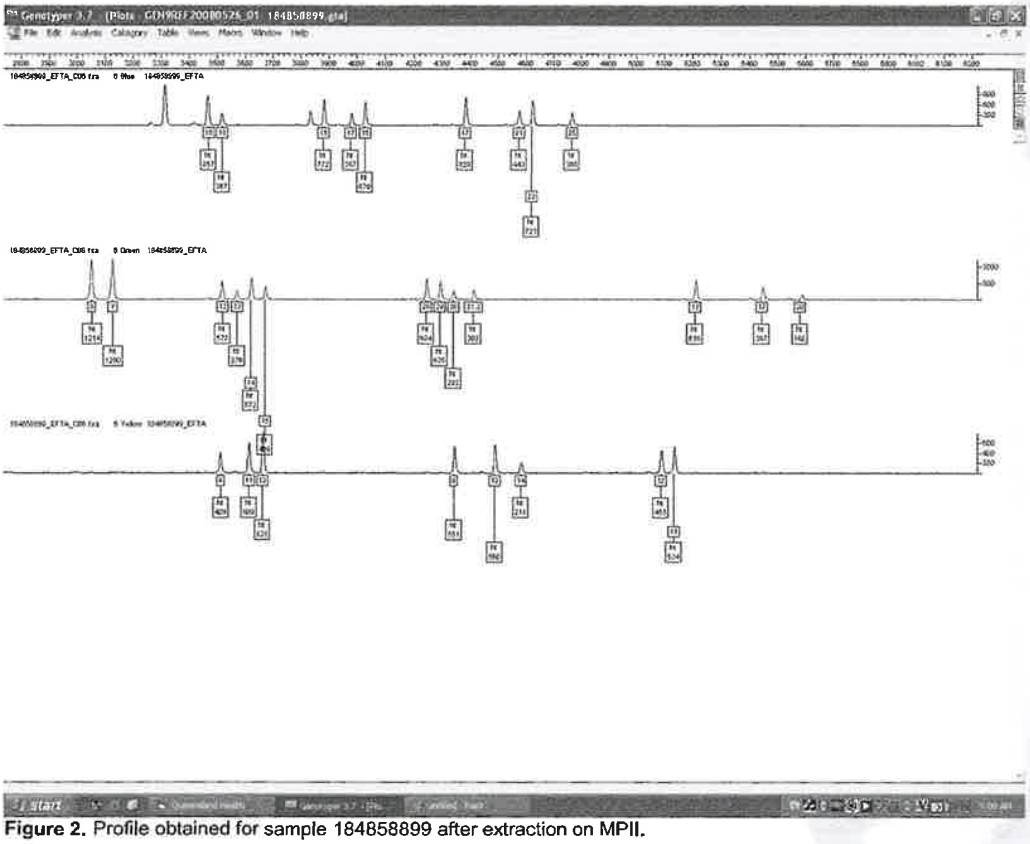

Figure 2. Profile obtained for sample 184858899 after extraction on MPII.

The sample 184858899 was reprepared and re-run on the 3130*xl* Genetic Analyser and re-analysed through Genescan software. The mixed DNA profile was shown to be reproducible.

The DNA extract was re-amplified on amplification batch 9AMPR200820080527_01 (to become batch GEN9REF20080602_02). The mixed DNA profile was shown to be reproducible as shown in Figure 3 below.

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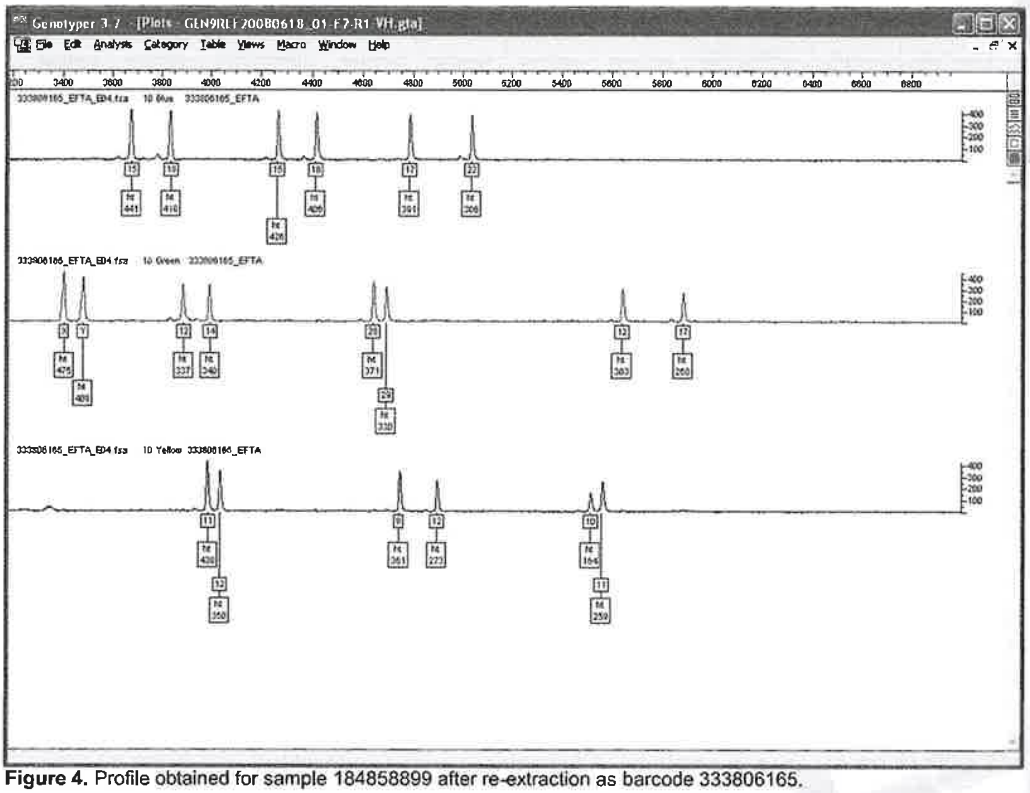

Figure 4. Profile obtained for sample 184858899 after re-extraction as barcode 333806165.

This profile was consistent with the original partial profile shown in Figure 1 above.

The mixed DNA profile from barcode 184858899 was then separated into the known DNA profile and the contaminating profile contributing to the mixture. This was then searched against all profiles obtained from samples on extraction batch RFIQEXT20080515_01. A match was found with sample 308802586. This is shown in Table 1 below.

Table 1. Summary or results for FTA sample 184858899.

Sample	Processing	Amel	D3	D8	D5	vWA	D21	D13	FGA	D7	D18
184858899	FTA	X, Y	15, 18	12, 14	11, NR	15, 18	28, 29	9, NR	17, 22	NSD	NSD
184858899	DNA IQ extraction	X, Y	15, 18, 19	12, 13, 14, 15	9, 11, 12	14, 15, 17, 18	28, 29, 30, 31.2	9, 12, 14	17, 21, 22, 25	10, 11	12, 17, 20
333806165*	DNA IQ extraction	X,Y	15,18	12,14	11,12	15,18	28,29	9,12	17,22	10,11	12,17
Mixture Contributor		X, Y	15,19	13, 15	9, 12	14, 17	30, 31.2	12, 14	21, 25	10, 11	12, 17, 20
308802586	DNA IQ extraction	X,Y	15,19	13,15	9,12	14,17	30,31.2	12,14	21,25	10,11	12,20

NR = no reportable allele, NSD = No Sizing Data (no alleles detected), *333806165 was a re-extraction of 184858899

A representation of the plate layout for extraction batch RFIQEXT20080515_01 is shown in Figure 5 below, with the relative positions of samples 184858899 and 308802586 indicated in yellow and green respectively.

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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. This adverse event has been documented as OQI#19767 in the quality system.