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Investigation into [redacted]e Control with peaks (barcode [redacted]

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Abstract

On the 23 May, 2008, during the importing of results for Genotyper batch GEN9CW2008[redacted] was noted by the scientist that sample [redacted] (negative control from extraction batch CWIQLYS20080502_02 and CWIQEXT20080506_01), was found to have a partial profile result. An investigation under OQI # 19768 comm[redacted] determine the cause for the contamination of the negative contro[redacted]

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) Breifly, sample extracted through the Promega DNA IQ™ 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 further extracted on a PerkinElmer MultiPROBE® II PLUS HT EX with Gripper™ Integration platform using the Pro[redacted]A IQ™ kit. After extraction DNA extracts were stored frozen (-20[redacted]nc™ 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) PerkinE[redacted] HT EX with Gripper™ Integration platform. The real-time PCR was then carried out o[redacted]ied Biosystems Prism® 7500 Sequence Detection System. Once the DNA quantification value had been obtained an appropriate [redacted] DNA template to be added to the STR amplification reaction was determined by [redacted]cal calculation as programmed in the AUSLAB laboratory information management [redacted]e 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, samples storage was tracked using AUSLAB storage functionality, each sample was stored frozen (-20°C) whilst waiting for processing. For the preparation of PCR reactions, [redacted]bes were uncapped and recapp[redacted]™ RECAP 96M automated

Investigation

Initially, negative extraction control sample [redacted] was extracted as outlined above on batches CWIQLYS20080502_02 and CWIQEXT20080506_01. The DNA extract quantified displaying a quant value of 0.00544 ng/µL. This value was above the limit of detection (0.00426n[redacted]reporting (0.0128ng/µL) previously determined by in-

house validation. The DNA extract was [redacted]fied at maximum volume (20µL) and the PCR product analysed on capillary electrophoresis batch CEPCW20080509_01, this was further analysed as Genotyper batch GEN9C[redacted]using methods described above. A single peak at the Amelogenin locus was observed above the peak detection threshold (75RFU) but below the reporting threshold (150RFU) for casework samples. This is shown in Figure 1 below.

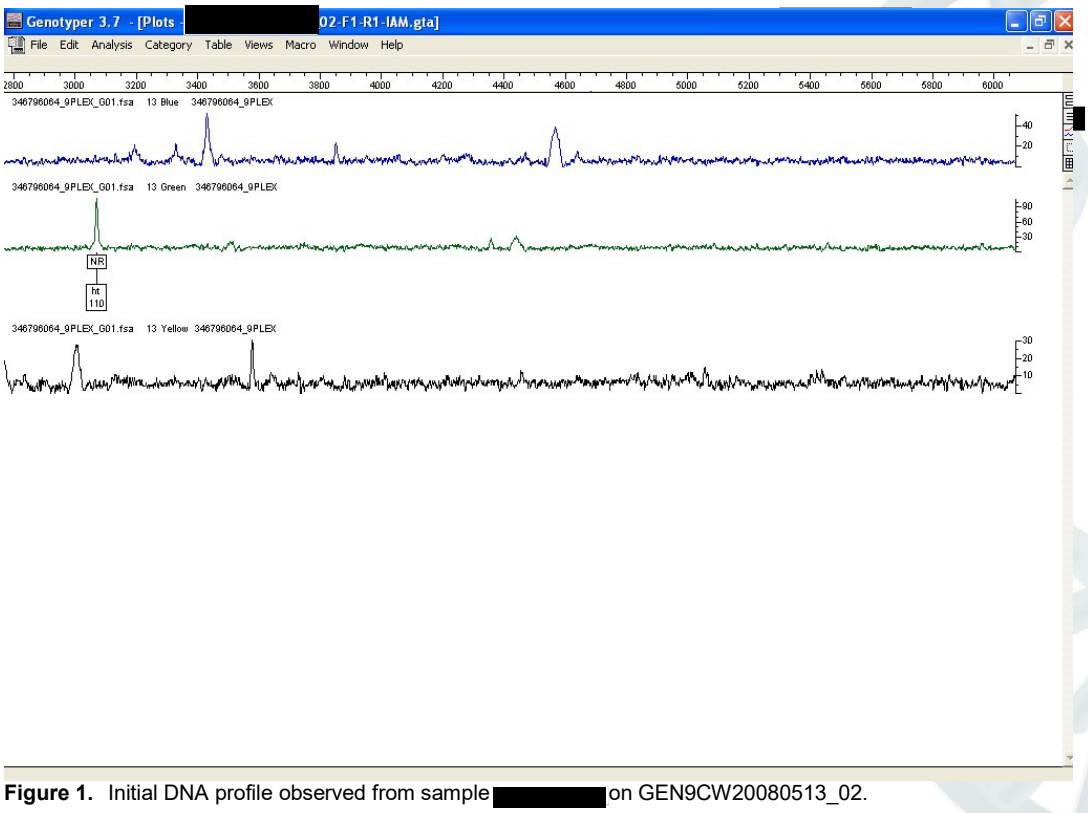

Figure 1. Initial DNA profile observed from sample [redacted] on GEN9CW20080513_02.

The DNA extract was re-amplified at 20µL with no peaks visible above peak detection [redacted] however potential peaks were visible below threshold. The DNA extract was then [redacted]ed using centrifugal filtration through a Microcon YM-100 filter membrane (Millipore) according to standard laboratory procedures. The DNA extract was reduced from approximately 50-60µL in volume to approximately 5µL in volume. This extract was then amplified and no DNA profile was observed. This was thought to be erroneous and the NUNC tube containing the DNA extract was visually reviewed. 3µL of DNA extract was shown to be remaining. This was most likely due to a failure of the MPII pipetting small volumes (see discussion below). The DNA extract was re-amplified (this time the DNA extract was added manually after master mix was added to the reaction well by the pre-PCR MPII) and a partial DNA profile was observed. The partial DNA profiles obtained from the original amplification, the re-amplification and the repeat amplification of the concentrated extract were the re-analysed using a lowered peak detection threshold of 30RFU. These profiles are shown in [redacted]3, and 4 below.

[redacted]

[redacted]

[redacted]

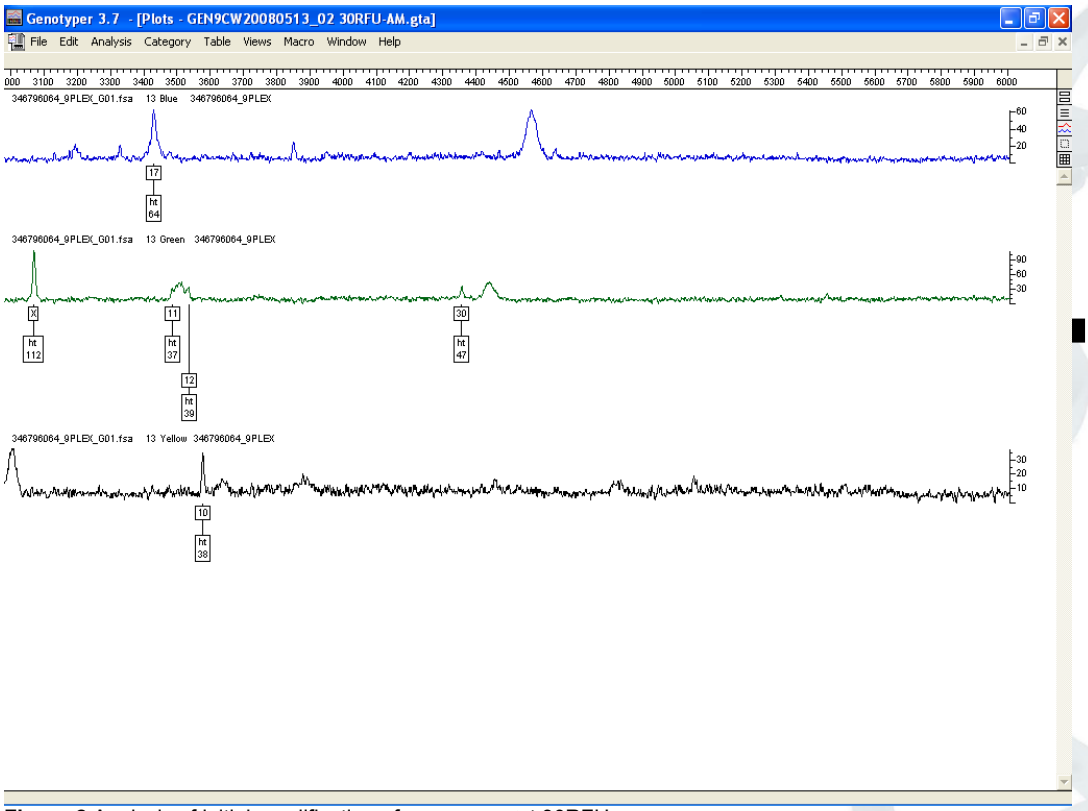

Figure 2 Analysis of initial amplification of [redacted] at 30RFU

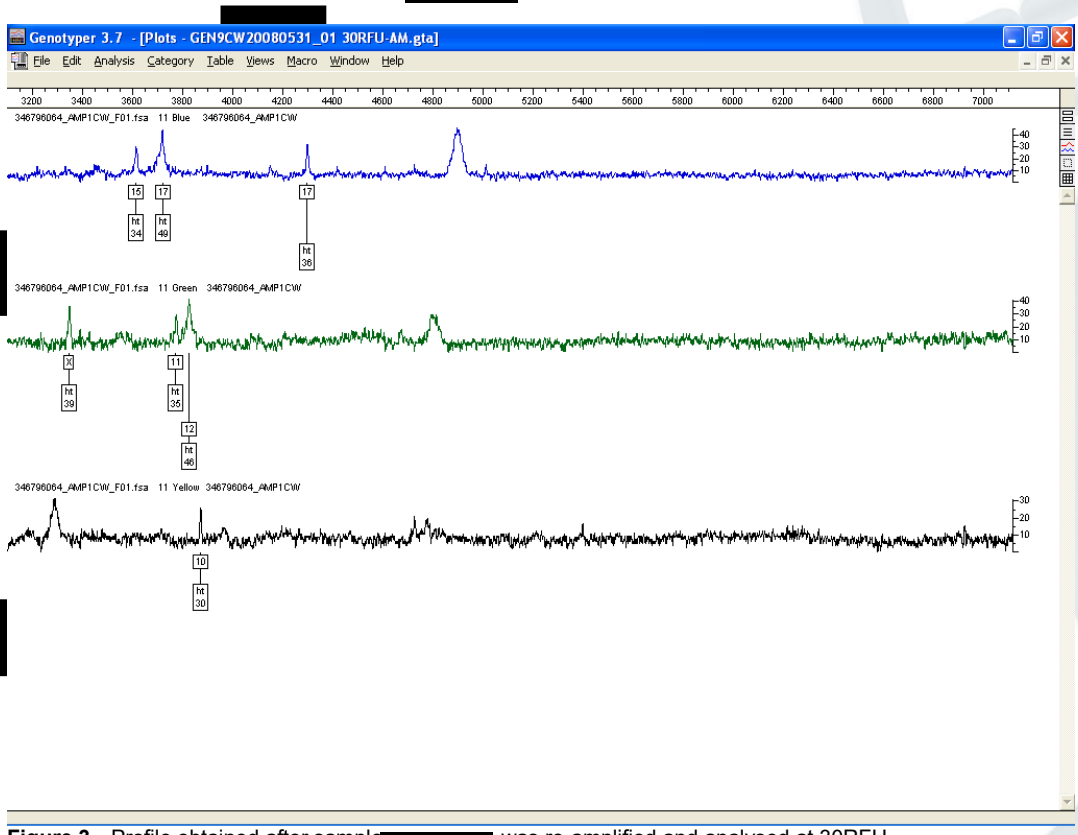

Figure 3. Profile obtained after sample [redacted] was re-amplified and analysed at 30RFU

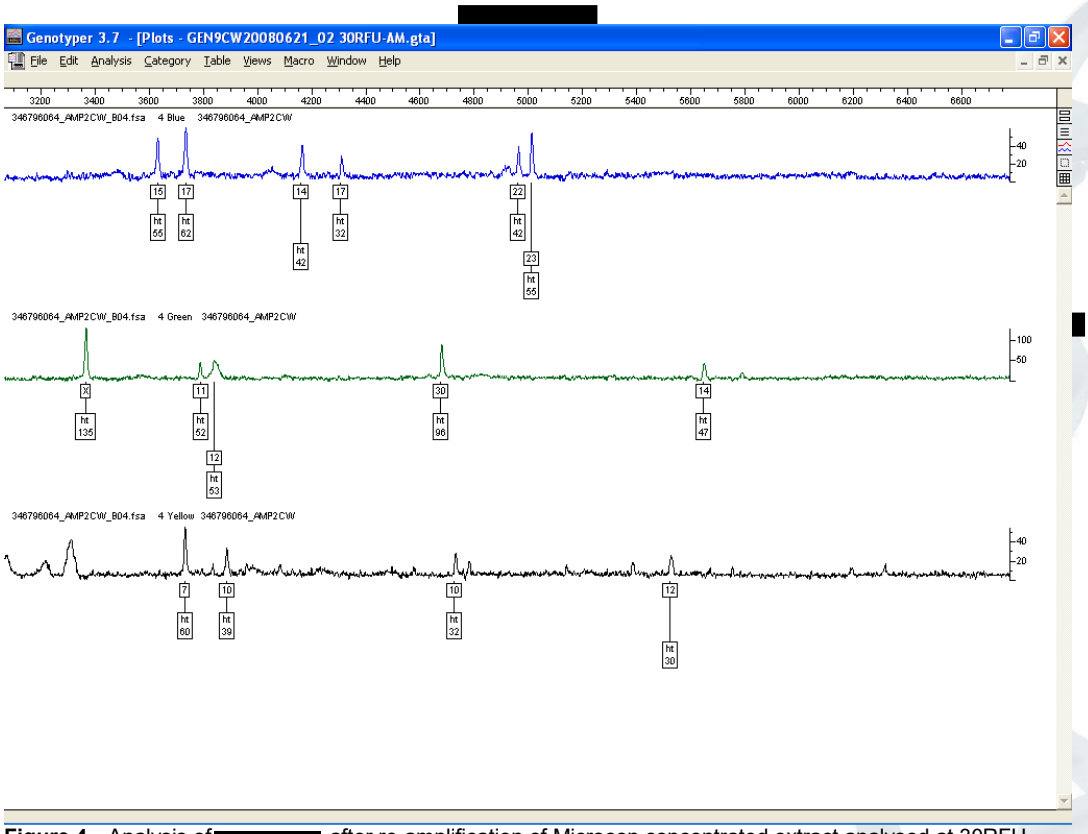

Figure 4. Analysis of [redacted] after re-amplification of Microcon concentrated extract analysed at 30RFU

The profile shown in Figure 4 above was then searched against all profiles obtained from samples on the same extraction batch (CWIQEXT20080506_01). Matches were made to two different samples, [redacted]. These results are shown in Table 1 below

Table 1. Profiles matching to profile obtained from extraction negative control 346796064

Sample ID	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
[redacted]	15,17	14,17	22,23	X,X	11,12	30,30	14	7,10	10	12
	15,17	14,17	22,23	X,X	11,12	30,30	14,17	7,10	10,11	9,12
	15,17	14,17	22,23	X,X	11,12	30,30	14,17	7,10	10,11	9,12

* Profile obtained after analysis at 30RFU

Further investigation was then carried out to determine at what processing step the contamination was likely to have occurred. The AUSLAB audit trails for each of the samples listed in Table 1 were reviewed. The quantification and amplification batches that each of these samples were processed on after extraction are shown in Table 2 below.

Table 2. Processing batches

Sample ID	Initial quantification		Initial amplification	
	Batch	Position	Batch	Position
[redacted]	QUACW20080508_01	25	[redacted]	7
	QUACW20080508_02	86		72
	QUACW20080508_02	87		74

Conclusion

The results of the investigation show that th[redacted]ation of negative extraction control 346796064 must have occurred prior to quantification and amplification of the DNA extract. All three samples above (Table 2) were processed together during the extraction process. Samples [redacted] progressed through to a different quantification and

amplification batches to that of sample [redacted], therefore, confirming that the contamination of this negative control sample most probably occurred during the extraction batch process. As the positive extraction cont[redacted] on the batch) and no other samples (excepting those shown in Table 1 above) show no evidence of the contaminating profile, contamination of the reagents and plastic-ware is unlikely.

The most likely cause of contamination was during the MPII processing of the extraction batch, however [redacted]k lysis component cannot be excluded. [redacted]k lysis component is least likely as this is a manual process, during which only one tube is opened at a time and samples are processed sequentially, as negative extraction control [redacted] was the first sample on the lysis batch (position 1) and samples [redacted] and [redacted] were in positions 31 & 32 respectively, a large number of samples were processed in between.

This has been documented in the FSS quality system as OQI #19768. This contamination event has occurred whilst other contamination events have been investigated. These events (namely OQI's #19330, 19349, 19477, & 19767), when viewed in conjunction with this event, have constructed a picture of what is likely a systematic quality failure of automated extraction process.

A full process audit (Audit #8227 – DNA IQ) has been commissioned to thoroughly review all facets of the automated extraction process. This had been planned as a post implementation review but has been brought forward in view of events mentioned above. An extra-ordinary meeting of the DNA Analysis management team 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 like[redacted] to platform A)
- Processing of [redacted]ework samples on Extraction platform B in a checkerboard pattern with extraction reagent blanks (layout shown in Figure 6 below).
- Urgent progression of audit mentioned above and investigation into findings
- A full information review of results from automated extractions with documented quality ev[redacted] 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	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 5. Checkerboard arrangement of samples and extraction blanks on extraction batches during investigation period

[redacted]ngs from Audit #8227 have hig[redacted]ting steps within the automated extraction process as being of particular concern. A second extra-ordinary meeting of the DNA Analysis management team was held on 28/07/2008 and a decision was made to cease processing of samples through the automat[redacted]n process until problems identified could be rectified to the satisfaction of the management team.

[redacted]

This event has been discussed in an A[redacted]m meeting and will constitute part of the ongoing investigations and discussions around the automated extraction procedure.

AUSLAB audit entries for extraction batch CWIQEXT20080506_01, and AUSLAB specimen notes, and notation in the comments section of the 9PLEX page to refer to specimen notes were made for all samples on this extraction batch. Additionally, OQI #19768 was also entered into the UR notes of all samples.

Pipetting of low volumes on the pre-PCR MPII

Prior to aspiration of DNA extract by the MPII, the NUNC tubes a[redacted]ged to [redacted] the DNA extract to the bottom of the tube. What appears to happen is that the entire sample may form a discrete drop on the side or corner of the NUNC tube. As there is variation between probes, tubes, tube racks and disposable tips, the pipette tip may not always be centred on the individual tube. This may cause the disposable tip to miss the sample and not actually aspirate the sample (or not aspirate the sample fully). See Figure 6 below.

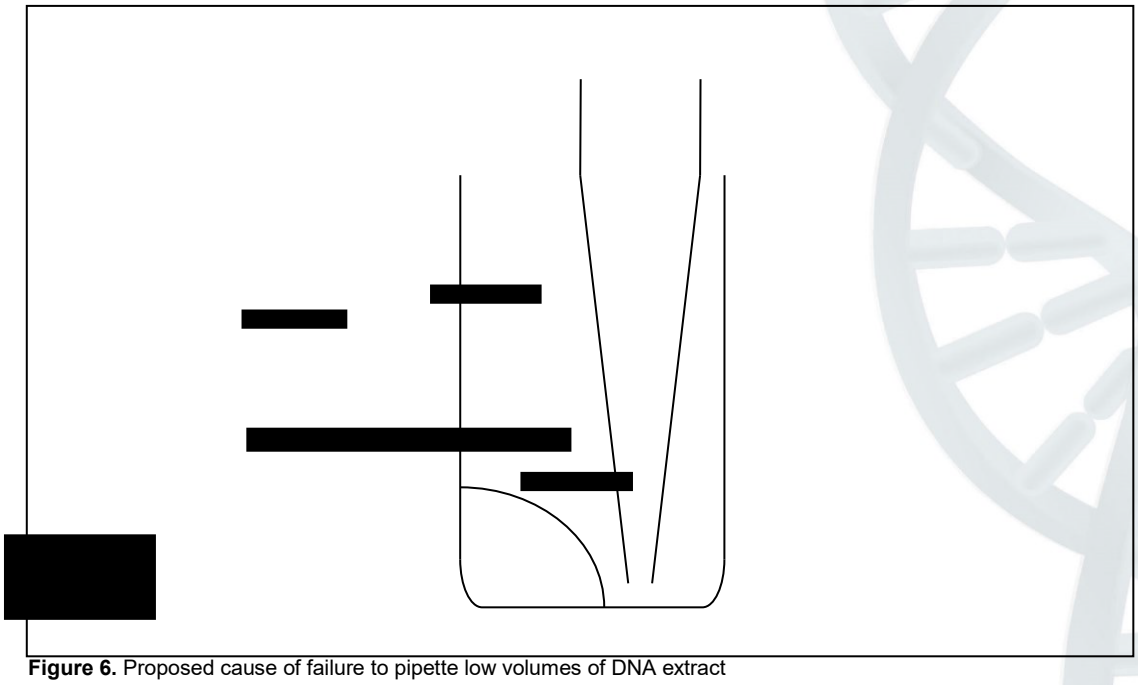

Figure 6. Proposed cause of failure to pipette low volumes of DNA extract

This would not necessarily be obvious as the MPII does not accurately liquid level sense at low volumes, and therefore by default is programmed to “go to bottom” i.e. aspirate at the pre-programmed bottom of the tube.

This would not necessarily affect all samples where there is very little sample remaining in the tube, as the sample may actually be aspirated. This does not affect samples with sufficient [redacted] cover the base of the NUNC tu[redacted]

[redacted] ing solution was proposed and [redacted] last step of the Microcon procedure, if the remaining DNA extract is <20µL, then an appropriate volume of TE buffer (the same that is used as a diluent in the amplification procedure) can be added to the NUNC tube so that the MPII has 20µL of DNA extr[redacted]ate. This therefore negates the above-mentioned problem. This problem has been raised in the quality system as OQI#20113.

[redacted]