Database Samples Warranting a Closer Look and Examination of the D8S1179 Locus

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ABSTRACT

In a field where precision is of the utmost importance, the generation of DNA profiles is essential. Two samples that originally produced reportable profiles required additional testing to further examine the D8S1179 locus after being questioned by the analyst. Attendees will see that in addition to standard practices employed at the Marshall University Forensic Science Center (MUFS), steps were needed to determine the true genotypes for each of these samples in question.

This presentation will impact the forensic science community by enlightening attendees that analysts should be very aware that profiles produced may require additional examination due to inconsistencies or questionable calls. It is imperative to ensure that the true DNA profiles for each sample will be uploaded into local, state, and federal databases.

INTRODUCTION

Hundreds of thousands of database samples are processed through state systems on an annual basis. The forensic science community should be very aware that profiles produced may require additional examination due to discrepancies. It is imperative to ensure that the true DNA profiles for each sample will be uploaded into local, state, and federal databases.

On a second amplification using PowerPlex® 16 HS of Sample A: The above electropherogram represents the amplification using PowerPlex® 16 HS employed at the MUFS. Initially, these samples could have passed according to protocol. Upon closer examination by the analyst, these two database samples required more lab work due to discrepancies at the D8S1179 locus. Sample A and B (generic sample names are used in place of identifying barcodes used in processing) needed extensive processing to determine the true genotypes.

RESULTS

The National Institute of Standards and Technology (NIST) was able to sequence Sample A with a confirmed result of an 8,13 at the D8S1179 locus. It was determined that PowerPlex® 16 HS was migrating to a 12.3 because of an A→G single nucleotide polymorphism 48 base pairs downstream of the repeat. The true genotype is an 8,11 and not an 8,12,3.

Sample B was determined to have a 12,14 genotype. The sample had to be extracted and amplified using PowerPlex® 16 HS, PowerPlex® 16, and AB® AmpFISTR® Identifiler® Plus to obtain the true genotype.

DISCUSSION

Sample A and Sample B were extracted and amplified using PowerPlex® 16 HS. PowerPlex® 16, and AmpFISTR® Identifiler® Plus. Sample A was initially direct amplified using the PowerPlex® 16 HS kit resulting in an OL peak at the D8S1179 locus with a height of 704 RFU, a size of 225.08, and a calculated call of a 12.3. When comparing the sample with the ladder the calculations of the adjacent alleles on the ladder were 0.71 and 3.31 which resulted in an allele call of a 12.3. This allele has been seen at the MUFS six times and on STRBase three times leading an analyst to believe this could be a valid allele. The resultant call and calculations seemed to vary from the normal OL/microvariant calculation. Typically, when calculating the OL/microvariants, they are closer to a “T” nucleotide difference. The locus would appear to result with allele calls of 8,12,3 at D8S1179.

Similar results were obtained from a cutting that was extracted on the EZ2® BioRobot resulting in the 8 and 12,3 at D8S1179. Sample A was finally amplified with AmpFISTR® Identifiler® Plus with a 1.0 µl load of the neat extract for a target of 1.933 ng. Here the allele falls into the 13 bin, resulting in an 8,13 for the D8S1179 locus with the height of 1688 RFU, a size of 143.84, and an allele call of a 13. This led to a discrepancy. Additional processing of Sample A was completed by the assistance of the National Institute of Standards and Technology (NIST) to obtain a profile that could be submitted to the West Virginia State Police for upload into the Combined DNA Index System (CODIS).

The final and true allele calls for Sample A were an 8 and 13 allele at the D8S1179 locus. Sample B originally appeared to be homozygous at the D8S1179 locus with a 12,12 but close up examination raised the question of potential dropout due to a slight “blip” in the 14 allele position. This sample would normally pass because the homozygous allele passed the stochastic threshold at the MUFS. More laboratory work concluded that this sample did in fact have a sister allele of a 14 at this locus. This sample had to be extracted and amplified using PowerPlex® 16 HS, PowerPlex® 16, and AmpFISTR® Identifiler® Plus to obtain the true genotype.

CONCLUSIONS

Sample A and Sample B were successfully genotyped after extensive additional processing. Multiple amplifications and sequencing were necessary to get a final profile that would be suitable for uploading into CODIS. Access to different amplification kits using different primers helped to troubleshoot the issues observed. Ultimately, it should be pointed out that access to a wide variety of resources, including NIST, allowed for the true profiles to be produced. Analysts should take into consideration that using just one amplification kit or technology may restrict their laboratory and may need to consider outside resources or partners to work with that have access to such additional technologies.

Each sample genotyped should be thoroughly examined before passing it along for upload into any database. There may be null alleles and discrepancies that may unknowingly pass through the system without notice or second glance.

REFERENCES

10. http://www.youtube.com/watch?v=biqXsNhY2uQ