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Prevalence and impact of PCR artifacts from soil samples using the Applied Biosystems™ GlobalFiler™ PCR amplification kit: Interference of non-human artifacts in DNA profiles from a homicide investigation

Toni M. Diegoli*, Tyiesha Moore, Steven Weitz, Todd W. Bille

Bureau of Alcohol, Tobacco, Firearms and Explosives Forensic Science Laboratory, 6000 Ammendale Road, Beltsville, MD, 20705, United States

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ABSTRACT

Non-human artifacts have been observed in a range of forensic typing methods. This is primarily due to the condition and potential environmental contamination of many evidentiary samples. Here, a case example of non-human artifact peaks is presented to illustrate ways in which to recognize potential artifact peaks as well as mitigate their effect on interpretation.

1. Introduction

Forensic samples are rarely pristine, and may be collected from a variety of substrates containing unknown contaminants such as bacterial, fungal, plant, or animal DNA. Unexpected artifacts are occasionally detected in commercial short tandem repeat (STR) amplification kits despite incorporation of human-specific primers. Such artifacts are typically characterized during developmental validation to the extent to which interfering contaminants are available for testing; however, additional artifacts specific to samples contaminated by soil, mold, and other environmental sources have been detected during routine use of the Applied Biosystems™ GlobalFiler™ PCR Amplification Kit at the Bureau of Alcohol, Tobacco, Firearms and Explosives Forensic Science Laboratory. When these artifacts appear in unknown samples in allelic bins or within the calling range, interpretation of single-source and, in particular, mixed samples is complicated. The following case example describes such a situation and steps that were undertaken to recognize and manage possible non-human artifacts.

2. Materials & methods

Relevant evidence from a homicide investigation consisted of a moldy curtain with a torn edge, cable ties, and a cigarette butt.

Samples were collected from the evidence using the double swab method [1] and DNA was extracted using a modified version of the QIAamp® DNA Micro extraction method (Qiagen®, Germantown, MD). Extracts were then quantified using the Applied Biosystems™ Quantifiler™ HP DNA Quantification Kit (ThermoFisher Scientific, Waltham, MA) and concentrated to a final volume of 15 μL using Microcon-30 kDa Centrifugal Filter Units (Millipore Sigma, St. Louis, MO). DNA typing was performed using the Applied Biosystems™ GlobalFiler™ and AmpFLSTR™ Identifiler® PCR Amplification Kits, and the Applied Biosystems™ 3130 Genetic Analyzer. Applied Biosystems™ GeneMapper™ *ID-X* v.1.4 and ID v.3.2.1 were used for data analysis.

3. Results

Initial GlobalFiler™ typing results from one cable tie, two ends of the torn edge of the curtain, and the cigarette butt together indicated the presence of potential non-human artifact peaks in addition to human alleles (Fig. 1). The profile from the cigarette butt was not suspected to contain artifacts in the TAZ™ (red) dye channel based primarily on quality of the resultant profile. The profiles from both ends of the torn edge of the curtain appeared as two-person mixtures with a clear major except at locus D7S820. One end additionally exhibited an off-ladder allele with abnormal peak morphology at locus SE33. A low-level DNA profile was obtained from the cable tie, with human peak heights ranging from below analytical threshold to 84 rfu. An additional possible non-human peak was present at 992 rfu in virtual allele bin 7.1 for locus D7S820.

Two substrate controls were then taken from the curtain in an attempt to isolate the non-human artifacts and exclude them from further interpretation. However, additional artifact and human peaks were observed in both. Instead, the original locations were resampled and amplified with the Identifiler® kit. The resultant profiles did not include the artifacts observed in the GlobalFiler™ profiles and did not contain

* Corresponding author.
E-mail address: toni.diegoli@atf.gov (T.M. Diegoli).

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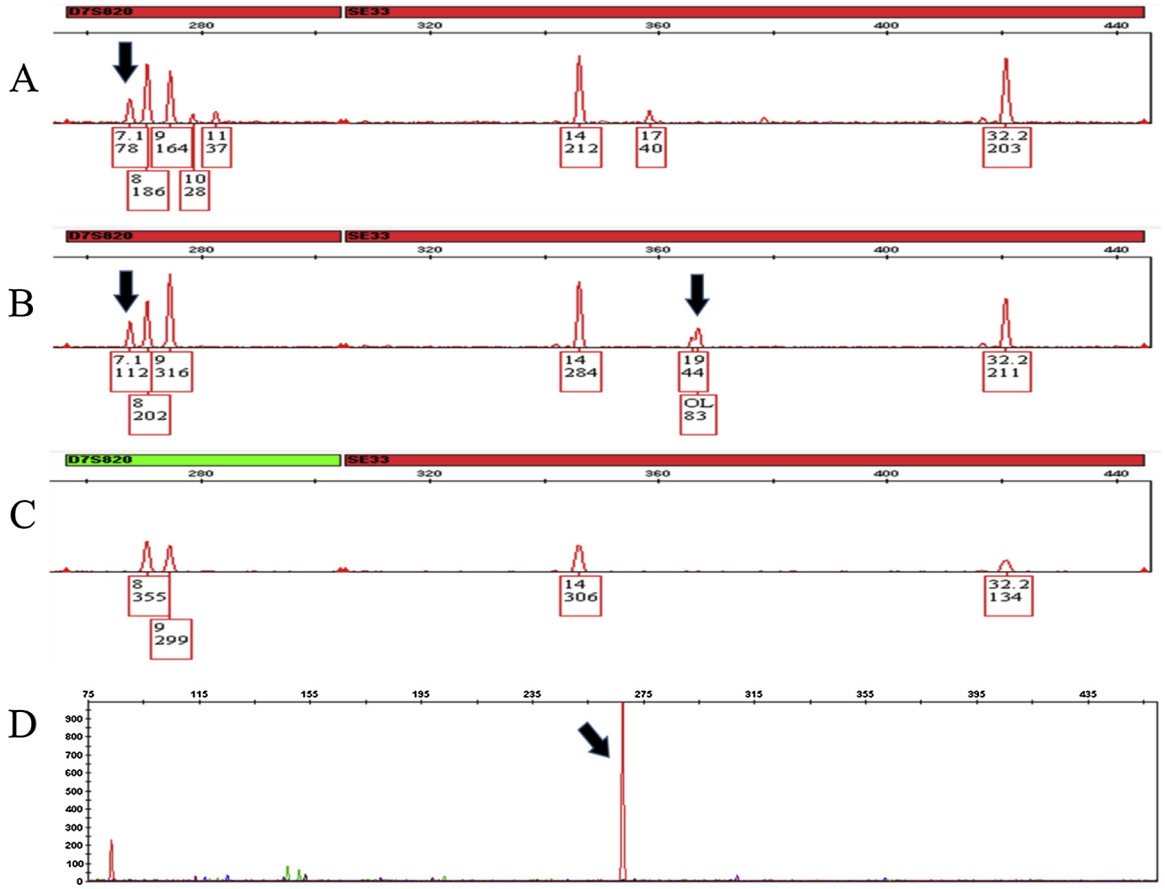

Fig. 1. Indications of possible non-human artifacts. Loci D7S820 and SE33 from GlobalFiler™ profiles of one end (A) and the other end (B) of the torn edge of the moldy curtain and the cigarette butt (C). GlobalFiler™ results from a cable tie (D) show a low-level human profile containing the same possible non-human artifact falling in the 7.1 virtual allele bin at D7S820 (large red peak). Possible non-human artifact peaks are indicated with arrows (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

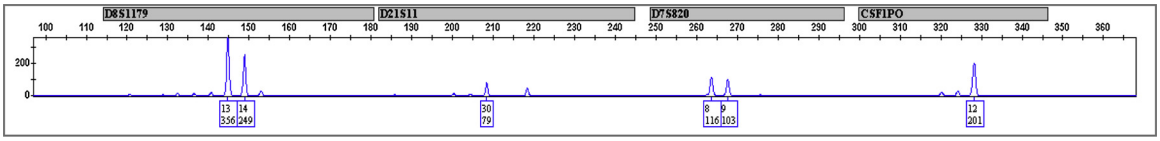

Fig. 2. Identifiler® profile showing no indications of possible non-human artifacts. 6-FAM™ (blue) channel data generated from one end of the torn edge of the moldy curtain. Potential non-human artifacts seen in GlobalFiler™ profiles at D7S820 are not present (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

any peaks suspected to be non-human (Fig. 2).

4. Discussion & conclusions

Strategies are needed to both identify non-human artifact peaks and to mitigate their impact on data interpretation. Here, a case example illustrates several indicators of possible non-human peaks as well as possible avenues to reduce their impact. In some instances, non-human artifacts tend to present with a higher relative peak height when low levels of human DNA are present. Given that non-human DNA present in soil samples (as a model for environmental contaminants) can be in excess of 20 ng per mg of soil (manuscript in preparation), this observation is likely due to preferential amplification of non-human DNA present in a greater relative quantity. Abnormal peak morphology and/or a pattern of peaks is another indication of a potential non-human artifact. Lastly, non-human peaks are often off-ladder or fall in rare allele or virtual bins. All of these indicators were present in the current case.

The impact of non-human artifact peaks may be dependent upon the circumstances. In some situations, it may be enough to resample to obtain a profile that is free of non-human artifacts. Background or substrate control samples could also be taken to rule out peaks attributable solely to non-human sources. In addition, loci suspected to contain possible non-human artifacts may be ignored during statistical calculation. Alternative kits might also be employed to eliminate suspected non-human artifacts. A combination of these approaches were taken in this case example.

More broadly, laboratories might find it useful to catalog known and suspected non-human artifacts in a database that could be referred to when possible non-human artifacts are encountered. The manufacturers of the GlobalFiler™ kit have created such a document for this particular kit based on community input [2]. In the future, next generation sequencing technologies might also play a role in confirming artifacts in specific samples as well as identifying the source of known artifacts in order to better characterize the types of samples in which they might be expected to occur.

Declaration of Competing Interest

None.

References

[1] D. Sweet, M. Lorente, J.A. Lorente, A. Valenzuela, E. Villanueva, An improved method to recover saliva from human skin: the double swab technique, J. Forensic Sci. 42 (1997) 320–322.

[2] Artifacts Identified Post-developmental Validation: GlobalFiler™ PCR Amplification Kit, Version February 12, ThermoFisher Scientific Technical Note, 2019 available at: https://www.thermofisher.com/order/catalog/product/4476135.