Forensic Science International: Genetics 6 (2012) 697–707

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Assessment of mock cases involving complex low template DNA mixtures: A descriptive study

Corina C.G. Benschop, Hinda Haned, Tanja J.P. de Blaeij, Alexander J. Meulenbroek, Titia Sijen *

Department of Human Biological Traces, Netherlands Forensic Institute, P.O. Box 24044, 2490 AA The Hague, The Netherlands

ARTICLE INFO

Article history:
Received 23 January 2012
Received in revised form 22 April 2012
Accepted 24 April 2012

Keywords:
Forensic science
Mixed DNA profiles
Low template DNA
Profile comparison
Case assessment
Likelihood ratios

ABSTRACT

Complex DNA mixtures with low template (LT) components provide the most challenging cases to interpret and report. In this study, we designed such mixtures and we describe how reporting officers (ROs) at the Netherlands Forensic Institute (NFI) assess these when embedded in a mock case setting. DNA mixtures containing LT DNA from two to four contributors, sporadic contamination (mimicked by adding 6 pg of DNA, which represents once cell equivalent) and/or DNA of relatives (brothers), were amplified four-fold using the AmpF*l*STR® NGM™ PCR Amplification Kit. Consensus profiles were then generated which included the alleles detected in at least half of the replicates. Four mock cases were created by including reference profiles of a hypothetical victim and suspect. The mock cases were assessed by eight ROs following the stepwise interpretation approach currently in use at the NFI. With this approach, the results of the comparisons between the DNA profiles of the evidentiary trace and the reference profiles are classified into four categories of evidential value [1]. The interpretations by the ROs were compared to the likelihood ratios (LRs) obtained from a probabilistic model that allows a calculation of LRs to assist the interpretation of LT DNA evidence and both were compared to the true composition of the designed mixtures.

1. Introduction

Forensic DNA analysis and interpretation of samples containing multiple contributors is especially challenging when some components are low template (LT). A decade ago, forensic DNA typing mainly dealt with (as what we would now call) high template DNA samples. Since the development of low copy number (LCN) DNA typing methods (*e.g.* [2–8]), and the release of highly sensitive short tandem repeat (STR) typing kits [9–11] we are approaching analysis down to the level of single cells. Drawbacks of STR typing of minimal amounts of DNA are the occurrence of stochastic amplification artifacts and alleles of sporadic contaminant(s). Stochastic amplification artifacts are well-defined and include allele drop-in, elevated stutter, heterozygote peak imbalance, allele drop-out and locus drop-out [2,12]. A common strategy to deal with LT artifacts is to use replicate analyses coupled with a consensus approach to infer the genotype(s) of the individual(s) that contributed to the DNA profile, though the inferences may be imperfect (*e.g.* have drop-outs) [2,13–16]. The consensus profile is then compared to reference profiles provided within the case. In some instances, the profile is searched against a DNA database. Both profile interpretation and profile comparison become more complicated when dealing with LT DNA components due to the stochastic amplification artifacts. Such artifacts may further provoke interpretation bias by ROs. Therefore, stepwise guidelines were proposed that ensure that profile interpretation occurs without prior knowledge of the reference DNA profile of, *e.g.* the suspect [1,17–21].

At the Netherlands Forensic Institute (NFI) the stepwise approach described in Meulenbroek et al. [1] is used to ensure unbiased interpretation of DNA-based evidence. This approach distinguishes four successive steps that are modified from Clayton et al. [17]: (1) STR profiling and analysis of the peaks, (2) interpretation of DNA profiles, (3) comparison of DNA profiles with categorization of the evidential value, and (4) considering the findings in the context of other facts within the criminal case. In step 1, the alleles in the DNA profile are assigned by applying a detection threshold and removing technical artifacts, such as spikes, bleed-through signals and blobs (dye residues). In step 2, the coherence of the alleles is established by estimating the (minimum) number of contributors and the contribution of different individuals and deducing, if possible, the genotype of the major and minor contributors. In step 3, the interpreted DNA profile of the evidentiary trace is compared to the reference DNA profile of a person of interest (if available), which in many cases

* Corresponding author. Tel.: +31 708886666; fax: +31 708886555.
E-mail addresses: c.benschop@nfi.minvenj.nl (Corina C.G. Benschop), h.haned@nfi.minvenj.nl (H. Haned), t.de.blaeij@nfi.minvenj.nl (Tanja J.P. de Blaeij), l.meulenbroek@nfi.minvenj.nl (A.J. Meulenbroek), t.sijen@nfi.minvenj.nl (T. Sijen).

1872-4973/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fsigen.2012.04.007

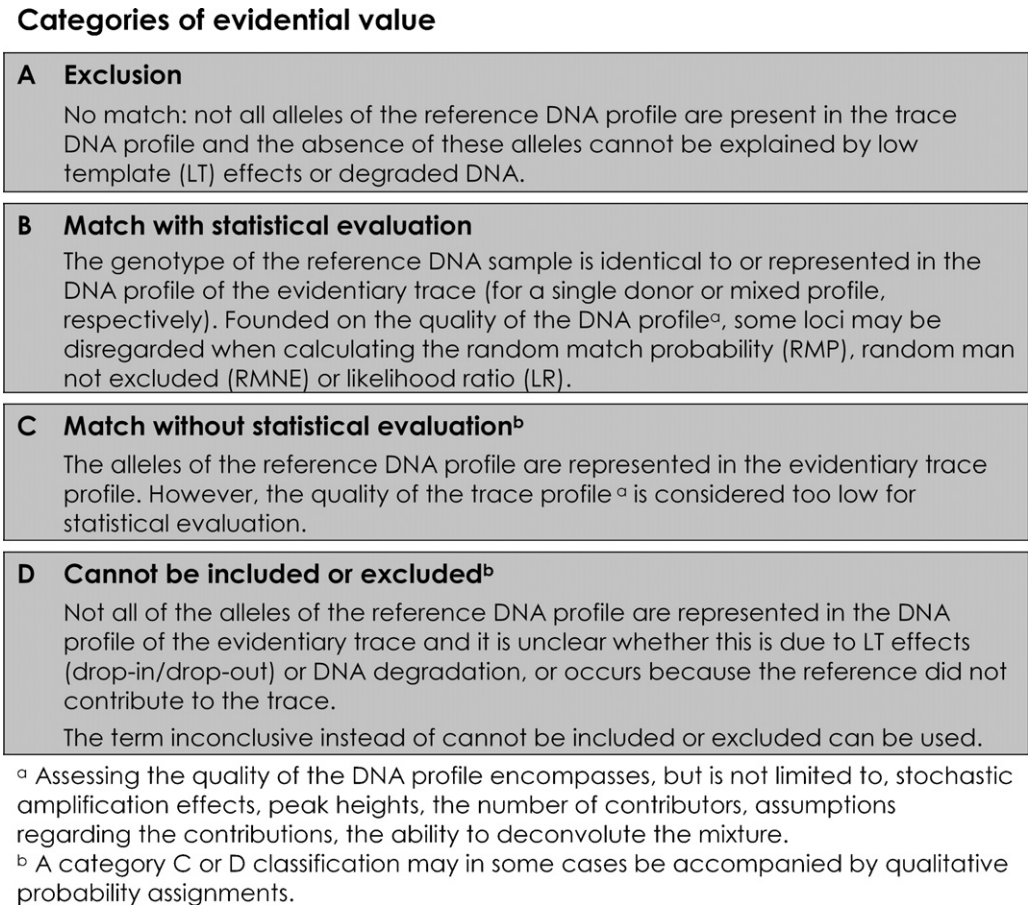

Fig. 1. General guidelines for the classification of the categories of evidential value.

concerns the suspect. A database search may have preceded this manual comparison. To avoid interpretation bias, step 3 starts when step 2 is completed. In practice, the evidentiary profile(s) may be re-evaluated based on the reference DNA profile of an assumed contributor prior to comparison to the reference DNA profile of the suspect. At the NFI, the results of comparisons between the profile of the evidentiary trace and the reference profile(s) are classified into four categories of evidential value [1]: (A) exclusion; (B) match with statistical evaluation; (C) match without statistical evaluation and (D) cannot be included or excluded (Fig. 1). Ideally, the results are placed in one category only. However, for complex DNA profiles and comparisons there may be arguments for two categories. This holds specifically for categories B and C ('match with statistical evaluation' and 'match without statistical evaluation'), C and D ('match without statistical evaluation' and 'cannot be included or excluded'), but also A and D ('exclusion' and 'cannot be included or excluded'). There can be clear differences in the juridical impact of for instance 'exclusion' or 'cannot be included or excluded'. Nevertheless, in case of complex LT STR profiles there can be arguments for both these categories. Basically the expert opinion in this evaluation process concerns the question 'are the alleles of the suspect that are not seen in the DNA profile of the evidence related to absence of DNA of this donor or a result of allele drop-out?'. With steps 1–3 the reporting officers (ROs) aim to infer which donor(s) contributed to the evidentiary trace. Hence, these steps are source level-driven. The fourth step is activity level-driven; the ROs consider the results of the profile comparisons in the context of the case and evaluate two competing propositions invoking actions that have led to deposition of the cell material. Qualitative probability assignments are generally used to present the results of this evaluation [22,23].

In this study, we report on the assessment of designed complex mock cases involving LT DNA mixtures by ROs of the NFI. Designed mixtures present the opportunity to weigh results against their actual composition and assess whether interpretation of complex low template mixtures is feasible, which is not possible with casework profiles. We prepared four challenging DNA mixtures, amplified these in four-fold using the AmpF*l*STR® NGM™ PCR Amplification (NGM) kit and generated consensus profiles that included alleles detected in at least half of the replicates (denoted the '*n/2 consensus approach*' [15]). Based on these DNA profiles, four mock cases were created by including reference DNA profiles of (an) assumed contributor(s) (*e.g.* victim, partner) and a hypothetical suspect. The four mock cases were handed to eight ROs at the NFI. They individually assessed the complex DNA profiles and examined whether the suspect may have contributed to the DNA mixture. Then they classified the outcomes in the above-described categories. In addition, for each case likelihood ratios (LRs) were obtained with LRmix [24], which implements a probabilistic model that allows a calculation of LRs to assist the interpretation of LT DNA evidence [25]. Since we used designed samples that have the advantage that the individual contributors to the mixtures are known, the ROs conclusions and the LRs can be verified. The outcomes are reported case by case.

2. Materials and methods

2.1. Samples

DNA mixtures were prepared using established amounts of pristine or diluted high template single donor DNA extracts of unrelated or related (brothers) donors with known STR profiles as