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Research paper

Production of high-fidelity electropherograms results in improved and consistent DNA interpretation: Standardizing the forensic validation process

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ABSTRACT

Samples containing low-copy numbers of DNA are routinely encountered in casework. The signal acquired from these sample types can be difficult to interpret as they do not always contain all of the genotypic information from each contributor, where the loss of genetic information is associated with sampling and detection effects. The present work focuses on developing a validation scheme to aid in mitigating the effects of the latter. We establish a scheme designed to simultaneously improve signal resolution and detection rates without costly large-scale experimental validation studies by applying a combined simulation and experimental based approach. Specifically, we parameterize an *in silico* DNA pipeline with experimental data acquired from the laboratory and use this to evaluate multifarious scenarios in a cost-effective manner. Metrics such as $\text{signal}_{1\,\text{copy}}$-to-noise resolution, false positive and false negative signal detection rates are used to select tenable laboratory parameters that result in high-fidelity signal in the single-copy regime. We demonstrate that the metrics acquired from simulation are consistent with experimental data obtained from two capillary electrophoresis platforms and various injection parameters. Once good resolution is obtained, analytical thresholds can be determined using detection error tradeoff analysis, if necessary. Decreasing the limit of detection of the forensic process to one copy of DNA is a powerful mechanism by which to increase the information content on minor components of a mixture, which is particularly important for probabilistic system inference. If the forensic pipeline is engineered such that high-fidelity electropherogram signal is obtained, then the likelihood ratio (LR) of a true contributor increases and the probability that the LR of a randomly chosen person is greater than one decreases. This is, potentially, the first step towards standardization of the analytical pipeline across operational laboratories.

1. Introduction

Samples containing low-copy numbers of DNA are routinely encountered in casework and are challenging to interpret because of the inherent complexity associated with determining contributing genotypes. Interpretation of DNA evidence is often carried out within the likelihood ratio (LR) framework, which assesses the weight of evidence by comparing the probability of observing the data under two different hypotheses. In the forensic context, the hypotheses compare the probability of observing the evidence, E, given that a specific person of interest was a contributor, H_1, versus the probability of evidence given that an unknown person contributed, H_2, and is expressed as

$$LR = \frac{\Pr(E|H_1)}{\Pr(E|H_2)} \quad (1)$$

In recent years, a number of probabilistic genotyping systems have been developed [1–7] and perform this computation, where E is presumed to consist of information obtained through the amplification and electrophoresis of a set of forensically relevant short tandem repeats (STRs). This evidence contains information related to the length of the amplified fragments as well as the number of DNA target molecules amplified as measured by an electropherogram (EPG), wherein each peak in the EPG may originate from any combination of three sources: 1) true allele; 2) instrument noise; and 3) artefact. Previous work has demonstrated that the level of detail incorporated into probabilistic

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models used for inference can substantially impact interpretation [8–10].

Core to any inference is, of course, the quality and information content of the EPG. Consequently, many strategies to improve EPG information content have been evaluated. They include the application of enhanced injection parameters [11], additional PCR cycles [11,12], upgraded amplification chemistries [13], purification of post-PCR product [14], and more sensitive instrumentation [15]. These strategies all result in an increase in signal with respect to the target number of DNA molecules, which we call α; this is the sensitivity of the process.

We utilize the recommendations set forth by the International Union of Pure and Applied Chemistry in distinguishing 'sensitivity' from the terms 'analytical threshold', 'limit of detection (LOD)' and 'signal-to-noise resolution'. Here, sensitivity is defined as

$$\alpha = \left(\frac{dS}{dT_c}\right) \tag{2}$$

where α is the slope of the tangent at nominal amplicon quantity T_c of an analytical curve, which is constant if the curve is linear. Consequently, a method may be extremely sensitive (*i.e.* large change in signal with quantity), but baseline noise may also be high leading to poor signal-to-noise resolution and a relatively large LOD. For discussions on sensitivity, LOD and signal-to-noise we refer the readers to [16–20].

In general, valuable EPG information can be garnered by lowering the LOD, or the number of molecules that can be detected. Along with improving sensitivity, another relatively simple way to accomplish this is to decrease the signal threshold (S_T) at which signal detection is demarcated [21]. The extreme version of this is to set S_T to the lowest possible level allowed by the analytical process and evaluate the signal in its entirety [22,23]. The S_T that is implemented into casework is often referred to as the analytical threshold (AT), and we reserve AT for that purpose.

There is an inherent relationship between α, S_T and the information content contained in the evidence *E*. Even in cases where no S_T is applied, the sensitivity of the laboratory process, which is dependent upon parameters such as PCR cycle number and injection conditions, determines the amount of information available for import into inference systems. Thus, development of a tenable DNA validation system that informs laboratories of α and S_T effects on the information content contained in *E* is warranted.

If no DNA target molecules are present at the start of amplification, then the signal is due to random noise or artefact. If one or a few target copies of a given allele are present then, depending on the laboratory conditions and sensitivity of the process, allele signal may be difficult to distinguish from noise. Alternatively, low-level signal may fall below some pre-defined S_T. In general, current practice relies upon the application of a S_T to the EPG signal, *S*, such that the probability of the signal observed due to noise surpasses that of S_T does not exceed a given γ [24],

$$\Pr(S > S_T | T_{c=0} = 0) \leq \gamma. \tag{3}$$

Here, $T_c = 0$ is the number of DNA copies present at the start of amplification and γ can be any value defined by the laboratory. It may be based on the post-analysis interpretation scheme where a larger risk of detecting signal in the absence of target copies may be tolerated if a probabilistic system that models noise is utilized, while a small γ may be appropriate in cases where binary or manual interpretation techniques prevail.

With the advent of probabilistic genotyping, large signal thresholds that reduce the risk of noise detection to negligible levels may not be desirable since there is a tradeoff between the false detection of noise and allele dropout. There are two main reasons for allele dropout: 1) detection effects [21]; and 2) sampling effects [25–27]. The former occurs when DNA target molecules are successfully amplified but there are too few amplicons produced to yield fluorescent signal that exceeds baseline levels. The latter arises when the DNA molecule is not available for amplification. This may occur when the DNA template is fragmented or when the DNA target molecule did not survive the pre-PCR steps and, as a result, is not present in the amplification tube. From a signal detection perspective, the risk of false non-detection of true signal, as defined in [24], is slightly modified for forensic DNA detection purposes such that we focus on defining the probability of signal not exceeding S_T when one copy of amplifiable DNA is present at the start of amplification,

$$\Pr(S < S_T | T_{c=0} = 1) \leq \beta \tag{4}$$

Therefore, the S_T is the level at which the target substance is decidedly considered to be part, or not part, of *E*. Note that although Eq. (4) is related to the probability of allele dropout, it is not equivalent to it. Previous work has demonstrated that detection of a single DNA molecule is possible [28] as long as the sensitivity is modified to a degree that ensures allelic signal surpasses the pre-defined S_T [11].

Historically, forensic DNA detection has relied upon the implementation of an AT that minimizes γ [29]. As with the authors of [30], we distinguish allele drop-in from noise detection, focusing on the signal detection problem in an attempt to design a full laboratory procedure that will lead to good signal-to-noise resolution and an LOD of one copy of DNA. Despite its unexpected nature, allele drop-in is, therefore, categorized as allele signal rather than noise and an AT designed to detect most allele signal will, by definition, also detect allele drop-in. As such, allele drop-in and the propensity of the laboratory to observe drop-in ought to be considered at the interpretation stage. With the implementation of probabilistic genotyping programmes [1,5] there has been renewed interest in re-evaluating the practice of minimizing γ in favor of utilizing lower thresholds [10]. Despite this, we emphasize that evaluating the tradeoff between the false non-detection of alleles and the false detection of noise without first considering sensitivity and signal-to-noise resolution would inevitably lead to large overall signal detection error rates.

Recently a stochastic model of EPG generation encompassing all aspects of the entire forensic DNA laboratory process, from quantification to peak detection, was described [31], where it was shown that good signal to noise resolution was readily achieved by the application of slight modifications to the laboratory conditions under which the DNA samples are processed. We extend that work here by developing a validation strategy to determine an optimized AT for the forensic DNA process. This AT is established by careful evaluation of the false detection rates only after good noise to $\text{signal}_{1\text{copy}}$ (*i.e.*, RFU signal obtained from 1 copy of amplifiable DNA) resolution is acquired. To determine the laboratory parameters that result in a sensitivity, *α,* that produces adequate resolution, we generate simulated capillary electrophoresis (CE) signal from $T_{c=0} = 1$ and $T_{c=0} = 0$ copies of DNA *via* the stochastic model described in [31]. For each laboratory condition, we compute the false positive and false negative detection rates for various S_T values for the large synthesized dataset to acquire a good approximation of the detection error rates. The PCR cycle number, injection parameters and S_T that minimize the false positive and negative detection rates while still maintaining a reasonable dynamic range (*i.e.*, large mass range of DNA that can be analyzed before the detector is saturated) are chosen as the validated laboratory process. If appropriate, the AT may be conditioned on the false positive rate.

We evaluate the validation protocol and confirm that it is applicable across two CE platforms resulting in signal detection capabilities that are at the single-copy level, regardless of instrument. We confirm that the optimized S_T values acquired from simulation coincide with those obtained from experimental samples and show the utility of this validation procedure by demonstrating that improving the information content through optimized detection strategies aids probabilistic interpretation. That is, we show that high-fidelity EPGs garnered from 1-, 2- and 3-person samples result in fewer LRs favoring the prosecution's