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Evolving Paradigms in Low-Template DNA Analysis: From Fixed Analytical Thresholds to Probabilistic Modeling

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Abstract. Inferring conclusions in low-template DNA (LT-DNA) profiles is a very difficult task in forensic genetics. The analytical threshold (AT), which distinguishes allele signals from noise, is the most important parameter for the quality of these profiles. This paper recounts the development of the AT, beginning as a fixed value and progressing to a parameter in complex probabilistic models. It explains how the intrinsic difficulty of LT-DNA, especially the stochastic nature, revealed the ineffectiveness of fixed-threshold schemes, that suffer from an information recovery vs noise introduction tradeoff. This challenge led to the development of the probabilistic genotyping systems (PGS), which evaluate evidence through continuous models, taking all signal information into account. While PGS has proven to be the powerful tool for the interpretation of difficult co-mingled DNA, it has added complexity when: software validation; inter-system variability; and court room communication are considered. In this review, it aims to highlight that signal interpretation can be redefined by next-generation sequencing (NGS) and machine learning (ML), where NGS and ML are transforming a view on the cellular state to bring the closer to a threshold free analysis. The development of the AT is indicative of the maturation of the field of trace evidence towards a greater focus on accuracy and serves to demonstrate that rigorous validation and standardization protocols are necessary to guide the appropriate use of this valuable forensic technology.

Keywords: Forensic Genetics; Low-Template DNA (LT-DNA); Analytical Threshold (AT); Probabilistic Genotyping Systems (PGS); DNA Mixture Interpretation; Forensic Science.

1. Introduction

Forensic DNA typing technology has revolutionized crime scene investigation with the unparalleled power of DNA analysis for positive personal identification. The cornerstone of this field is the analysis of short tandem repeats (STRs), which is highly polymorphic genomic regions functioning as unique genetic loci [1]. The typical practice is to amplify STR loci by polymerase chain reaction (PCR) and analyze the fluorescently labelled products by capillary electrophoresis (CE) to produce an electropherogram (EPG) [2]. The reliability of the generated DNA profile depends on the correct interpretation of this EPG, specifically the need to differentiate true allelic signals from instrumental baseline noise and artifacts.

For this purpose, laboratories define an analytical threshold (AT), the minimum signal intensity in relative fluorescence units (RFU) that is necessary to call a peak for an allele [3]. A low value is not acceptable to AT which will lead to fatal mistakes: an undershot threshold would result in noise being added, while an overshot threshold would lead to the loss of sensible low-level information, which is also known as allelic dropout [4]. Despite its efficiency with high-quality samples, this system faces difficulties with low-template DNA (LT-DNA), which includes both a low amount of DNA (<100-200 pg) and degradation as well as the presence of common complex mixtures [5]. Analysis of LT-DNA is affected by stochastic effects, with a high allelic dropout rate, and can lead to wrong assignment of a heterozygote genotype as a homozygote [6].

The challenges of LT-DNA testing revealed the inadequacy of only one fixed AT and gave rise to a need for the forensic community to critically review its interpretational framework. This article discusses the efforts to accommodate forensic challenge and the changes in the analytical concept

over time, moving from simple fixed-threshold models to dual-threshold structures, to the current step change in paradigm towards probabilistic genotyping systems (PGS) [7]. This paper will review the methods for defining those thresholds, the influence of international recommendations and the new chances and problems related to more sophisticated interpretation models.

2. Fundamentals of STR Interpretation and the Role of the AT

The interpretation of an STR DNA profile is a complex, multi-step exercise, and the first thing the analyst should do is to evaluate the electropherogram in a systematic manner. An EPG is a graph in which the size of DNA fragments (in the x-axis) is directly compared with RFU (on the y-axis) according to an internal size standard. A sound and defendable interpretation is dependent on the analyst's capacity to discriminate between true alleles in the blood sample vs byproducts of the analytical process. This requires a deep understanding of the biochemistry of PCR and the physics of electrophoresis.

2.1. Signal, Artifact, and Noise in an EPG

In an optimal EPG in single-source high-quality DNA, one or two characteristic peaks would be observed at each STR locus reflecting the individual's homozygous or heterozygous genotype. However, EPGs in the real-world are more complex, with a combination of signals from different sources. The peaks of interest are the allelic peaks, which correspond to the actual alleles amplified from the source DNA. Their size (measured in RFU) is usually relative to the amount of input DNA, although not when below or far above specific template concentrations [8].

In addition to the true signals, EPGs harbor also artifacts (i.e. non-allelic peaks coming from the PCR or CE), which require an accurate interpretation in order to avoid erroneous data interpretation. Stutter peaks are a common artifact, presenting as small peaks that are typically one repeat unit smaller (n-1) than the true parent allele and arise from polymerase strand slippage during amplification [9]. Forward stutter (n+1) may less commonly be present. Stutter filters are defined empirically (as a percentage change from the parent allele) at each locus to help in the calling of these artifacts. Pull-up artifact is the other common type, it is seen in multiplex systems when high signals in one fluorescent dye channel are incorrectly deconvolved into another due to less than perfect spectral deconvolution [10]. This spectral overlap is automatically corrected mathematically by a matrix, but this correction may not be sufficient when the signal is very strong (often off-scale). Other signals not related to an allele include so-called dye blobs created by unincorporated fluorescent dye molecules that appear as broad peaks and also sharp, high narrow spikes as a result of instrument issues such as electrical surges, air bubbles or microcrystals in the polymer that usually simultaneously occur in all dye channels at a common position [11]. Finally, all of this is added to baseline noise, that is the flat random low-level signal fluctuations that arise from the CE instrument's detection system, predominantly due to the photomultiplier or camera. This sampling noise is what the AT is explicitly meant to separate out from everything else.

2.2. The AT as a Gatekeeper

The AT is the main quality control device in terms of data interpretation and has an objective, validated threshold for role-based detection of signals that are significantly different from the instrumental baseline. Its purpose is well defined: to give confidence that a signal is not just white noise. Guidelines of major international bodies like the scientific working group on DNA analysis methods (SWGDAM) and the European network of forensic science institutes (ENFSI) say that any peak lower than the AT is not analytically reliable and should be disregarded during interpretation [12]. Conversely, any peak above this AT is treated as a "real" signal and has to be systematically classified as a true allele or a particular artifact according to its morphology, position and its ratio with the other peaks.

Generation of a laboratory-specific AT is a required component of internal validation for any DNA typing procedure. It should be informed by a high volume of empirical data, namely, the analysis of many negative controls (i.e., reagent blanks and amplification negatives) processed on a given instrument with a given chemistry [13]. This painstaking operation ensures that the threshold itself is adjusted to the peculiar noise characteristic of the entire laboratory analysis system. It is an important feature of these guidelines that the threshold value, AT, should be set so that it discriminates signal from noise only, and should never be deliberately manipulated to be higher for the more convenient reason that it also removes biological contaminants like stutter. This would be invalid scientifically as introducing artefactual sensitivity into the fundamental sensitivity of the assay and result in un-recoverable loss of weak but true allelic data, particularly within the context of LT-DNA [14].

3. The Challenge of LT-DNA

The well-accepted set of rules for STR interpretation, based on predicted (and predictable) signal behavior and obvious (and easy to discern) differences between signal and noise, is undermined by LT-DNA evidence. These samples are often the forensic linchpins of an investigation; minute amounts of biological material left behind at a crime scene, such as skin cells on a weapon grip, steering wheel, or item of clothing.

3.1. Inherent Characteristics of LT-DNA

Key features in several aspects, the LT-DNA samples have certain characteristics that in synergy are complicating the analysis. The reasons are first their minuscule sample size (below the optimization input range (approximately <100–200 pg DNA, equivalent of DNA from < 30 cells) [15]. At this degree, only a very few template molecules of each allele are incorporated into the PCR. Second, such samples are commonly highly degraded. Both hydrolytic and oxidative damage to DNA are generated in the environment by heat, humidity, UV radiation, and microbes, resulting in random strand breaks. As PCR is able to amplify only intact DNA segments between the primer binding sites, this fragmentation leads to an increased preferential amplification of smaller STR loci, and thereby the stepwise incomplete amplification of larger loci, resulting in the characteristic "ski-slope" appearance of the EPG and potential for multiple alleles failing to be amplified at loci. Finally, most trace samples comprise DNA mixtures of two or more individuals. In an LT-DNA scenario, estimating the number of contributors (NOC) is particularly challenging, yet it is a prerequisite for an accurate profile deconvolution. Their DNA may vary also severely out of proportions, complicating the interpretation.

3.2. Stochastic Effects in LT-DNA Analysis

When the number of template DNA molecules is very low, the initial cycles of PCR, where the template is copied, are subject to significant random sampling variation. This is because the distribution of individual DNA molecules into the PCR tube approximates a Poisson distribution. The resulting amplification imbalances are known collectively as stochastic effects, which are the hallmark of LT-DNA profiles [16]. The most critical of these is allelic dropout, where one of the alleles at a heterozygous locus, by chance, is not sampled into the PCR reaction or fails to amplify to a detectable level. This causes a true heterozygote to be misinterpreted as a homozygote and can lead to false exclusions of true contributors.

Likewise, severe peak height imbalance can occur, resulting in extremely low peak height ratios (PHRs) where the two alleles of a heterozygote, expected to amplify to near equal amounts (PHR ~ 1.0), are highly imbalanced (e.g., PHR < 0.60) due to one allele's having been preferentially amplified in the early cycles. This complicates interpretation of mixtures where relative peak heights are used to distinguish contributors. Analysts can also observe allelic drop-in (the presence of transient low-level alleles not from the original sample) as well. These are frequently attributed to trace levels of contamination from laboratory consumables or the environment which are magnified to traceable

levels by the high sensitivity protocols (e.g., augmented numbers of PCR cycles) involved in LT-DNA amplification [14]. These issues, especially the high rate of dropout and drop-in, indicated that a single analytic cut-point produced a very inadequate basis for sound interpretation.

4. The Evolution of Threshold-Based Interpretation

In response to the evident and immediate dangers associated with misinterpreting stochastic effects, the forensic community has established more sophisticated interpretation frameworks that transcend a singular analytical threshold. This development signifies an important recognition that not every detectable signal possesses an identical level of certainty.

4.1. Methodologies for Establishing the AT

It is with the aim of striking a balance between sensitivity and specificity that laboratories have used various empirical approaches to establish these cut off points for the AT. The two most typical methodologies involve statistical analysis of negative controls and the examination of DNA dilution series. One is to analyze a large quantity of negative control samples, and checking the RFU of all baseline noise peaks. The AT is then fixed "in the noise" such that it significantly exceeds this noise, for instance by the average of the noise plus 10x the standard deviation (SD) or a multiple (e.g. 2x) of the highest noise peak ever observed [17]. This method is perfectly suited to the objective of mitigating false positive signals due to instrumental noise. The second approach consists of analyzing known levels of DNA at lower and lower concentration. When plotted as RFUs versus the amount of DNA input, the signal strength at the limit of detection (LoD) can be visually observed and a threshold can be set (AT) that corresponds to the lower limits of being able to reliably generate signal from known allele(s) [18]. This rather complex regression-based strategy would be advantageous in examining the entire process, however, and relies heavily on accurate quantification. The fundamental principles and trade-offs of these approaches are enumerated in Table 1.

Table 1. Comparison of Methodologies for Determining AT.

Method Category	Principle	Required Data	Advantages	Limitations & Disadvantages
Based on Negative Controls (Statistical Method)	Sets AT based on the statistical distribution of baseline noise in multiple negative control samples (e.g., mean + 3-10x SD of noise, or a multiple of the highest observed noise peak).	Data from numerous negative control (reagent blank, amplification negative) electropherograms.	Simple to calculate; statistically clear rationale; directly reflects instrument and reagent background noise.	Ignores that noise levels can increase with high DNA concentration; may be too conservative (low) for high-template samples; can be skewed by rare, high-noise events.
Based on Positive Samples (Regression Method)	Establishes a linear regression model between known DNA input quantity and resulting RFU signal intensity from a dilution series of a standard sample.	Data from precisely quantified and serially diluted standard DNA sample electropherograms.	Reflects the dynamic performance of the entire analytical process (incl. PCR); based on true allele signals rather than just noise.	More complex and costly to perform; requires highly accurate DNA quantification; relationship is non-linear at very low (stochastic) and very high (saturation) concentrations.

Both approaches are correct, but both illustrate one major question: not all types of samples may be served with one, set AT. For example, baseline of high-quantity DNA often is “noisier” than that of the negative control, which indicates that AT based on negatives alone may be too low for high-template samples. Furthermore, modifying an analytical parameter to enhance sensitivity, such as increasing the number of PCR cycles, will amplify both signal and noise. Consequently, such a change mandates a complete re-validation of the process, which typically includes establishing a higher AT.

4.2. Introduction of the Stochastic Threshold (ST)

The most serious deficiency of the AT is that it can only report the probability of a signal being noise, not the probability of a 'true allele' not being observed as dropout. To address this particular form of risk, the idea of the stochastic threshold (ST) was proposed. The ST is a higher RFU value, empirically determined by a laboratory, above which allelic dropout is considered highly unlikely [19]. While its definition is primarily based on observing dropout events in known heterozygous samples, its practical application is to provide greater confidence in genotyping calls. Specifically, it allows an analyst to more reliably interpret a single detected peak above the ST as a true homozygous genotype, because the risk of its heterozygous partner having dropped out is minimal.

The ST is also empirically determined, usually by analyzing multiple samples that are known to be heterozygous at several different concentrations within the stochastic region [20]. The analyst notes all areas of dropout and calls the peak height of the remaining partner allele. The ST is then placed above the maximum surviving-peak high across all dropouts. The introduction of the ST produced a double-threshold setup with three interpretational zones in the EPG. The lower band of the AT was designated as unreliable noise. There are signals that are not unique above the ST that can be used for genotyping. The most difficult to map region was the “gray zone” between AT and ST. A peak in this intermediate range is a legitimate allele, but it occurs in the region where the stochastic processes predominate. Thus, a single peak in this region cannot be confidently designated as a true homozygote. This interpretational indeterminacy frequently necessitated the application of more conservative statistical measures (e.g., combined probability of inclusion/exclusion) which leave most of the strength of the profile unsampled. Although the new legislation refined the AT/ST, the binary approach, and the loss of information that it entailed, opened the door to a new way of thinking.

5. Paradigm Shift: PGS

The inherent limitations that force an all-or-nothing decision for each peak in fixed-threshold methods catalyzed a shift in paradigm. Therefore, the PGS have emerged. It is this system that replaces the binary decision rule with integrated continuous statistical models for evaluating the DNA evidence, thus changing the role and importance of thresholds.

5.1. From Binary Decisions to Continuous Models

Traditional STR interpretation as implemented is a semi-continuous approach as it takes into account the qualitative presence of alleles (once cleared above the AT) but largely ignores the richer quantitative information contained in peak heights, apart from simple PHR calculations and a few other nearby retention/enrichment operations. PGS, on the other hand, is based on a completely continuous model [21]. Rather than posing the yes/no question—"Is this peak an allele?" PGS asks a probabilistic question: "Based on a set of assumptions about the number of contributors, what is the probability of observing the entire electropherogram, including all of its quantitative differences, if a certain individual's DNA were in the mixture? "

The PGS software involves computation-intensive algorithms like Markov Chain Monte Carlo (MCMC) which enable the search of the large space of all possible sets of genotypes that would account for the observed EPG [22]. These biological models factor in the likelihoods of a number of phenomena given the validation data of the laboratory, including allelic dropout, allelic drop-in,

variable stutter ratios, and peak height variation. By treating the presence of these events as series of continuous probabilities, rather than as hard filters, PGS can utilize all of the quantitative data in a profile—most importantly, data from peaks below the traditional AT. The AT can be used as in the past as an initial data reduction filter removing the most blatant instrumental noise, but not as a definitive veto for a candidate to be included in the overall statistical evaluation.

The result of a PGS test is also not a "hit" or "non-hit," but rather a likelihood ratio (LR) value. The LR is a robust measure that quantifies the weight of evidence by comparing the probability of observing the evidence under two competing propositions: that of the prosecution (Hp: the suspect is a source of the evidence) and that of the defense (Hd: an unknown, unrelated individual is the source of the evidence). For example, an LR of 10,000 means that the observed DNA evidence is 10,000 times more likely under the hypothesis that the suspect contributed DNA than the hypothesis that an unknown, unrelated individual contributed DNA. This enables a quantitative statement about the quality of evidence also in complex and low-level samples.

5.2. Implications and New Challenges

It is PGS that has made it possible to interpret substantially more complex and low-level DNA mixtures in the last years than it was feasible in the past and to save evidence that would have been declared inconclusive in old, threshold-based methods. But this awesome new tool comes with its own set of big problems. Internal validation of a PGS software is at least an order of magnitude more difficult than the validation of an AT. Labs should perform a thorough set of performance tests that are representative of their specific laboratory sample types (contributor numbers, mixture ratios, template amounts, and degradation levels) to help define the limits where the results are empirically supported and accurate [23].

Additionally, there is inter-software variability for the same reason that various PGS software (e.g., STRmix™, TrueAllele®) apply different mathematical modeling and algorithms [24]. This may in turn result in different LR outcomes for the same evidence file, and for the same value the differences can be orders of magnitude, a fact that has been a cause for considerable dispute in courts. The proprietary and often opaque nature of the algorithms has also prompted the "black box" argument in the courtroom, where criminal defense lawyers argue that if they cannot thoroughly cross-examine the source code and logic of the software, it runs afoul on a defendant's constitutional right to confront the evidence against them. Finally, the large numbers needed to communicate the implications of a large LR to lay people, are a considerable challenge, with a significant risk of misunderstanding; the "prosecutor's fallacy". As a consequence, the switch to PGS has shifted the 'hot seat' away from validating a 'yes/no' threshold to validate and transparently use an entire complex bio-statistical system. A summary of two major platforms for PGS is listed in Table 2.

Table 2. Comparative Analysis of Mainstream Probabilistic Genotyping Systems (STRmix™ vs. TrueAllele®).

Feature	STRmix™	TrueAllele®	Key Differences & Judicial Impact
Underlying Statistical Model	Bayesian methods, utilizing Markov Chain Monte Carlo (MCMC) algorithms.	Bayesian methods, also utilizing MCMC algorithms.	While both are Bayesian, specific model parameters (e.g., for stutter, degradation) and prior assumptions differ, which can lead to different LR results from the same data.
Data Utilization	Employs a fully continuous model, using all quantitative peak height and area information.	Also employs a fully continuous model, using all quantitative information.	Both models maximize data usage over semi-continuous methods. Differences lie in how they weigh and model the quantitative data.
Output	A Likelihood Ratio (LR) representing the weight of evidence for competing propositions.	A Likelihood Ratio (LR) representing the weight of evidence.	The LR is the standard output, but the numerical value can differ significantly between systems for the same case, potentially causing confusion in court.
Source Code Accessibility	Generally made available to defense experts under protective court orders for case-specific review.	Proprietary and generally not made available for external review.	This is a major point of legal contention. The "black box" nature of proprietary code raises defense challenges regarding due process and the right to confront evidence.
Courtroom Admissibility	Widely validated and admitted in courtrooms globally.	Also widely validated and admitted in courts, primarily in the United States.	Both have extensive admissibility histories, but cases with conflicting results between platforms or challenges to validation have highlighted the complexity of their use as evidence.

6. Future Horizons: Redefining the Signal

The evolution of DNA interpretation is not over. Emerging technologies are poised to once again redefine the fundamental concepts of signal, noise, and thresholds in forensic genetics.

6.1. Next-Generation Sequencing (NGS)

Next-generation sequencing (NGS), or massively parallel sequencing (MPS), moves analysis beyond fragment length to the direct reading of DNA base sequences. In the context of STR analysis, NGS has several pluses. NGS can identify isoalleles (alleles of the same length but with different internal sequences) and detect single nucleotide polymorphisms (SNPs) within and flanking the STR regions. This additional genetic information greatly enhances the discriminatory power of the assay and significantly aids in the deconvolution of complex mixtures.

Importantly, signal strength on NGS is not measured in RFU, but sequence reads. The criterion of an intensity-based AT is outdated. New quality metrics and filters appropriate for the handling of sequencing errors and other artifacts are needed, but NGS data is inherently digital and discrete, providing a distinct framework for statistical analysis [25]. The technology may compensate for a number of the stochastic effects observed with CE by enabling better amplification and/or filtering of PCR stutter and other artifacts.

6.2. Machine Learning and Artificial Intelligence

Machine learning (ML) or Artificial intelligence (AI) is the next level. These technologies open the way for end-to-end automatic self-learning interpretation systems that may move beyond any human-based rules and thresholds. An ML model might be trained on thousands of EPGs to capture the subtle, multi-dimensional pattern that differentiates genuine alleles from a range of artifacts, e.g. complex stutter, pull-up and baseline noise [26].

A system might evolve in which signals are variably interpreted in a context-dependent manner in adopting the peak or no-peak classification, in which the "threshold" for declaring a peak positive is not a hard RFU number but a probability density score based on consideration of the entire profile. While the opportunity is massive, the use of ML in the justice system will face even more “black box” scrutiny than PGS. For their introduction in casework the question of transparency, fairness and explainability of such algorithms will have to be tackled.

7. Conclusion

The evolution of analytical threshold in forensic DNA analysis represents the microcosm of the development pathway of forensic science toward more precise measurement of information-rich evidence. Although originally a simple, necessary tool, the fixed AT was inappropriate for LT-DNA samples, since its use led to a conflict between loss of information and noise inclusion that could not be resolved. This limitation prompted a major paradigm change towards PGS in an attempt to incorporate the strengths of probabilistic reasoning instead of binary rules which utilizes a continuous model to weigh all data statistically and express uncertainty through the concept of a LR. In this construct, the AT was relegated from a critical gatekeeper to a trivial parameter, refocusing quality assurance efforts from verifying a simple line to verifying a complex interpreter. From here on, the quest is on to reach novel horizons, in which the encounter between technologies such as next-generation sequencing and machine learning might open up the future to an intelligent, possibly threshold-free, interpretation. But the real challenges remain the same, no matter the technology: that the best methods are thoroughly validated but also transparent and communicated effectively to the justice system. The ultimate goal is not to search for a perfect threshold, but rather to develop systems that can provide the most faithful and robust representation and communication of uncertainty in trace evidence analysis.

References

[1] Butler J M. Forensic DNA typing: biology, technology, and genetics of STR markers. Elsevier, 2005.

[2] Moretti T R, Baumstark A L, Defenbaugh D A, et al. Validation of STR typing by capillary electrophoresis. Journal of Forensic Sciences, 2001, 46(3): 661-676.

[3] Rakay C A, Bregu J, & Grgicak C M. Maximizing allele detection: effects of analytical threshold and DNA levels on rates of allele and locus drop-out. Forensic Science International: Genetics, 2012, 6(6): 723-728.

[4] Gonzalez-Bao J, Mosquera-Miguel A, Casanova-Adan L, et al. Performance comparison of a previously validated microhaplotype panel and a forensic STR panel for DNA mixture analysis. Forensic Science International: Genetics, 2025, 74: 103144.

[5] Pfeifer C M, Klein-Unseld R, Klintschar M, et al. Comparison of different interpretation strategies for low template DNA mixtures. Forensic Science International: Genetics, 2012, 6(6): 716-722.

[6] Hedell R, Dufva C, Ansell R, et al. Enhanced low-template DNA analysis conditions and investigation of allele dropout patterns. Forensic Science International: Genetics, 2015, 14: 61-75.

[7] Greenspoon S A, Schiermeier-Wood L, & Jenkins B C. A tale of two PG systems: a comparison of the two most widely used continuous probabilistic genotyping systems in the United States. Journal of Forensic Sciences, 2024, 69(5): 1840-1860.

[8] Butler J M, & Hill C R. Biology and genetics of new autosomal STR loci useful for forensic DNA analysis. Forensic Science Review, 2012, 24(1): 15.

[9] Daunay A, Duval A, Baudrin L G, et al. Low temperature isothermal amplification of microsatellites drastically reduces stutter artifact formation and improves microsatellite instability detection in cancer. Nucleic Acids Research, 2019, 47(21): e141-e141.

[10] Goor R M, Hoffman D, & Riley G R. Novel method for accurately assessing pull-up artifacts in STR analysis. Forensic Science International: Genetics, 2021, 51: 102410.

[11] Al-Shuhaib M B S, & Hashim H O. Mastering DNA chromatogram analysis in Sanger sequencing for reliable clinical analysis. Journal of Genetic Engineering and Biotechnology, 2023, 21(1): 115.

[12] Hares D R, Kneppers A, Onorato A J, et al. Rapid DNA for crime scene use: Enhancements and data needed to consider use on forensic evidence for State and National DNA Databasing–An agreed position statement by ENFSI, SWGDAM and the Rapid DNA Crime Scene Technology Advancement Task Group. Forensic Science International: Genetics, 2020, 48.

[13] Chen D, Tan M, Xue J, et al. Optimizing analytical thresholds for low-template DNA analysis: insights from multi-laboratory negative controls. Genes, 2024, 15(1): 117.

[14] Parys-Proszek A, Marcińska M, Branicki W, et al. Examination of LT-DNA traces–literature overview and general recommendations of the Polish Speaking Working Group of the International Society for Forensic Genetics (ISFG-PL). Archiwum Medycyny Sądowej i Kryminologii/Archives of Forensic Medicine and Criminology, 2020, 70(2): 103-123.

[15] Benschop C C, Haned H, Yoo S Y, et al. Evaluation of samples comprising minute amounts of DNA. Science & Justice, 2015, 55(5): 316-322.

[16] Berti A, Barni F, Pilli E, et al. A new ultradeep LT (low template) DNA profiling approach based on an emulsion-based clonal amplification of an STRs multiplex PCR product followed by massive pyrosequencing. ISFG, 2011.

[17] Reimann C, Filzmoser P, & Garrett R G. Background and threshold: critical comparison of methods of determination. Science of the Total Environment, 2005, 346(1-3): 1-16.

[18] Simonsen A T, Hansen M C, Kjeldsen E, et al. Systematic evaluation of signal-to-noise ratio in variant detection from single cell genome multiple displacement amplification and exome sequencing. BMC Genomics, 2018, 19: 1-9.

[19] Bregu J, Conklin D, Coronado E, et al. Analytical thresholds and sensitivity: establishing RFU thresholds for forensic DNA analysis. Journal of Forensic Sciences, 2013, 58(1): 120-129.

[20] Gill P, Puch-Solis R, & Curran J. The low-template-DNA (stochastic) threshold—its determination relative to risk analysis for national DNA databases. Forensic Science International: Genetics, 2009, 3(2): 104-111.

[21] McNevin D, Wright K, Barash M, et al. Proposed framework for comparison of continuous probabilistic genotyping systems amongst different laboratories. Forensic Sciences, 2021, 1(1): 33-45.

[22] Coble M D, & Bright J A. Probabilistic genotyping software: an overview. Forensic Science International: Genetics, 2019, 38: 219-224.

[23] Gleicher N, Kushnir V A, & Barad D H. How PGS/PGT-A laboratories succeeded in losing all credibility. Reproductive Biomedicine Online, 2018, 37(2): 242-245.

[24] Riman S, Iyer H, & Vallone P M. Examining performance and likelihood ratios for two likelihood ratio systems using the PROVEDIt dataset. PLoS One, 2021, 16(9): e0256714.

[25] Hwang D H, Garcia E P, Ducar M D, et al. Next-generation sequencing of cytologic preparations: an analysis of quality metrics. Cancer Cytopathology, 2017, 125(10): 786-794.

[26] O'Donnell L. Clustering single-cell electropherograms by genotype through unsupervised machine learning. National University of Ireland, 2021.