Analysis of allelic drop-out using the Identifiler® and PowerPlex® 16 forensic STR typing systems

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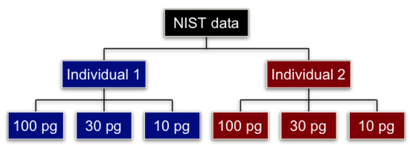
Abstract

Low-template (LT) DNA profiles continue to present interpretational challenges to the forensic community. Whether the LT contribution comprises the main profile, or whether it is present as the minor component of a mixture, ambiguity arises from the possibility that alleles present in the biological sample may not be detected in the resulting DNA profile. This phenomenon is known as allelic drop-out. This ambiguity complicates both the assessment of the potential number of contributors and estimation of the weight of the DNA evidence for or against specific propositions. One solution to estimating the weight of the evidence is to use a likelihood ratio (LR) that incorporates the probability of allelic dropout $P(D_0)$ estimated for the specific evidence sample under consideration. However, although a vast repository of data exists, few empirical studies to determine allelic drop-out probabilities have been performed to date. Here we characterized patterns of allelic drop-out in single-source samples using both universal and run-specific analytical thresholds. Not surprisingly, we found fewer instances of apparent drop-out when using a lower (run-specific) detection threshold. Also, unsurprisingly, a positive correlation exists between allele drop-out and allele length, even in good quality samples. We used logistic regression to model the fraction of alleles that dropped out of a profile as a function of the average height of the detected peaks. The equation derived from the logistic regression model allowed us to estimate the expected drop-out probability for an evidentiary sample based on the average peak height of the profile. We show that the LRs calculated using the estimated drop-out probabilities were similar to those calculated using the benchmark drop-out probabilities, suggesting that the estimates of the drop-out probability are accurate and useful. This trend holds even when using the data from the PowerPlex ® 16 typing system to estimate the drop-out probability for an Identifiler® profile, and vice versa. Thus we demonstrate that use of a LR that incorporates empirically estimated allelic drop-out probabilities provides a reliable means for extracting maximum information from LT forensic DNA profiles.

Profiles Analyzed

60 single-source DNA profiles generated by the group at NIST were amplified using a standard protocol for both Identifiler® and PowerPlex® 16. (www.cstl.nist.gov/strbase/LTDNA.htm).



10 replicate amplifications of each dilution for a total of 60 profiles for each genetic analysis system

Data Analysis

Logistic regression was performed to calculate the relationship between peak height and proportion of alleles dropped out at both 50 RFU and 30 RFU for each individual and each system.

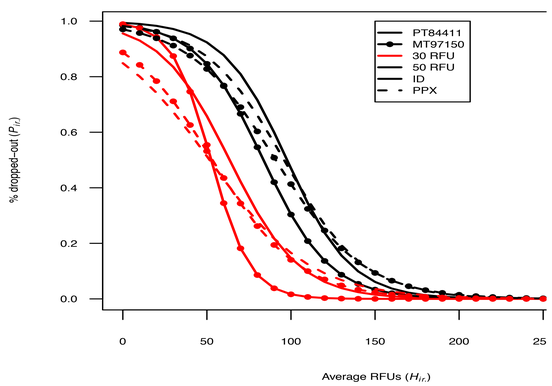


Fig. 2. Logistic curves showing the relationship between the probability of drop-out and average RFUs for different detection thresholds (30 RFUs and 50 RFUs), individuals (PT84411 and MT97150), and typing systems (Identifiler® and PowerPlex® 16).

Analytical Threshold — 50 RFU vs. 30 RFU

As expected, the apparent drop-out was lower for the profiles analyzed at 30 RFU that for the profiles analyzed at 50 RFU. A substantial amount of data was recovered by reducing the analytical threshold to an empirically determined AT of 30 RFU. No false positives allele calls were incurred at 30 RFU.

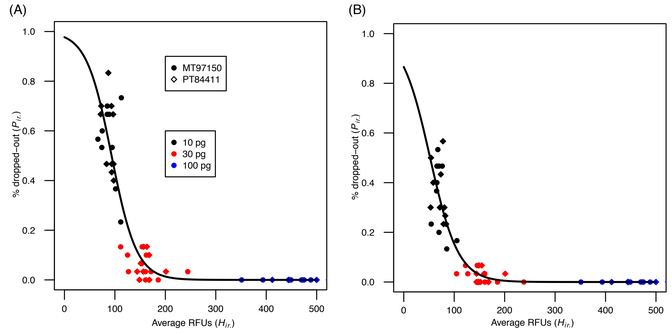


Fig. 1.
Relationship between the proportion of alleles that dropped out of each Identifiler® profile (P_{ir}) and the average RFUs of all detected peaks in the profile (H_{ir}). (A) 50 RFU detection threshold, and (B) 30 RFU detection threshold. The logistic regression curves were fit to all 30 profiles from individual MT97150 and 30 profiles from individual PT8441. The Identifiler® data are depicted. Note: the plotting area was truncated at 500 RFUs. Several points fell beyond this region.

Probability of Drop-out — $P(D_o)$

We used the logistic regression curve calculated from the data for Individual 1 to estimate the $P(D_o)$ for Individual 2 and then compared the estimated $P(D_o)$ to the benchmark $P(D_o)$ calculate for Individual 2.

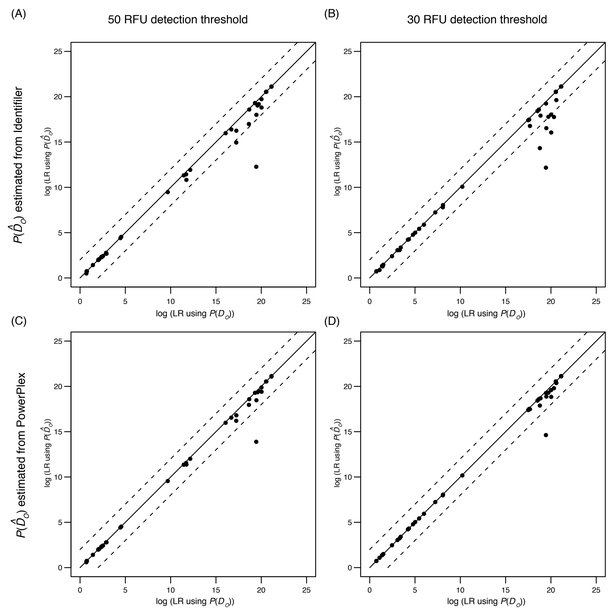


Fig. 4. LRs calculated from the Balding and Buckleton program when the hypothesized contributor was the true donor of the Identifiler® LT DNA profile. Each dot represents the log(LR) for a particular LT DNA profile calculated using the estimated drop-out probability vs. the benchmark drop-out probability. The solid line is the diagonal and the dashed lines denote 2 orders of magnitude in either direction around the diagonal. (A) 50 RFU detection threshold, where $P(D_o)$ was estimated from the other individual's Identifiler® data. (B) 30 RFU detection threshold, where $P(D_o)$ was estimated from the other individual's Identifiler®. (C) 50 RFU detection threshold, where $P(D_o)$ was estimated from the other individual's PowerPlex® 16 data. (D) 30 RFU detection threshold, where $P(D_o)$ was estimated from the other individual's PowerPlex® 16 data.

Comparison to Known Non-Contributors (KNC)

A useful metric to understand the import of a LR calculated for any particular profile is to compare the result to a population of KNCs. LRs were calculated using 5000 simulated European individuals as the suspected contributors to the LT profiles. For the majority of the comparisons (>80%), the LRs were <1, and in nearly all of the comparisons (>99%), the LRs were <2, indicating that, in most instances, the LR approach correctly rejects the proposition of KNCs as true donors of the evidence. Interestingly, the 30 RFU detection threshold led to a greater number (about 8%) of LRs < 1 than those computed using the 50 RFU detection threshold. The 30 RFU detection threshold allows the detection of additional alleles, some of which were exclusionary, correctly supporting the hypothesis that a KNC in fact is not a contributor to the profile. Further, the lower detection threshold reduced the proportion of LRs >1 for KNCs using the estimated $P(D_o)$ when the benchmark $P(D_o)$ gave an LR <1. This result supports using data-driven thresholds that rescue low-level, but informative, data.

Table 3
Distribution of the LRs calculated comparing 60 LT Identifiler® profiles to 5000 simulated individuals.[a]

Detection Threshold (RFU)	Drop-out Probability[b]	%LRs <1	% LRS <2	% LRs >100	%LRs >1000	99.9% of LRs are < than…	Max LR
50	Benchmark *P(Do)*	84.2	99.4	0.053	0.010	34	6.6×10^4
	Esimated using Identifiler®	84.3	99.5	0.031	0.001	27	2.4×10^3
	Estimated using PPX	84.2	99.5	0.03	0.001	27	2.4×10^3
30	Benchmark *P(Do)*	92.9	99.6	0.041	0.005	31	1.5×10^6
	Esimated using Identifiler®	92.8	99.6	0.012	0.001	17	3.2×10^6
	Estimated using PPX	92.8	99.6	0.012	0.001	18	3.4×10^5

[a] LT profiles containing 0 alleles were left in this analysis, but the LRs were set to 1

[b] Denotes the approach used to estimate the drop-out probability. The benchmark *P(Do)* is the best drop-out probability for each locus at each profile.
The benchmark *P(Do)* was 0, if 2 alleles were detected at a locus. The benchmark *P(Do)* was 0.5 of a single allele was detected at a locus. The benchmark *P(Do)* was 1 if 0 alleles were detected at a locus. Estimated using Identifiler® refers to drop-out probabilities estimated using the logistic regression equations fit to the Identifiler® data from the 30 samples from the other individual. Estimated using PPX (PowerPlex®16) refers to drop-out probabilities estimated using the logistic regression fit to the PowerPlex®16 data from the 30 samples from the other

Conclusion

We have modeled the relationship between the average peak height and the proportion of allelic drop-out in LT DNA samples using logistic regression.

Using these results, we have evaluated the difference between using a standard, policy-driven allelic detection threshold of 50 RFUs and using a data-derived threshold of 30 RFUs. Not unexpectedly, we found fewer instances of apparent drop-out when using the lower detection threshold. This finding indicates that useful genetic information, both inclusionary and exclusionary, is discarded by ignoring alleles that fall below a conventional 50 RFU threshold.

We have shown that, when estimated drop-out probabilities were used to compute LRs, these LRs were very similar to the LRs calculated using the benchmark drop-out probability. Similarly, the drop-out probabilities estimated for Identifiler® profiles using the logistic regression equation fit to the PowerPlex® 16 data gave LRs very similar to those obtained using the benchmark drop-out probability. The same pattern was observed for the opposite situation. This suggests that any differences between the two systems are relatively minor compared to the overall variability inherent in LT DNA evidence. Further, the fact that the logistic regression equations could be used interchangeably across systems speaks to the robustness of the estimates. An estimate of the drop-out probability for a particular sample is sufficiently accurate, even if the conditions used for the analysis of that sample are not exactly the same as those used to estimate the parameters of the logistic regression model.

In conclusion, our results indicate that it is possible to accurately compute LRs using drop-out probabilities estimated from empirical data. This lays the groundwork for wider application of probabilistic approaches to forensic casework using LRs that incorporate an estimate of the drop-out probability. Combining the appropriate detection threshold with LRs that incorporate empirically estimated drop-out probabilities should provide a sensitive and statistically rigorous framework to reliably interpret LT DNA evidence.