Title	**Population Genetic Issues for Forensic DNA Profiles.**
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Accomplishments

Major Goals of the Project

This project was planned to conduct research on the population genetic issues a ecting the interpretation of forensic DNA profiles. The particular topics addressed were:

- Interpretation of forensic evidence.
- Interpretation of DNA sequence data.
- Interpretation of lineage marker evidence.
- Interpretation of DNA mixtures.
- Probabilistic genotyping.
- Other topics.

During the award period we published 42 papers addressing these topics, and they are summarized in the next section. (The sequence numbers, author names, and citation details are shown below in the list of publications.)

Summaries of Publications

Interpretation of Forensic Evidence.

8. Bright, Buckleton, Taylor, 2021 It is a typical practise in forensic laboratories that once the weight exceeds a threshold (such as 99 %), then they can be considered to be resolved enough to interpret (for example to load onto a database). They found that unless an individual is a clear major (or minor) contributor, the genotype weights do not typically exceed 99 % for any genotype.

LRs have not been traditionally assigned for the Amelogenin locus and are small compared to an LR assigned for a modern day STR multiplex where, for a more discriminatory locus, the per locus LR for a resolved contributor could be in the order of tens or hundreds.

The method described uses per-contributor template values for a previously interpreted profile. The discrimination power is restricted, with a maximum possible LR of 2 for a fully resolved genotype, due to the limited number of alleles and hence genotypes and assuming equal proportions of genders in the population. However, it has a good power to exclude when the component is well resolved and non-concordant with a Person of Interest.

10. Buckleton, Bright, Taylor, et al, 2022 Recently Riman et al. published a comparison of the two PG software - STRmix V2.6 and EuroForMix V2.1.0 (EFM) - using STR profiles generated using the GlobalFiler kit from the open source PROVEDIt dataset. Whilst both software perform well, if used properly, there are a number of technical deficiencies to consider when assessing di erent results from analyses of the same DNA profile with each software. Some of the technical faults are sufficiently significant to render the data valueless. Others may have minimal consequences. We discussed these faults and their impact.

8. Buckleton, Kalfut, Curran, 2022 Rigorous mathematical treatment, given by Slooten and others, appears to o er strong guidance for setting propositions. This treatment assumes that the prior probabilities for conditioning, or not conditioning, on any individual are not extreme. It, is when these prior probabilities appear ambiguous that the decision to condition or not can appear to be problematic. This is often the situation found in casework. We attempted to show that such situations may benefit most from following such guidance. A lower bound on the Bayes factor (BF) can be obtained by finding the highest LR that includes the person of interest (POI) and dividing by the highest LR that does not include the POI. These two highest LRs may be found with and without the disputed conditioning profile. The resultant lower bound is on the BF for the inclusion of the POI without directly assuming the disputed conditioning profile. Adopting this approach would both minimize adventitious inclusions and approximate an exhaustive set of propositions.

20. Hicks, Buckleton, Castella, et al. 2022 The forensic community has devoted much e ort over the last decades to the development of a logical framework for forensic interpretation, which is essential for the safe administration of justice. We reviewed the research and guidelines that have been published and provide examples of how to implement them in casework (Hicks et al., 2022). After a discussion on uncertainty in the criminal trial and the roles that the DNA scientist may take, we presented the principles of interpretation for evaluative reporting. We showed how their application helps to avoid a common fallacy and present strategies that DNA scientists can apply so that they do not transpose the conditional. We then discussed the hierarchy of propositions and explain why it is considered a fundamental concept for the evaluation of biological results and the di erences between assessing results given propositions that are at the source level or the activity level. We showed the importance of pre-assessment, especially when the questions relate to the alleged activities, and when transfer and persistence need to be considered by the scientists to guide the court. We concluded with a discussion on statement writing and testimony. This provided guidance on how DNA scientists can report in a balanced, transparent, and logical way.

23. Kalafut T, Bright JA, Taylor D, et al Recently, W.C. Thompson reported his perception of a US federal case in which he advised the prosecution. The case involved a Daubert hearing from which no ruling was given but did not proceed to trial. The evidence discussed was the interpretation of a low-level mixed DNA profile on a plastic bag containing drugs. A probabilistic genotyping (PG) software, STRmix, had been used to interpret this mixture. This produced an LR that supported an exclusion of the POI. A Daubert hearing was requested by the prosecution to suppress the DNA evidence. This is the first time, of which we are aware, that the prosecution has sought to suppress PG evidence.

There are sufficient doubts about the storage and handling of the bag that any source-level conclusion is of limited value at the activity level. However, the source level is exactly what the discussion centered upon. Subjectively, using the sub-threshold peaks, the data support the exclusion of the POI. Thompson's paper covers a very broad range of topics. To retain some focus to our comments arising from this we discussed only the following: 1. Whether the analytical threshold (AT) should be varied in casework "To learn more about the consequences of applying a threshold." 2. Why TrueAllele and STRmix give di erent answers. 3. The number of contributors (NoC) should automatically be varied across the plausible range. 4. The upper bound is the correct bound to report if the LR is below one. 5. What do you do with multiple LRs.

27. Kriujver, Kelly, Cheng, et al The interpretation of DNA profiles typically starts with an assessment of the number of contributors. In the last two decades, several methods have been proposed to assist with this assessment. We describe a relatively simple method using decision trees, that is fast to run and fully transparent to a forensic analyst. We use mixtures from the publicly available PROVEDIt dataset to demonstrate the performance of the method. We show that the performance of the method crucially depends on the performance of filters for stutter and other artefacts. We compare the performance of the decision tree method with other published methods for the same dataset.

28. Kruijver, Bright, 2022 Simulation studies play an important role in the study of probabilistic genotyping systems, as a low cost and fast alternative to in-vitrost udies. With ongoing calls for further study of the behavior of probabilistic genotyping systems, there is a continuous need for such studies. In most cases, researchers use simplified models, for example ignoring complexities such as peak height variability due to lack of availability of advanced tools. In Kruijver and Bright (2022) we filled this void and described a tool that can simulate DNA profiles in silico for the validation and investigation of proba-bilistic genotyping software. Contributor genotypes are simulated by randomly sampling alleles from selected allele frequencies. Some or all contributors may be related to a pedigree and the genotypes of non-founders are obtained by random gene dropping. The number of contributors per profile, and ranges for parameters such as DNA template amount and degradation parameters can be configured. Peak height variability is modeled using a lognormal distribution or a gamma distribution. Profile behavior of simulated profiles was shown to be broadly similar to laboratory generated profiles though the latter shows more variation. Simulation studies do not remove the need for experimental data. The tool has been made available as an R-package named simDNAmixtures.

35. Laurent, Fischer, Oldt et al, 2022 The identification of human remains belonging to missing persons is one of the main challenges for forensic genetics. Although other means of identification can be applied to missing person investigations, DNA is often extremely valuable to further support or refute potential associations. When reference DNA samples cannot be collected from personal items belonging to a missing person, a direct DNA identification cannot be carried out. However, identifications can be made indirectly using DNA from the missing person's relatives. The ranking of likelihood ratio (LR) values, which measure the fit of a missing person for any given pedigree, is often the first step in selecting candidates in a DNA database. Although implementing DNA kinship matching in a national environment is feasible, many challenges need to be resolved before applying this method to an international configuration. In Laurent et al. (2002) we presented an innovative and intuitive method to perform international DNA kinship matching and facilitate the comparison of DNA profiles when the ancestry is unknown or unsure and/or when di erent marker sets are used. This straightforward method, which was based on calculations performed with the DNA matching software BONAPARTE, Worldwide allele frequencies and tailored cuto log(10)LR thresholds, allows for the classification of potential candidates according to the strength of the DNA evidence and the predicted proportion of adventitious matches. This is a powerful method for streamlining the decision-making process in missing person investigations and DVI processes, especially when there are low numbers of overlapping typed STRs. Intuitive interpretation tables and a decision tree will help strengthen international data comparison for the identification

of reported missing individuals discovered outside their national borders.

38. Taylor, Buckleton, 2021 Cold case re-investigations are a common occurrence. Occasionally some of the original work was conducted up to 30 years ago using profiling systems of the early 1990s, which targeted HLA-DQA1, ApoB, D1S80 and D17S5. When contemporary work is carried out, if a suspect is identified they will be profiled in contemporary profiling kits such as GlobalFiler. It would be common to then also attempt to profile the evidence profiles in the same contemporary profiling kit. Imagine a scenario where two evidence samples, E1 and E2, had previously produced single-source profiles, but only E2 had any DNA extract left to re-profile with GlobalFiler. At the old loci E1 matched E2, and at the new loci E2 matched the suspect reference. Of interest to the investigation was whether anything could be said about the suspect being a donor of DNA to E1 even though the reference of the suspect and the profile from E1 had no loci in common, by using the information from the profile of E2.

41. Weir, 2022 We reviewed the book "Probability and Forensic Evidence: Theory, Philosophy and Applications" by Ronald Meester and Klaas Slooten (Cambridge University Press, 2021). The three introductory chapters covered probability and likelihood ratios within the forensic science context. The authors adopt an epistemic view: "which intends to assign probabilities based on one's information or knowledge." This is clearly beyond the simple statement of the axioms of probability found in introductory statistics courses but it provide a coherent framework for evidence interpretation. Chapters 4,5,6 covered forensic identification and a Bayesian framework in the legal setting. The central role of likelihood ratios continued to be stressed. As expected, and welcomed, the book has four chapters devoted to DNA evidence. Chapter 7 stressed that likelihood ratios require genetic profile (or profile constituent) probabilities, whereas all we have are observed proportions in samples from populations. The remaining four chapters returned to general evidence types. The book will likely become an essential text for graduate programs in forensic science and an essential reference for forensic laboratories.

Interpreting DNA Sequence Data.

1. Aalbers, Khan, Weir, 2023 With the introduction of next generation sequencing (NGS) technology in the forensic field, it will be of interest to assess if forensic scientists feel equipped to interpret and present DNA evidence for sequence data. Here, we describe perceptions of sixteen U.S.-based forensic scientists on statistical models, sequence data, and ethical implications for DNA evidence evaluations.

To get an in-depth understanding of the current situation, we used a qualitative research approach with a cross-sectional study design. Semi-structured interviews (N = 16) were conducted with U.S. forensic scientists working with DNA evidence. Open-ended interview questions were used to explore participants views and needs surrounding the use of statistical models and sequence data for forensic purposes. We conducted a conventional content analysis using ATLAS. ti software and employed a second coder to ensure reliability of our results. Eleven themes emerged: 1) a statistical model that maximizes the value of the evidence is preferred; 2) a high-level understanding of the statistical model used is generally sufficient; 3) transparency is key in minimizing the risk of creating black boxes; 4) training and education should be an ongoing e ort; 5) the e ectiveness of presenting results in court can be improved; 6) NGS has the potential to become revolutionary; 7)

some hesotations surrounding the use of sequence data remain; 8) there is a need for a concrete plan to alleviate barriers to the implementation of sequencing techniques; 9) ethics plays a major part in the role of a forensic scientist; 10) ethical barriers for sequence data depend on the application; 11) DNA evidence has its limitations. The results of this study give insight into the perceptions of forensic scientists regarding the use of statistical models and sequence data, providing valuable information in the move towards implementing sequencing methods for DNA evidence evaluations.

2. Aalbers, Weir, 2023a Population data have become available for sequence data to aid forensic investigations and prepare the forensic community in the move towards implementing NGS methods. This comes with a need for updated population genetic parameters estimates to allow DNA evidence evaluations using sequence data. Initial work has been done on a small sample and here we expand this work by providing estimates of population structure and relatedness for autosomal STR data generated by sequencing technologies. We also discuss the e ect of inbreeding on forensic calculations and discuss why the use of genotypic-based estimates may be preferred over allelic-based estimates.

3. Aalbers, Weir, 2023b Forensic genetics is concerned with the matching of two genetic profiles of interest. Here, we demonstrate the e ect of sequence data on match probabilities, a measure integral to DNA evidence evaluations. Results show that empirical matching proportions become less conservative the more markers we include and that this problem is exacerbated with sequence-based data compared to length-based data. While a theta-correction can be invoked to compensate for multi-locus dependencies, we caution against the combination of markers across di erent systems due to the occurrence of dependencies even for unlinked loci.

9. Buckleton, Taylor, Bright, et al, 2021 We sought to develop a rational approach to forming propositions when little information is available from the outset, as this often happens in casework. If propositions used when evaluating evidence are not exhaustive (in the context of the case), then there is a theoretical risk that an LR greater than one may be associated with a proposition in the numerator that if all meaningful propositions had been considered would in fact have a lower posterior probability after consideration of the evidence.

Ideally, all propositions should be considered. However, with multiple propositions, some terms will be larger than others and for simplification very small terms can be neglected without changing the order of magnitude of the value of the evidence (i.e. LR). Our analysis shows that mathematically a contributor's DNA can be assumed to be present under both prosecution and alternative propositions (Hp and Ha) if there is a reasonable prior probability of their DNA being present and their inclusion is supported by the profile. This is because the terms associated to these sub-propositions will dominate our LR. For example, in the absence of specific information, when considering two persons of interest (POI) as potential contributors to a mixed DNA profile we suggest the assumption of one when examining the presence of the other, after checking that both collectively explain the profile well. This represents more meaningful propositions and allows better discrimination.

Slooten and Caliebe have shown that the overall LR is the weighted average of LRs with the same number of contributors (NoC) under both propositions. The weights involve both an assessment of

the probability of the crime scene DNA profile and the probability of this NoC given the background information.

36. Liu, Bright, Taylor, et al, 2023 We describe the estimation of theta (theta) values from autosomal STR sequencing data for five metapopulations. The data were compiled from 20 publications and included 39 datasets comprising a total of 7005 samples. The estimates are suitable for use within the calculation of match probabilities in forensic casework. We also have constructed a phylogenetic tree using this data that aligns with our understanding of human evolution.

Interpreting Lineage Marker Evidence.

13. Buckleton, Hall, Bright, et al, 2023 We examined 31,011 PPY23 profiles at the subpopulation, metapopulation and world levels. Many haplotypes appear only once but a few have higher values , including a set of 23 matching profiles in Delhi, India and a set of 16 matching profiles in Burkina Faso with one additional matching American African profile. We estimate "theta" values ( ) for use in match probability calculations, following the method we used in our earlier survey of autosomal STR data. Match probability estimates using or the κ method of Brenner for a previously unseen profile are similar but di er for any profile previously seen.

33. Kruijver, Taylor, Buckleton, 2022 Frequency estimation for Y-STR haplotypes is a challenging problem because limited data are available and complex dependencies exist within the data. As a result, various statistical methods have been proposed for frequency estimation. The discrete Laplace method has been recommended in some contexts by the DNA commission of the ISFG. This method is limited to haplotypes with single integer repeat alleles only at all loci. We propose a generalisation of the method that handles duplicated loci such as DYS385 and less common alleles that are not integer repeats. The extension is implemented in an experimental R package called disclapmix2.

34. Kruijver, Taylor, Buckleton, 2023 The discrete Laplace method can be used to estimate the frequency of a Y-chromosomal STR haplotype using a random sample from the population. Two limitations of the method are the assumptions that each profile has exactly one allele at every locus and that this allele has an integer repeat number. We relax these assumptions to allow for multi-copy loci, partial repeats and null alleles. We show how the parameters to the extension of the model can be estimated by numerical optimisation using an o -the-shelf solver. Concordance with the discrete Laplace method is obtained when the data satisfy the more stringent assumptions of the original method. We also investigate the performance of the (extended) discrete Laplace method when used to assign match probabilities for haplotypes. A simulation study shows that as more loci are used, match probabilities are underestimated more severely. This is consistent with the hypothesis that the discrete Laplace method cannot model the matches that arise by being identical by descent (IBD). As the number of loci increases the fraction of matches that are IBD increases. Simulation provides support that the discrete Laplace can model those matches that arise from identity by state (IBS) only.

Interpretation of DNA Mixtures.

4. Alfieri, Coble, Conroy, et al, 2022 A new calculation module within the PopStats module of the CODIS software package, based on the underlying mathematics presented in the MixKin software package, was developed for assigning the Likelihood Ratio (LR) of DNA mixture profiles (Alfieri et al., 2022). This module uses a semi-continuous model that allows for population structure and allelic drop-out and drop-in but does not require allelic peak heights or other laboratory-specific parameters. This new implementation (named SC Mixture), like MixKin, does not specify or estimate a probability of drop-out. Instead, each contributor to a mixture has an independent drop-out rate, and the probability of the mixture profile for a specified proposition concerning the contributors is integrated over the range of possible drop-out rates. The allelic drop-in rate and the population structure parameter, theta, used by the software are specified by the user. The user can examine up to five contributors to a mixture, however, conditioning on assumed contributors and limiting the number of unknowns in both numerator and denominator hypotheses greatly improves performance. We reported results from an extensive validation study performed for ten mixtures with each of one (single source), two, three, four, or five contributors, with four combinations of drop-in rate and a population structure parameter. Each mixture was run as a complete profile or with the random removal of alleles to simulate drop-out. All 1620 combinations were evaluated with PopStats, MixKin, and LRmix and considerable consistency was found among the results with all three packages.

5. Allen, Pugh, Bright, et al DNA mixtures will have multiple donors under both the prosecution and alternate propositions when assigning a likelihood ratio for forensic DNA evidence. These donors are usually assumed to be unrelated to each other. In this paper, we make a small, preliminary examination of the potential e ect of relaxing this assumption. We consider the simple situation of a two-person mixture with no dropout and a two-person major/minor mixture with dropout of the minor contributor. We make no adjustment for subpopulation e ects. Mixtures were simulated under two assumptions: 1. that the donors were siblings 2. or that they were unrelated. Both unresolvable and major/minor mixtures were considered. We compared the likelihood ratio assuming sibship with the likelihood ratio assuming no relatedness. The LR for hypotheses assuming no relatedness is less than the LR assuming relatedness approximately 95% of the time when relatives are present in the mixture.

6. Bille, Coble, Kalafut, et al, 2022 T he National Institute of Standards and Technology has released a document entitled DNA Mixture Interpretation: A NIST Scientific Foundation Review for public comment. This has become known as the Draft NIST Foundation Review. It contains the statement: "Across these 69 data sets, there were 80 false negatives and 18 false positives reported from 110,408 possible responses (27,602 participants $\times$ two evidence items $\times$ two reference items). In the past five years, the number of participants using PGS has grown." In Bille et al. (2022) we examined a set of proficiency test results to determine if these NIST statements could be justified. The summary reports for each relevant forensic biology test (Forensic Biology, Semen, and Mixture) in the years 2018-2021 were reviewed. Data were also provided to us by CTS upon our request. None of the false positives or negatives could be attributed to the mixture interpretation strategy and certainly not to the use of PGS.

19. Hicks, Kerr, Pugh, et al, 2021 In casework, laboratories may be asked to compare DNA mixtures to multiple persons of interest (POI). Guidelines on forensic DNA mixture interpretation recommend that analysts consider several pairs of propositions; however, it is unclear if several likelihood ratios (LRs) per person should be reported or not. The propositions communicated to the court should not depend on the value of the LR. As such, we suggest that the propositions should be functionally exhaustive. This implies that all propositions with a non-zero prior probability need to be considered, at least initially. Those that have a significant posterior probability need to be used in the final evaluation. Using standard probability theory we combined various propositions so that collectively they are exhaustive. This involves a prior probability that the sub-proposition is true, given that the primary proposition is true. Imagine a case in which there are two possible donors: i and j. They focused their analysis first on donor i so that the primary proposition is that i is one of the sources of the DNA. In this example, given that i is a donor, we would further consider that j is either a donor or not. In practice, the prior weights for these subpropositions may be difficult to assign. However, the LR is often linearly related to these priors and its behaviour is predictable. They also believed that these priors are unavoidable and are hidden in alternative methods. They termed the likelihood ratio formed from these context-exhaustive propositions LRi/i. LRi/i is trialed in a set of two- and three-person mixtures. For two-person mixtures, LRi/i is often well approximated by LRij/ja, where the subscript ij describes the proposition that i and j are the donors and ja describes the proposition that j and an alternate, unknown individual (a), who is unrelated to both i and j, are the donors. For three-person mixtures, LRi/i is often well approximated by LRijk/jka where the subscript ijk describes the proposition that i, j, and k are the donors and jka describes the proposition that j, k, and an unknown, unrelated (to i, j, and k) individual (a) are the donors. In our simulations, LRij/ja had fewer inclusionary LRs for noncontributors than the unconditioned LR (LRia/aa).

21. Kalafut, Bright, Taylor, et al, 2022 The interpretation of mixtures containing related individuals can be difficult due to allele sharing between the contributors. Challenges include the assignment of the number of contributors (NoC) to the mixture with the under assignment of NoC resulting in false exclusions of true donors. Non-donating relatives of the true contributors to mixtures of close relatives can result in likelihood ratios supporting their adventitious inclusion within the mixture. We examined the e ect of non-donor likelihood ratios on mixtures of first order relatives. Mixtures of full siblings and parent-child were created by mixing the DNA from known family members in vitro, or by in silico simulation. Mixtures were interpreted using the probabilistic genotyping software STRmix (TM) and likelihood ratios were assigned for the true donors and non-donors who were either further relatives of the true donors or unrelated to the true donors. The two donor balanced mixtures deconvoluted straightforwardly when analysed as NoC (Number of Contributors) = 2 giving approximately the experimental design 1:1 ratio. When analysed as NoC = 3 a very large number of non-donor genotypes produced LRs close to 1 including many instances of adventitious support. The in vitro three donor balanced mixtures proved difficult to assign as NoC = 3 by a blind examination of the profile. It is likely that many of these would be misassigned as NoC = 2. The analysis of the in vitro and in silico mixtures assuming NoC = 3 with no use of a conditioning profile or with the use of a conditioning profile but without informed priors on the mixture proportions (Mx priors) was ine ective. If the profile can be assigned as NoC = 3 then assignment of the Mx priors is straightforward. This analysis gave no false exclusions.

Adventitious support did happen for relatives with high allele sharing. Adventitious support was not observed for any unrelated non-donors. The analysis of the three-person mixtures as NoC = 2 produced many false exclusions and fewer instances of adventitious support. The three donor unbalanced mixtures could all be assigned as NoC= 3. Analysis without Mx priors produced an alternate genotype explanation.

22. Kalafut, Pugh, Gill, et al, 2022 Semaan et al. (J Forensic Res, 2020, 11, 453) discussed a mock case "where eight di erent individuals [P1 through P8] could not be excluded in a mixed DNA analysis. Even though horizontal ellipsis expert DNA mixture analysis software was used." Two of these are the true donors. The LRs reported are incorrect due to the incorrect entry of propositions into LRmix Studio. This forced the software to account for most of the alleles as drop-in, resulting in LRs 60-70 orders of magnitude larger than expected. P1, P2, P4, P5, and P8 can be manually excluded using peak heights. This has relevance when using LRmix which does not use peak heights. We extended the work using the same two reference genotypes who were the true contributors as Semaan et al. (J Forensic Res, 2020, 11, 453). We simulated three two-donor mixtures with peak heights using these two genotypes and analyze using STRmix (TM). For the simulated 1:1 mixture, one of the non-donor's LRs supported him being a contributor when no conditioning was used. When considered in combination with any other potential donors (i.e., with conditioning), this non-donor was correctly eliminated. For the 3:1 mixture, all results correctly supported that the non-donors were not contributors. The low-template 4:1 mixture LRs with no conditioning showed support for all eight profiles as donors. However, the results from pair-wise conditioning showed that only the two ground truth donors had LRs supporting that they were contributors to the mixture. We recommend the use of peak heights and conditioning profiles, as this allows better sensitivity and specificity even when the persons share many alleles.

24. Kelly, Coble, Kruijver, et al, 2022 Relatives tend to have more DNA in common than unrelated people. The closer the biological relationship, the higher the chance of alleles being identical by descent between the individuals. Therefore, when considering a mixed DNA profile, close relatives of the true contributor may not always be excluded as a possible contributor to a mixture due to allele sharing. In these situations, it might be more appropriate under the alternate proposition to consider that the DNA could have originated from a relative of the person of interest rather than an unrelated individual. The probabilistic genotyping software STRmix (TM) automatically provides LRs considering close biological relatives as alternate sources of the DNA. In Kelley et al. (2002), we investigated the support for siblings of the true contributor to a mixture (who are not present in the mixture themselves). We interpreted the mixtures and assign LRs using STRmix (TM) and investigated whether the resulting LRs could be used to indicate whether the true contributor could be a sibling of the POI. Most siblings will have one or more alleles that are not observed in the mixture profile. Support for siblings to have contributed can only occur when allelic dropout is a possibility at the loci where the siblings have alleles that are not observed in the profile. In these data, that was only observed in components with assigned template of 588 rfu or less.

25. Kelly, Bright, Kruijver et al, 2022 The assignment of the number of contributors (N) to a forensic DNA profile is undertaken as part of the interpretation process. There is no requirement

for N to be the same for both propositions within the likelihood ratio framework. ISFG recommendations on mixture interpretation suggest that there may be times where prosecution and defense both specify their own, di erent N. In Kelly et al. (2022) we investigated how this a ects the likelihood ratio (LR) for 100 mixed DNA profiles. We showed that the addition of a superfluous unknown contributor within a proposition tends to increase the likelihood because the profile can always be better described with more contributors but the priors on the additional parameters and an extra genotype can ultimately reduce the likelihood ratio. Additionally, we found that choosing improbable values for N given di erent propositions can lead to misleading LRs. Specifically, we found that LRs can both increase and decrease compared to the value obtained using the ground truth N. Choosing a single N which maximizes the probability of the observations for each party tends to approximate an exhaustive stratified LR that takes into account di erent Ns with di erent prior probabilities.

26. Kruijver, Taylor, Bright, 2021 Forensic DNA profiling is used in various circumstances to evaluate support for two competing propositions with the assignment of a likelihood ratio. Many software implementations exist that tackle a range of inference problems spanning identification and relationship testing. We proposed a flexible likelihood ratio framework that caters to inference problems in forensic genetics. The framework allows for investigation of the degree of support for the contribution of multiple persons to multiple samples allowing for persons to be related according to a pedigree, including inbred relationships. We explained how a number of routine as well as more complex problems can be treated within this framework.

29. Kruijver, Curran, 2022 The maximum allele count (MAC) across loci and the total allele count (TAC) are often used to gauge the number of contributors to a DNA mixture. Computational strategies that predict the total number of alleles in a mixture arising from a certain number of contributors of a given population have been developed. Previous work considered the restricted case where all of the contributors to a mixture are unrelated. In KriWe relax this assumption and allow mixture contributors to be related according to a pedigree. We introduce an efficient computational strategy. This strategy based on first determining a probability distribution on the number of independent alleles per locus, and then conditioning on this distribution to compute a distribution of the number of distinct alleles per locus. The distribution of the number of independent alleles per locus is obtained by leveraging the Identical by Descent (IBD) pattern distribution which can be computed from the pedigree. We explain how allelic dropout and a subpopulation correction can be accounted for in the calculations.

31. Kruijver, Bright, 2023 When evaluating support for the contribution of a person of interest (POI) to a mixed DNA sample, it is generally assumed that the mixture contributors are unrelated to the POI and to each other. In practice, there may be situations where this assumption is violated, for instance if two mixture contributors are siblings. The e ect on the likelihood ratio of (in)correctly assuming relatedness between mixture contributors has previously been investigated using simulation studies based on simplified models ignoring peak heights. We revisit this problem using a simulation study that applies peak height models both in the simulation and mixture interpretation part of the study. Specifically, we sample sets of mixtures comprising both related and unrelated contributors and evaluate support for the contribution of the mixture donors as well

as unrelated persons with and without incorporating an assumption of relatedness. The results show, consistent with earlier studies, that including a correct assumption of relatedness increases the capacity of the probabilistic genotyping system to distinguish between mixture donors and unrelated persons. Any e ect of the relatedness is found to depend strongly on the mixture ratio. We further show that the results do not change materially when a sub-population correction is applied. Finally, we suggest and discuss a likelihood ratio approach that considers relatedness between mixture contributors using a prior probability.

32. Kruijver, Kelly, Taylor, et al, 2023 Evidential value of DNA mixtures is typically expressed by a likelihood ratio. However, selecting appropriate propositions can be contentious, because assumptions may need to be made around, for example, the contribution of a complainant's profile, or relatedness between contributors. A choice made one way or another disregards any uncertainty that may be present about such an assumption. To address this, a complex proposition that considers multiple sub-propositions with di erent assumptions may be more appropriate. While the use of complex propositions has been advocated in the literature, the uptake in casework has been limited. We provide a mathematical framework for evaluating DNA evidence given complex propositions and discuss its implementation in the DBLRTM software. The software simultaneously handles multiple mixed samples, reference profiles and relationships as described by a pedigree, which unlocks a variety of applications. We provide several examples to illustrate how complex propositions can efficiently evaluate DNA evidence. The addition of this feature to DBLRTM provides a tool to approach the long-accepted, but often impractical suggestion that propositions should be exhaustive within a case context.

42. Wivell, Kell,. Kokosszka, et al, 2023 Simple propositions are defined as those with one POI and the remaining contributors unknown under H-p and all unknown contributors under H-a. Conditional propositions are defined as those with one POI, one or more assumed contributors, and the remaining contributors (if any) unknown under H-p, and the assumed contributor(s) and N unknown contributors under H-a. In this study, compound propositions are those with multiple POI and the remaining contributors unknown under H-p and all unknown contributors under Ha. We study the performance of these three proposition sets on thirty-two samples (two laboratories x four NOCs x four mixtures) consisting of four mixtures, each with N = 2, N = 3, N = 4, and N = 5 contributors using the probabilistic genotyping software, STRmix (TM). In this study, it was found that conditional propositions have a much higher ability to di erentiate true from false donors than simple propositions. Compound propositions can misstate the weight of evidence given the propositions strongly in either direction.

Probabilistic Genotyping

7. Bille, Coble, Bright, 2022 Previous studies examining whether splitting the DNA extract for replicate amplification versus maximizing the template available for a 'one-shot' amplification either examined the benefits of using replicates (without a comparison to a single amplification), or used semi-continuous probabilistic software that ignores peak height information. In this study, we use a fully continuous probabilistic genotyping software to compare the e ectiveness of amplifying a single sample compared to splitting the sample and conducting a joint analysis of replicate amplifications. We show that the one-shot approach is marginally better than splitting the DNA extract across

a range of contributor numbers and template amounts. Where there is unexpected peak height variability or drop-in within the profile not modelled during interpretation, a replicate approach may be better.

12. Buckleton, Susik, Curran, et al, 2023 There is interest in comparing the output, principally the likelihood ratio, from the two probabilistic genotyping software EuroForMix (EFM) and STRmix (TM). Many of these comparison studies are descriptive and make little or no e ort to diagnose the cause of di erence. There are fundamental di erences between EFM and STRmix (TM) that are causative of the largest set of likelihood ratio di erences. This set of di erences is for false donors where there are many instances of LRs just above or below 1 for EFM that give much lower LRs in STRmix (TM). This is caused by the separate estimation of parameters such as allele height variance and mixture proportion using MLE under H-p and H-a for EFM. This can result in very di erent estimations of these parameters under H-p and H-a. It results in a departure from calibration for EFM in the region of LRs just above and below 1.

14. Cheng, Lin, Moreno, et al, 2021 We described an adaption of Bright et al." work modeling peak height variability in CE-DNA profiles to the modeling of allelic aSTR (autosomal short tandem repeats) read counts from NGS-DNA profiles, specifically for profiles generated from the ForenSeq (TM) DNA Signature Prep Kit, DNA Primer Mix B. Bright et al.'s model consists of three key components within the estimation of total allelic product-template, locus-specific amplification efficiencies, and degradation. In this work, we investigated the two mass parameters-template and locus-specific amplification efficiencies-and used MLE (maximum likelihood estimation) and MCMC (Markov chain Monte Carlo) methods to obtain point estimates to calculate the total allelic product. The expected read counts for alleles were then calculated after proportioning some of the expected stutter product from the total allelic product. Due to preferential amplicon selection introduced by the sample purification beads, degradation is difficult to model from the aSTR outputs alone. Improved modeling of the locus-specific amplification efficiencies may mask the e ects of degradation. Whilst this model could be improved by introducing locus specific variances in addition to locus specific priors, our results demonstrate the suitability of adapting Bright et al.'s allele peak height model for NGS-DNA profiles. This model could be incorporated into continuous probabilistic interpretation approaches for mixed DNA profiles.

15. Cheng, Bleka, Gill, et al, 2021 Likelihood ratios (LR) di erences between the probabilistic genotyping software EuroForMix and STRmix (TM) were examined. After considering di erences in the allele probabilities, the LRs from both software for an unambiguous single-source profile were identical (to four significant figures). LRs from both software for an unambiguous single-source profile with alleles previously unseen in the allele frequency database (rare alleles) were the same (to three significant figures) for theta was 0.01. Due to di erences in the minimum allele frequencies, the LRs di ered by three orders of magnitude when theta was 0. For both software, the LRs for a single-source dilution series decreased as the input amount decreased. The LRs from both software were within an order of magnitude for known contributors. The largest di erence was where the target input amount was 0.0156 ng: The LREuroForMix was 2.1×10^{25}) and the LRSTRmix was 8.0×10^{24}. Both software show similar LR behavior with respect to mixture ratio. For two person mixtures the LR increases for both the major and the minor as the ratio moves away from 1:1.

The LR for the major stabilizes at about 3:1 whereas the LR for the minor reaches its maximum at about 3:1 and then declines. Greater di erences in LR were observed between EuroForMix and STRmix (TM) for mixtures. One-hundred and twenty-nine mixtures from the PROVEDIt dataset were compared. LRs for 84% of the comparisons for known contributors without rare alleles were within two orders of magnitude. Five divergent results were investigated, and a manual intervention approach was applied where appropriate.

16. Cheng, Bright, Kelly, et al, 2021 Cheng: We describe the developmental validation of the probabilistic genotyping software - STRmixTM NGS - developed for the interpretation of forensic DNA profiles containing autosomal STRs generated using next generation sequencing (NGS) also known as massively parallel sequencing (MPS) technologies. Developmental validation was carried out in accordance with the Scientific Working Group on DNA Analysis Methods (SWGDAM) Guidelines for the Validation of Probabilistic Genotyping Systems and the International Society for Forensic Genetics (ISFG) recommendations and included sensitivity and specificity testing, accuracy, precision, and the interpretation of case-types samples. The results of developmental validation demonstrate the appropriateness of the software for the interpretation of profiles developed using NGS technology.

17. Gill, Benschop, Buckleton, et al, 2021 Probabilistic genotyping has become widespread. EuroForMix and DNAStatistX are both based upon maximum likelihood estimation using a gamma model, whereas STRmix (TM) is a Bayesian approach that specifies prior distributions on the unknown model parameters. A general overview is provided of the historical development of probabilistic genotyping. Some general principles of interpretation are described, including: the application to investigative vs. evaluative reporting; detection of contamination events; inter and intra laboratory studies; numbers of contributors; proposition setting and validation of software and its performance. This is followed by details of the evolution, utility, practice and adoption of the software discussed.

30. Kruijver, Kelly, Bright, et al, 2023 It is common practice to evaluate DNA profiling evidence with likelihood ratios using allele frequency estimates from a relevant population. When multiple populations may be relevant, a choice has to be made. For two-person mixtures without dropout, it has been reported that conservative estimates can be obtained by using the Person of Interest's population with a theta value of 3%. More accurate estimates can be obtained by explicitly modelling di erent populations. One option is to present a minimum likelihood ratio across populations; another is to present a stratified likelihood ratio that incorporates a weighted average of likelihoods across multiple populations. For high template single source profiles, any di erence between the methods is immaterial as far as conclusions are concerned. We revisit this issue in the context of potentially low-level and mixed samples where the contributors may originate from di erent populations and study likelihood ratio behaviour. We first present a method for evaluating DNA profiling evidence using probabilistic genotyping when the contributors may originate from di erent ethnic groups. In this method, likelihoods are weighted across a prior distribution that assigns sample donors to ethnic groups. The prior distribution can be constrained such that all sample donors are from the same ethnic group, or all permutations can be considered. A simulation study is used to determine the e ect of either assumption on the likelihood ratio. The likelihood ratios are also compared to the minimum likelihood ratio across populations. We demonstrate

that the common practise of taking a minimum likelihood ratio across populations is not always conservative when F_{ST}. Population stratification methods may also be non-conservative in some cases. When $F_{ST} > 0$ is used in the likelihood ratio calculations, as is recommended, all compared approaches become conservative on average to varying degrees.

37. Taylor, Bright, Scandretti et al, 2021 Slooten described a method of targeting major contributors in mixed DNA profiles and comparing them to individuals on a DNA database. The method worked by taking incrementally more peak information from the profile (based on the peak contribution), and using a semi-continuous model, calculating likelihood ratios for the comparison to database individuals. We described the performance of this ?top down approach? to profile interpretation within probabilistic genotyping software employing a fully continuous model. They interpreted both complex constructed profiles where ground truth is known and casework profiles from non-suspect crimes. The interpretation of constructed four- and five person mixtures demonstrated good discrimination power between contributors and non-contributors to the mixtures. Not all known contributors linked, and this is expected, particularly for minor contributors of DNA to the profile, or when the DNA from contributors was in relatively equal contributions. This finding was also reported by Slooten for the semi-continuous application of the approach. The maximum observed LR was shown to not exceed the LR obtained after a standard interpretation approach outside of that expected due to Monte Carlo variation. The interpretation of 91 complex profiles from no-suspect casework demonstrated that approximately 75% of profiles returned a link to someone on a database of known individuals. With a yearly average of 110 no-suspect cases that fall into this too-complex category at Forensic Science SA, the top down analysis, if applied to all such profiles, would represent an increase of 83 links per year of investigative information that could be provided to investigators.

39. Taylor, Buckleton, 2023 Standard processing of electrophoretic data within a forensic DNA laboratory is for one (or two) analysts to designate peaks as either artefactual or non-artefactual in a process commonly referred to as profile 'reading'. Recently, FaSTRTM DNA has been developed to use artificial neural networks to automatically classify fluorescence within an electropherogram as baseline, allele, stutter or pull-up. These classifications are based on probabilities assigned to each timepoint (scan) within the electropherogram. Instead of using the probabilities to assign fluorescence into a category they can be used directly in the profile analysis. This has a number of advantages; increased objectivity in DNA profile processing, the removal for the need for analysts to read profiles, the removal for the need of an analytical threshold. Models within STRmixTM were extended to incorporate the peak label probabilities assigned by FaSTRTM DNA. The performance of the model extensions was tested on a DNA mixture dataset, comprising 2-4 person samples. This dataset was processed in a 'standard' manner using an analytical threshold of 50rfu, analyst peak designations and STRmixTM V2.9 models. The same dataset was then processed in an automated manner using no analytical threshold, no analysts reading the profile and using the STRmixTM models extended to incorporate peak label probabilities. Both datasets were compared to the known DNA donors and a set of non-donors. The result between the two processes was a very close performance, but with a large efficiency gain in the 0rfu process. Utilising peak label probabilities opens up the possibility for a range of workflow process efficiency gains, but beyond this allows full use of all data within an electropherogram.

Other Topics

18. Gra elman, Weir, 2022 The Hardy-Weinberg law was shown (Grafeelman and Weir, 2022) to be transitive in the sense that a multi-allelic polymorphism that is in equilibrium will retain its equilibrium status if any allele together with its corresponding genotypes is deleted from the population. Similarly, the transitivity principle also applies if alleles are joined, which leads to the summation of allele frequencies and their corresponding genotype frequencies. These basic polymorphism properties are intuitive, but they had apparently not been formalized or investigated. This article provided a straightforward proof of the transitivity principle, and its usefulness in genetic data analysis was explored, using high-quality autosomal microsatellite databases from the US National Institute of Standards and Technology. We addressed the reduction of multi-allelic polymorphisms to variants with fewer alleles, two in the limit. Equilibrium test results obtained with the original and reduced polymorphisms are generally observed to be coherent, in particular when results obtained with length-based and sequence-based microsatellites are compared. We exploited the transitivity principle in order to identify disequilibrium-related alleles, and showed its usefulness for detecting population substructure and genotyping problems that relate to null alleles and allele imbalance.

40. Wasser, Wolock, Kuhner, et al, 2022 Transnational ivory traffickers continue to smuggle large shipments of elephant ivory out of Africa, yet prosecutions and convictions remain few. We identified trafficking networks based on genetic matching of tusks from the same individual or close relatives found in separate shipments. Analyses were drawn from 4320 tusks sampled from 49 large ivory seizures totaling 111 metric tons, shipped out of East and West Africa between 1995 and 2019. Network analyses revealed a repeating pattern wherein large numbers of tusks from close relatives are found in separate seizures that were containerized in, and transited through, common African ports. The consistency of these repeating patterns suggested that the same traffickers are exporting dozens of shipments, with considerable connectivity between traffickers operating in di erent ports. These tools provide a framework to combine evidence from multiple investigations, strengthen prosecutions and support indictment and prosecution of transnational ivory traffickers for the totality of their crimes.

Opportunities for Professional Development

Nothing to report.

Dissemination of Results

The primary means of dissemination of results has been the publication of peer-reviewed papers, as listed below. In addition we made presentations at scientific conferences and taught courses, as now listed:

Scientific Presentations / Teaching

- Aalbers S Taught PHG302 “Forensic Genetics” undergraduate course at the University of Washington in the Spring Quarter of 2023 (April-June).

- Aalbers S, Weir BS. Taught "Forensic Genetics" short course at Summer Institute in Statistical Genetics, July 2021.
- Aalbers S, Weir BS. Led "Workshop on Statistical Genetics" for the International Society for Forensic Genetics, July 2021.
- Aalbers S, Weir BS. "Match Probabilities for NGS Data of Forensic Autosomal STR Markers." NIJ Forensic Science Graduate Research Program, September 2022.
- Aalbers S, Weir BS. "Match Probabilities for NGS Data of Forensic Autosomal STR Markers." ISHI, November 2022. during the first week of November 2022.
- Bright JA and Coble . "Practical Application of the Likelihood Ratio." International Symposium On Human Identification. 12 September 2021.
- Bright JA, Coble M. Workshop "Applying a Casework Assessment and Interpretation Approach to Probabilistic Genotyping Results", ISHI, November 2022.
- Bright JA, Cheng K. "Probabilistic interpretation of next generation sequencing data." Asian Forensic Sciences Network. Virtual Conference. 15 October 2021.
- Buckleton J, 2021. NIJ/NIST Expert Working Group on Human Factors in Forensic DNA Interpretation. January 21st 2021
- Buckleton J. 2021. "A comparison of EuroForMix and STRmix (TM) to Subgroup Analysis and Methods of the ENFSI DNA Working Group meeting 28th September 2021.
- Buckleton J. 2022. "Underperformance of the HPD MCMC component". New York State Forensic Science Commission DNA sub-committee. 6th February 2021.
- Buckleton J. 2023. "Developments in STRmix." 4th Annual Northeast STRmix User's Group Connecticut. 19th October, 2023.
- Buckleton J. 2023. "The continuing need for human expertise in forensic evidence interpretation." Adelaide Medal Lecture IAFS, 21st November 2023
- Curran J, Kruijver M. "The number of distinct alleles in mixed DNA profiles when contributors are related" New Zealand Statistical Conference, November 2022.
- Curran J, Kruijver M. "The number of distinct alleles in mixed DNA profiles when contributors are related" Australasian Applied Statistics, December 2022.
- Weir BS. Taught PHG302 "Forensic Genetics" undergraduate course at the University of Washington in the Spring Quarter of 2021 (April-June).
- Cheng L, Bleka U, Gill P, Curran J, Bright JA, aylor D, Buckleton J. 2022. Essentially, all models are wrong, but some are useful. A comparison of likelihood ratios obtained from EuroForMix and STRmix. QIAGEN Young Investigator Seminar Series at AAFS Annual Meeting, February.

- Cheng K, Bright JA, Curran J, Buckleton J. 2022. Results from a probabilistic genotyping software for the continuous interpretation of NGS aSTR mixtures. AAFS Annual Meeting, February.

- Cheng L, Bleka U, Gill P, Curran J, Bright JA, Taylor D, Buckleton J. 2022. Essentially, all models are wrong, but some are useful. A comparison of likelihood ratios obtained from EuroForMix and STRmix. QIAGEN Young Investigator Seminar Series at AAFS Annual Meeting, February.

- Cheng K, Bright JA, Curran J, Buckleton J. 2022. Results from a probabilistic genotyping software for the continuous interpretation of NGS aSTR mixtures. AAFS Annual Meeting, February.

- Weir BS. 2021 Taught PHG302 "Forensic Genetics" undergraduate course at the University of Washington in the Spring Quarter (April-June).

- Weir BS, Aalbers S. 2021, 2022, 2023. Short Course "Forensic Genetics", Summer Institute in Statistical Genetics, July.

- Weir BS, Buckleton J. 2022. Interpretation of Y-STR Evidence. NIJ Forensic Science R&D Symposium, February.

Publications Acknowledging NIJ award 2020-DQ-BX-0022

1. Aalbers SE, Khan AT, Weir BS. 2023. Perceptions of forensic scientists on statistical models, sequence data, and ethical implications for DNA evidence evaluations: A qualitative assessment. Forensic Science International: Synergy 6:100335.

2. Aalbers SK, Weir BS. 2023a. Sequence-based population structure, relatedness, and inbreeding estimates for forensic autosomal STR markers Forensic Science International: Genetics (accepted)

3. Aalbers SK, Weir BS. 2023b. The impact of DNA sequence data on match probabilities for di erent forensic marker systems. Forensic Science International: Genetics (submitted)

4. Alfieri J, Coble MD, Conroy C, Dahl A, Hares DR, Weir BS, Wolock C, Zhao E, Kingston H, Zolandz TW. 2022. A new implementation of a semi-continuous method for DNA mixture interpretatation. Forensic Science International: Reports 6:100281, https://doi.org.10.1016/j.fsir.2022.100281.

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15. Cheng K, Bleka O, Gill P, Curran J, Bright JA, Taylor D, Buckleton J. 2021. A comparison of likelihood ratios obtained from EuroForMix and STRmix (TM). Journal of Forensic Sciences 66:2138-2155.

16. Cheng K, Bright JA, Kelly H, Liu YY, Lin MH, Kuijver M, Taylor D, Buckleton J. 2023. Developmental validation of STRmix NGS, a probabilistic genotyping tool for the interpretation of autosomal STRs from forensic profiles generated using NGS. Forensic Science International:Genetics 62: DOI10.1016/j.fsigen.2022.102804

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Participants

Item	Description
Name	Bruce Weir
Project Role	Principal Investigator
Average Per Year Person Month Worked	5.4
Contribution	Directed and conducted research in the project. Published papers, gave presentations.
Funding Support	Not applicable.
Foreign Collaboration	Yes
Foreign Collaboration Country	New Zealand
Traveled to Foreign Country	Yes
Duration in Foreign Country	Two weeks

Item	Description
Name	John Buckleton
Project Role	co-Principal Investigator
Average Per Year Person Month Worked	2.4
Contribution	co-Directed and conducted research in the project. Published papers, gave presentationx.
Funding Support	Not applicable.
Foreign Collaboration	Yes
Foreign Collaboration Country	New Zealand
Traveled to Foreign Country	Yes
Duration in Foreign Country	New Zealand resident

Item	Description
Name	Jo-Anne Bright
Project Role	Investigator
Average Per Year Person Month Worked	1.2
Contribution	Conducted research in the project. Published three papers, gave presentations.
Funding Support	Not applicable.
Foreign Collaboration	Yes
Foreign Collaboration Country	New Zealand
Traveled to Foreign Country	Yes
Duration in Foreign Country	New Zealand resident

Item	Description
Name	James Curran
Project Role	Investigator
Average Per Year Person Month Worked	1.2
Contribution	Conducted research in the project. Published papers, gave presentations.
Funding Support	Not applicable.
Foreign Collaboration	Yes
Foreign Collaboration Country	New Zealand
Traveled to Foreign Country	Yes
Duration in Foreign Country	New Zealand resident

Other Partner Organizations

- Institute of Environmental Science and Research, New Zealand.
- University of Auckland, New Zealand.

Other Collaborators

- Sanne Aalbers, Biostatistics, University of Washington.
- Kevin Cheng, ESR New Zealand.
- Hannah Kelly, ESR New Zealand.
- Duncan Taylor, Forensic Science, South Australia.

Impact on Forensic Sciences

Our methods allow the quantification of DNA profile evidence in forensic science. They therefore support a central method for human identification, allowing the identification or exclusion of contributors to an item of evidence and the identification of human remains. Without quantification of the type following our methods, it is difficult to assign meaning to a DNA match between a suspect and a crime-scene stain, for example.

Our activities impact forensic science through the advice we o er on these bodies:

- Curran JM. American Statistical Association: Member of Committee on Forensic Statistics.
- Weir BS. Lineage Marker Committee, SWGDAM.
- Weir BS. American Statistical Association: Member of Committee on Forensic Statistics.
- Weir BS. CODIS consultant.
- Weir BS. Chair, DNA Subcommittee, New York Commission on Forensic Science.

Impact on Other Disciplines

Nothing to report.

Impact on Human Resource Development

Nothing to report.

Impact on Infrastructure

Nothing to report.

Impact on Technology Transfer

Nothing to report.

Impact on Society

Our work helps assure society that the guilty are identified and the wrongly accused or convicted are exonerated.

Dollar Amount Spent in Foreign Countries

$261,378.68

Changes in Approach

Nothing to report.

Delays

Nothing to report.

Changes in Expenditure

Nothing to report.

Changes in Human Subjects, Vertebrate Animals, Biohazards

Nothing to report.

Change of Primary Performance Site

Nothing to report.