DECONVOLUTION OF DNA MIXTURES USING REPLICATE SAMPLING AND TRUEALLELE® MIXTURE INTERPRETATION

by

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Dedication

Para mi madre. Tu fortaleza, consejo, apoyo, y amor incondicional han hecho posible este logro personal. No hay suficientes palabras que puedan transmitir plenamente lo agradecida que estoy por todos tus sacrificios al darme una vida brillante, floreciente, y exitosa. Gracias mami, te quiero muchisimo. (To my mother. Your strength, advice, support, and unconditional love have made this personal achievement possible. There are no words that can fully convey how grateful I am for all your sacrifices in giving me a bright, flourishing, and successful life. Thank you, mom, I love you so much.)

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List of Definitions/Acronyms

LT-DNA	Low template DNA
LR	Likelihood ratio
LF	Likelihood function
JLF	Joint likelihood function
logLR	$\log_{10}$(LR value) – additive value; standard weight of measure
DNA	Deoxyribonucleic acid
STR	Short tandem repeat
PG	Probabilistic genotyping
PCR	Polymerase chain reaction
bp	Base pair of DNA
EPG	Electropherogram
RFU	Relative fluorescence unit
PHR	Peak height ratio
MW	Mixture weight
MCMC	Markov chain Monte Carlo
NIST	National Institute of Standards and Technology

Abstract

Analysis of DNA mixture evidence does not always yield distinct profiles. This process is further complicated with low template DNA (LT-DNA) samples often seen in forensic casework. Traditional qualitative methods use thresholds to distinguish allele peaks from stutter peaks, noise, etc. resulting in data being omitted during analysis. In cases where LT-DNA is present, low peaks that could potentially be attributed to low contributor profiles may not be called due to these instituted thresholds. The probabilistic genotyping computer software program created by Cybergenetics (Pittsburgh, PA), TrueAllele® Casework, considers all data and performs quantitative analysis using probability to represent uncertainty. It objectively forms likelihood ratios (LR) that compare the probabilities of an evidentiary genotype with a suspect genotype relative to a reference population. A joint likelihood function (JLF) takes two or more independent sets of data and compares them jointly as opposed to a single event. The JLF can elicit more identification information proving useful in DNA mixture analysis. This project used TrueAllele® Casework to perform DNA mixture analysis on two sets of previously published mixture data provided by Cybergenetics. The first set comprised 40 two contributor mixture samples and the second set included four sets of 10 randomized mixtures with two, three, four, and five contributors, respectively. The selected samples were interpreted singly and jointly in three variable groups: mixture weight, template concentration, and complex mixtures. The differences between the match logLRs of the single and joint analyses were calculated and an information gain was seen in all three groups when the samples were analyzed jointly. Changing DNA collection and amplification procedures for touch and DNA mixture evidence samples will increase the amount of data available for DNA mixture analysis using probabilistic genotyping. These procedures can be modified so that multiple swabs and replicate amplifications produce

more data that TrueAllele can analyze using the JLF. Jointly analyzing each independent evidence data can lead to higher match statistics which will ultimately help in the identification of those who commit crimes.

Keywords: DNA mixtures, low template DNA, probabilistic genotyping, likelihood ratios, joint likelihood function

Introduction

Overview

Forensic casework is frequently composed of deoxyribonucleic acid (DNA) mixture evidence containing two or more contributors. Extracting genotypes from the evidence data and comparing them to suspect profiles in hopes of obtaining a DNA profile match can be a complicated process as the number of contributors in a mixture increase. The challenge is even greater when the evidence DNA is degraded and/or present in low quantities. Traditional mixture interpretation methods of DNA short tandem repeat (STR) analysis use a qualitative approach where thresholds are implemented to simplify the data into "all or nothing" allele events (Perlin & Sinelnikov, 2009). Pertinent quantitative data and its inherent patterns are discarded by these thresholds. Preservation of this invaluable and informative data can reduce the number of false inclusions, false exclusions, and inconclusive results.

For over a decade, probabilistic genotyping (PG), or computer interpretation, has gained favor and utility in crime laboratories. Numerous validation studies have demonstrated how mathematical modeling of quantitative data in its entirety is more productive, efficacious, and objective (Perlin et al. 2009, 2014, 2015). TrueAllele® Casework is an expert system that is already implemented in many forensic labs. It uses hierarchical Bayesian probability modeling to explain quantitative data. When there is uncertainty in the data as is usually the case with mixtures or LT-DNA, this uncertainty is represented in the probability distribution for each possible allele pair at all loci for each contributor. This probability distribution is the inferred evidence genotype, which is subsequently compared to suspect genotypes to determine whether a match exists. Computers provide the capacity to interpret complex data, infer evidence genotypes, and calculate match statistics without discarding any identification information.

These tasks can also be performed simultaneously on more than one set of data (Ballantyne et al., 2013; Perlin et al., 2011).

A joint likelihood function (JLF) takes two or more independent sets of data and compares them jointly as opposed to a single event. The additional data used in the JLF constrains the probability distribution for each locus. This in turn, results in higher probabilities assigned to the allele pairs that provide a good explanation for each set of data. When replicate amplifications are performed for an evidence item, the STR profiles in each replicate can be analyzed together with the JLF (Ballantyne et al., 2013). Replicate amplifications are typically performed on LT-DNA samples to compensate for the increased stochastic effects seen during PCR which result in allele drop-out, indistinguishable stutter peaks, etc. To account for the replicate data using manual interpretation, a consensus profile is formed from the data peaks that are reproduced in two out of three runs. If an allele is seen in only one of the replicates but dropped out in the other two, it is not considered for the consensus profile (Butler, 2015, Chapter 7). Like the traditional interpretation methods, the consensus approach discards information. PG methods use all the data and the JLF assigns a probability to those replicates that would otherwise be discarded. Qualitative approaches can be overwhelmed with additional data especially when high uncertainty is present. Replicate amplifications and the JLF can potentially provide more identification information leading to higher match statistics compared to match statistics from a single amplification. This can prove useful in DNA mixture analysis.

Importance

Increased scrutiny and high public expectations of forensic DNA evidence calls for accurate, reliable, and efficient DNA mixture interpretation methods. These methods must result

in more probative and reliable identification information that can exculpate the innocent and incriminate those responsible of committing crimes.

This project utilized TrueAllele® Casework to perform DNA mixture analysis and obtain match statistics in the form of logLR values. This analysis was performed on two sets of previously published data, varying in mixture weight and DNA quantity, that were provided by Cybergenetics. The first set comprised 40 two contributor mixture samples and the second set included two, three, four, and five contributor mixtures. The samples were interpreted singly and jointly in two ways: in duplicate and triplicate. Mixture weight and template concentration were two data variables that were considered independently. The data for each variable was analyzed separately to see how the uncertainty in each would affect the match log LR values obtained from joint and single analysis. The second set combined varying mixture weights, template concentrations, and number of contributors. It was demonstrated that replicate amplifications can result in higher match statistics. This supports the suggestion that DNA collection and amplification procedures in forensic cases be modified so that more data is available for DNA mixture analysis using probabilistic genotyping and the JLF.

Background

The use of DNA to uniquely identify an individual in forensic evidence has been a widely accepted and lauded method to identify perpetrators of violent crimes. A wide array of crime scene evidence samples is sent to forensic laboratories with hopes of retrieving highly coveted DNA profiles. Because genetic identity is naturally represented in an individual's genotype, identification information is represented in a DNA profile. Because DNA profiles are vital to criminal justice proceedings, correctly inferring genotypes from evidence DNA using sound

scientific and statistical approaches is undoubtedly a delicate and important task. (Perlin, Kadane, et al., 2009; Perlin & Sinelnikov, 2009; Perlin, 2015).

STR Genotyping

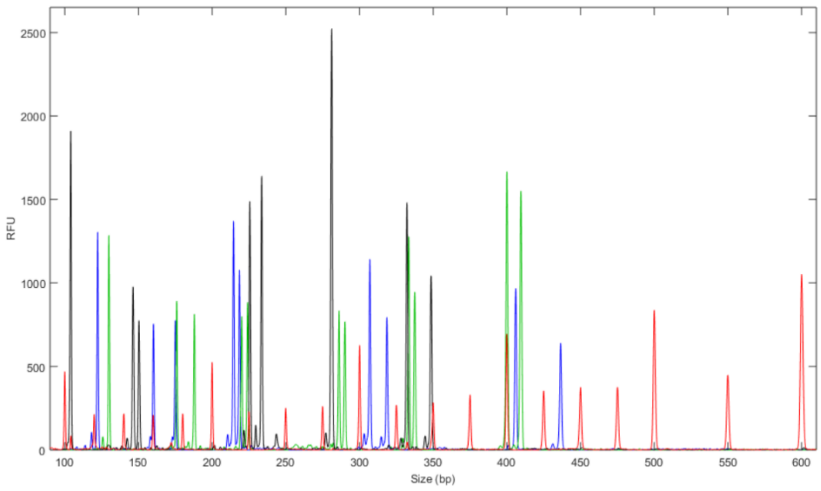
Biological evidence samples are subjected to a multi-step process where DNA is extracted from the biological specimens, quantified, and amplified via the polymerase chain reaction (PCR). Only specific non-coding regions of DNA containing short tandem repeats (STR) are targeted during amplification. These regions are referred to as STR loci or locus if referring to only one. These STR loci are composed of DNA segments where the sequence repeats itself. These segments are categorized by the number of base pairs (bp) in the repeating sequence and the number of repeating units in the locus. The number of repeats at a locus is given the term allele and a genotype is formed by two alleles at each locus: one passed down from the father and one from the mother. An individual is said to be homozygous at a locus when both alleles are the same value. A heterozygous individual has two different alleles. A vast amount of possible allele combinations exists for every locus which makes STR genotyping useful for human identification. The STR profile is the composite of these locus genotypes. In forensic science, commercial STR kits can target more than 20 STR loci that result in different genotype possibilities for a single individual (Butler, 2010, Chapter 8; Perlin, 2013).

Conversion of biological evidence to DNA begins with extraction. This process involves the release of DNA from cells, purification of the DNA through washes, and concentration of the DNA. The DNA is then quantified to assess how much was extracted. A template amount of 0.5 to 1 ng of DNA works best for most STR kits. If enough DNA is available for further processing, PCR amplification is performed. After PCR, millions of STR fragments are separated via capillary electrophoresis by size and represented as peaks in an electropherogram (EPG)

demonstrated in Figure 1 below (Butler, 2005, Chapters 1-3, 14). Peaks are a visual representation of DNA quantity and amount of PCR product measured by height in relative fluorescence units (RFU) and size in base pairs (bp). The amount of DNA present in a sample is indicated by the peak height. Peaks also represent alleles at each locus. An allele designation

Figure 1

EPG of all loci for reference sample H1



Note. The peaks represent different sized STR fragments.

is given to a peak based on its size. The size of a peak is determined by the number of base pairs (bp) the STR fragments contain. The greater the number of repeats in an STR fragment, the bigger the size of the peak. Larger sized alleles contain a greater number of bp and may not replicate as consistently during PCR leading to smaller amounts of PCR products. Because peak heights represent PCR product amount, larger sized alleles often have shorter peaks than smaller sized alleles. This proportionality is affected by DNA template concentration (Butler, 2010, Appendix 1; Butler, 2015, Chapter 4).

Computer programs make allele designations for every locus which help in determining an STR profile. DNA analysis and interpretation then compare the evidence and reference STR

profiles to each other which can result in a DNA match (Butler, 2010, Chapters 1, 9-10, Appendix 1; Perlin & Szabady, 2001).

DNA Mixture Interpretation

The last step of forensic DNA typing is interpretation of the STR data. This involves inferring genotypes from the data. Evidence genotypes are compared to suspect genotypes and if a match exists, the weight of this evidentiary match must be represented by a statistic (Perlin, Kadane, et al., 2009; Perlin & Sinelnikov, 2009). Identification information present in the genotypes is measured by the match statistic.

DNA mixture analysis requires additional steps. First, it must be determined that a mixture is present. This is accomplished by reviewing the EPG data for every locus. All loci are assessed for number of peaks, peak heights, and peak height ratios. A peak height ratio (PHR) is the measurement of the peak height of one allele over the peak height of another allele for a locus. PHRs are affected by locus size, effectiveness of fragment separation, and DNA template concentration. When an individual is homozygous at a locus, only one allele peak is present. A heterozygous individual will have two allele peaks at one locus and how well those alleles amplified is measured by the PHR. Heterozygous loci with relatively equal peak heights will have low PHR values. Peak height imbalance occurs when there is a large difference between the peak heights of heterozygous alleles indicated by a high PHR. A single source sample will usually produce one or two allele peaks at every locus with low PHR values. If the number of peaks at a locus is greater than two or the difference in peak height ratios is too great to originate from a single source (greater than 30%-40%), the STR data indicate the presence of a mixture (Butler, 2010, Appendix 1; Butler 2015, Chapter 4).

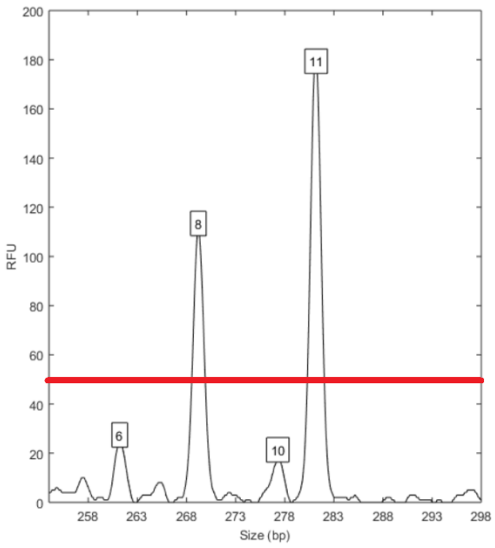
The mixture weight of each contributor is determined next. Different amounts of DNA are contributed by each individual present in a mixture. An individual that has contributed high quantities of DNA is said to be a major contributor and is identified by tall data peaks. Minor contributors are represented by shorter peaks indicating lower quantities of their DNA being present in the mixture. Once it has been determined that more than one contributor is present in a DNA evidence sample, their weight of contribution can be determined using PHRs (Butler, 2010, Chapter 10). Obtaining mixture weights for each contributor helps infer contributor genotypes which can be compared to a reference. If both the evidence and suspect genotypes match, a match statistic can be calculated, and the amount of identification information determined. Though simple in theory, DNA mixture analysis is known to be an intricate and difficult process.

Single source samples usually provide clean and ideal data which makes DNA analysis of these samples a straightforward task. This is not usually the case for STR profiles derived from crime scene evidence. DNA evidence from crime scenes can be low template, damaged, or a mixture of two or more individuals. When evidence of this nature is amplified, it can result in low peak heights, missing peaks, or several peaks indicative of a mixture. Low peak heights indicate low quantities of DNA which can lead to peak height imbalance at heterozygous loci. If damage to the DNA is extensive, there will be regions in the DNA that will not amplify during PCR. This results in allele drop-out, an extreme form of peak height imbalance. When more than two contributors are present in a mixture, the amount of DNA present for each can vary potentially resulting in low peak heights and peak height imbalance between major and minor contributors. Each of these elements in isolation or combined, makes it difficult to confidently infer locus genotypes. This results in uncertainty in the data, the inferred genotypes, and the resulting match statistics (Ballantyne et al, 2013; Perlin et al., 2011).

The goal of qualitative and quantitative mixture interpretation methods is to address data uncertainty. Qualitative methods implement thresholds to address uncertainty but in doing so, discard data peaks that can be useful in determining genotypes if they fall below the threshold (Greenspoon et al., 2015). Figure 2 provides a visual example of how thresholds can discard information at a locus. This decreases the identification information of a subsequent match statistic. Perlin and Sinelnikov (2009) determined that qualitative mixture interpretation methods are less informative than quantitative methods. This is due to the utilization of all the available data by quantitative methods. The variation present in the data is mathematically accounted for by assigning probabilities to all possible genotypes at each locus (Bauer et al., 2020; Gill et al., 2012; Perlin et al., 2014). Probabilities are used to explain the data peak patterns and preserve identification information.

Figure 2

EPG of TPOX for sample J4



Note. The analytical threshold (AT) is set to 50 RFUs.

Interpretation of STR data culminates in the comparison of an evidence genotype with a reference genotype to see if a match is obtained. There are different approaches to calculate a match statistic, but quantitative approaches use likelihood ratios (LR) which account for probabilities. An LR "is a calculation of the ratio of probabilities of observing the DNA under two alternative hypotheses" (Gill et al., 2012). These hypotheses are commonly referred to as the prosecutor's hypothesis and the alternative or defense hypothesis. The prosecutor's hypothesis assumes that the suspect did contribute their DNA to the evidence while the alternative hypothesis is that some other unrelated individual contributed their DNA to the evidence. When the LR is greater than one, it supports the hypothesis that the suspect contributed to the evidence. If the LR is less than one, support for the alternative hypothesis strengthens. The base 10 logarithm of the LR, logLR, is used to measure the change of information in the evidence, show how well the data preserves identification information, and can be expressed in ban units (Good, 1979, as cited in Ballantyne et al., 2013). The match logLR weighs the strength of the relationship between the evidence genotype and suspect genotype relative to a population. Because quantitative methods utilize all the available data in an STR profile and objectively infer evidence genotypes, preservation of identification information is captured best in the match logLR obtained from probabilistic genotyping (Perlin et al., 2014). How informative the match logLR is then measured by its value which also indicates how much identification information was preserved from the quantitative data. In criminal proceedings, LR match statistics that reach or surpass 6 ban (one in a million) tend to have the most persuasive effect on juries, but the method used to calculate the match logLR should also be an indicator of how informative and reliable a match statistic is (Koehler, 2001, as cited in Perlin & Sinelnikov, 2009). TrueAllele computes this match statistic using all the identification information present in the data.

TrueAllele® Casework

TrueAllele® Casework is a software program that uses a hierarchical Bayesian model to explain STR data and infer genotypes. The likelihood function (LF) in Bayes theorem accounts for genotype, mixture weight, PCR stutter, relative amplification, and DNA degradation that may be present in the data using the peak height variation and mixture variation between different loci (Perlin et al., 2011). A statistical search of the STR data using Markov chain Monte Carlo (MCMC) is conducted by the computer which tries different variable combinations to explain the data. Thousands of MCMC cycles sequentially test these variables using new combinations each cycle. The combinations that give a better explanation for the data are assigned a higher likelihood (giving higher probability values) and a lower likelihood is given to the pairs whose explanation is poor (leading to lower probability for the combination). The result is a posterior probability distribution for all allele pairs at every locus for each contributor from which genotypes are inferred. A match LR, expressed as a logLR, is calculated when the inferred evidence genotypes are compared to a reference genotype from a particular individual relative to a population. Because TrueAllele uses all the quantitative data available for interpretation, the match logLR is a reliable number conveying a sample's identification information (Ballantyne et al., 2013; Greenspoon et al., 2015; Perlin et al., 2011; Perlin et al., 2015; Perlin & Sinelnikov, 2009).

Independent sets of data can be analyzed and multiplied together using a joint likelihood function (JLF). The probabilities that result for each independent data set are multiplied together using the JLF which increases the posterior probability distribution of the locus allele pairings. When there is high uncertainty in STR data as seen with LT-DNA and mixtures, the posterior probability distribution will be diffuse for allele pairings across all loci. A diffuse probability is

indicative of uncertainty and will result in a lower match statistic. Joint interpretation of multiple data sets can lead to higher probabilities because the additional data is constrained by the variable combinations that give the best explanation jointly during MCMC sampling. A JLF can help tighten and increase those probability distributions if LT-DNA and mixture samples are amplified in replicates and interpreted jointly (Perlin, Kadane, et al., 2009). Replicate amplification of evidence highly suspected to contain LT-DNA or consist of a mixture, will provide a greater amount of data that TrueAllele can readily process. By applying the JLF, higher probabilities will naturally result in higher and more informative match statistics for replicates that come from the same piece of evidence or evidentiary material that are closely related.

Review of Literature

TrueAllele uses probabilistic modeling (Bayes theorem) and statistical sampling (MCMC) methods that have been established for decades and are used by different disciplines such as "nuclear physics, psychology, computer learning, economics, biological systems, and more recently, DNA analysis" to explain complex data patterns (Greenspoon et al., 2015). It has been established that mathematical modeling of complex evidence mixture data has produced consistently higher match statistics compared to manual review. Computer interpretation has proven to be objective, efficient, and reproducible in several studies. Perlin et al. (2009, 2011, 2013, 2014, 2015) have performed validation studies using Cybergenetics TrueAllele® Casework for mixture interpretation. Interpretation was performed on data obtained from premixed templates from the National Institute of Standards and Technology (NIST), adjudicated cases from the New York State Police Forensic Investigation Center (Perlin et al., 2011, 2013), mixture evidence in 72 cases from the Virginia Department of Forensic Science, and data created

by Sorenson Forensics for the Kern County Regional Crime Laboratory in these particular validation studies, respectively. The studies compared the traditional qualitative methods of inclusion probability and combined probability of inclusion to TrueAllele interpretation and concluded that TrueAllele preserved the most identification information. The preservation of identification information by TrueAllele has supported its use in criminal cases.

TrueAllele has been officially used in forensic casework since 2009 and it has been instrumental in many criminal trials such as *Commonwealth of Pennsylvania v. Kevin J. Foley* and *Commonwealth of Pennsylvania v. Glenn Lyons*. The United Kingdom (UK) has also used TrueAllele. Match statistics calculated by TrueAllele were pivotal in the case of the *Queen v. Duffy & Shivers* where Brian Shivers was convicted of murdering two soldiers who died in the 2009 terrorist attack of the Massereene Barracks in Northern Ireland (Perlin, 2013, 2015; Perlin & Galloway, 2012; Perlin & Sinelnikov, 2009). TrueAllele has also been used to identify victims from the World Trade Center disaster and in building the UK's National DNA Database. Crime labs in California, Louisiana, Maryland, South Carolina, Ohio, Georgia, and Virginia have already incorporated TrueAllele into their DNA analysis process (Bauer et al., 2020; Kwong, 2017).

Because previous validation studies established the reliability and objectivity of probabilistic genotyping and TrueAllele is already used in forensic casework, it is important to explore other applications of computer modeling in mixture interpretation. An earlier study by Ballantyne et al. (2013) looked at how a JLF can increase identification information with samples varying in mixture weight. Mixture weight is the measurement of how much template DNA was contributed to the sample by a single contributor to the mixture. (Perlin & Szabady, 2001). Peak height imbalance helps distinguish one contributor from another but when a mixture

contains equal contributor weights, the peak heights are also relatively equal. Equal weighted mixtures increase uncertainty in the inferred genotypes leading to a decrease in identification information. Ballantayne et al.'s 2013 study used binomial sampling of an equal weighted mixture to produce sub-samples containing different mixture weights to elicit more identification information. Several sample groupings were analyzed using the data from a single experiment, but two experiments were combined and analyzed using a JLF. TrueAllele was used to jointly analyze the two sets of data to test the hypothesis that joint analysis of multiple experiments will increase identification information. This study found that pairing one experiment with another generally increased identification information. Even though there were instances where no increase was seen and even a decrease occurred, the full sample genotype information was recovered using the joint likelihood thus preserving and increasing identification information. The study concluded by suggesting that using joint likelihoods with samples varying in mixture weight can obtain greater identification information in routine casework as well. Variations in mixture weight can be generated with "micro-geographical samplings" of physiological stain evidence and creation of sub-samples from limited dilutions of DNA extract of mixture evidence (Ballantyne et al., 2013). The resulting data from these sub-samples created from casework evidence can be interpreted jointly using TrueAllele resulting in more genetic information.

Materials and Methods

Materials

- TrueAllele® Casework
- TrueAllele® Visual User Interface (VUIer™)
- TrueAllele® VUIer™ manuals for Analyze, Data, Request, Review, and Report modules
- Microsoft Excel

- STR data from Perlin & Sinelnikov (2009)
    - Perlin & Sinelnikov (2009) data set is composed of DNA from two unrelated individuals. The set contains five different mixture weights of 10%, 30%, 50%, 70%, and 90% for DNA template concentrations of 1 ng, 0.5 ng, 0.25 ng, and 0.125 ng for each contributor. There were two sets which yielded 40 samples. Figure 3 shows the composition of each sample.

Figure 3

First sample set composition

		10%	30%	50%	70%	90%
A+G pair	**1.0 ng**	B1	C1	D1	E1	F1
	0.5 ng	B2	C2	D2	E2	F2
	0.25 ng	B3	C3	D3	E3	F3
	0.125 ng	B4	C4	D4	E4	F4
H+N pair	**1.0 ng**	I1	J1	K1	L1	M1
	0.5 ng	I2	J2	K2	L2	M2
	0.25 ng	I3	J3	K3	L3	M3
	0.125 ng	I4	J4	K4	L4	M4

There are two contributor pairings, female A with male G, and female H with male N. The culprit mixture weights are 10%, 30%, 50%, 70% and 90%. The total DNA quantities used in a 25 μl PCR volume were 1.0 ng, 0.5 ng, 0.25 ng and 0.125 ng.
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Note. (Perlin & Sinelnikov, 2009)

- STR data from Perlin et al. (2015)
    - This data set was composed of 40 randomized mixture items. These items were separated into four groups of two, three, four, and five contributors. Each group had a total of 10 mixtures. The mixtures were made from five known references: K01, K02, K03, K04, and ASB. Only four samples were used from this set for

analysis: mixtures 1.1, 2.8, 3.6, and 4.2. References K01 and ASB were present in all four samples.

Methods

After computer installation of TrueAllele® Casework and TrueAllele® VUIer™, familiarization with the manuals, and completion of the tutorial material, data for analysis was selected considering two common sources of data uncertainty: mixture weight and template concentration. Using the data from Perlin & Sinelnikov (2009), mixture weight and template concentration were considered as separate variables to see which elicited more information using the JLF. A second data set (Perlin et al., 2015) involved more complex mixtures which varied in mixture weight, template concentration, and number of contributors. All three variables of the second data set were intertwined in the joint analysis.

In order to observe how well a JLF recovers identification information from varying mixture weights, the 1 ng template samples were used from the Perlin & Sinelnikov (2009) data in different mixture weight combinations. Table 1 shows these combinations. Each sample was analyzed independently using TrueAllele® Casework. Then the samples were combined in duplicate and triplicate for joint analysis. This same pattern was also used for the low template samples.

Table 1

Mixture Weight Combinations for Single and Joint Interpretation

	References A1 and G1			References H1 and N1		
Mixture Weight	**Single**	**Duplicate**	**Triplicate**	**Single**	**Duplicate**	**Triplicate**
0.9/0.1	B1	B1+D1	B1+C1+D1	I1	I1+K1	I1+J1+K1
0.7/0.3	C1	C1+D1	D1+E1+F1	J1	J1+K1	K1+L1+M1
0.5/0.5	D1	B1+C1		K1	I1+J1	

	References A1 and G1			References H1 and N1		
Mixture Weight	**Single**	**Duplicate**	**Triplicate**	**Single**	**Duplicate**	**Triplicate**
0.3/0.7	E1	D1+E1		L1	K1+L1	
0.1/0.9	F1	D1+F1		M1	K1+M1	
		E1+F1			L1+M1	

Note. Mixture weight is indicated for the single samples.

To test whether a joint likelihood recovers genetic information from low template samples, only the sample groups that changed in concentration (as seen in Figure 3), but not in mixture weight, were combined for joint analysis. Table 2 shows these combinations. Table 3 shows the combinations for the second set of data that included more complex mixtures.

Table 2

Combinations of Template Concentrations for Single and Joint Interpretation

	References A1 and G1			References H1 and N1		
Template Concentration (ng)	Single	Duplicate	Triplicate	Single	Duplicate	Triplicate
0.5	B2 - F2	B3+B4	B2+B3+B4	I2 – M2	I3+I4	I2+I3+I4
0.25	B3 - F3	C3+C4	C2+C3+C4	I3 – M3	J3+J4	J2+J3+J4
0.125	B4 - F4	D3+D4	D2+D3+D4	I4 – M4	K3+K4	K2+K3+K4
		E3+E4	E2+E3+E4		L3+L4	L2+L3+L4
		F3+F4			M3+M4	

Note. Samples B2, C2, D2, E2, and F2 all have concentrations of 0.5 ng and each was analyzed singly. As were samples B3 - F3 and B4 - F4.

Table 3

Combinations of Complex Mixtures for Single and Joint Interpretation

	References ASB and K01		
Number of Contributors	**Single**	**Duplicate**	**Triplicate**
2	1.1	1.1 + 2.8	1.1 + 3.6 + 4.2
3	2.8	1.1 + 3.6	

References ASB and K01			
Number of Contributors	**Single**	**Duplicate**	**Triplicate**
4	3.6	1.1 + 4.2	
5	4.2	3.6 + 4.2	

Note. The number of contributors is indicated for the single samples.

TrueAllele® Casework Visual User Interface (VUIer™) contains six modules where different steps of the interpretation and analysis process are performed: Analyze, Data, Request, Review, Report, and Tools. The Data, Request, Review, and Report modules were primarily used for this research. The STR data files from Perlin & Sinelnikov (2009) and Perlin et al. (2015) were stored in a TrueAllele World, a database where files and interpretation requests are stored, from which they were accessed for subsequent analysis (Hornyak et al., 2015).

The Data module was used to access the TrueAllele World that contained the two sets of previously published data. The STR data files were downloaded and used to form interpretation requests for each single sample, duplicate combination, and triplicate combination for mixture weight, template concentration, and complex mixtures groups in the Request module. Each interpretation request indicated the maximum number of contributors for each sample being analyzed. The requests were reviewed and uploaded to a cloud server where they were processed.

TrueAllele uses MCMC statistical searching where different variables (genotype, mixture weight, etc.) are used to try to explain the STR data and separate the contributors in a mixture. Contributor genotypes are inferred from the probability distributions determined for each variable during MCMC sampling. The number of times the computer will subject the data to MCMC sampling is determined by the number entered for burn-in and read-out cycles in the Request module (Greenspoon et al., 2015; Hornyak et al., 2015; Perlin et al., 2011, 2013). The time it takes for an interpretation request to process depends on the number of cycles used for

each request. A high number of cycles will usually result in more information but will also lead to longer running times. Fewer cycles lead to shorter running times. When data is known to be complex and high uncertainty is expected, a greater number of burn-in and read-out cycles are used. Single source data or simple mixtures can be processed with lower cycle settings. The number of cycles that can be chosen vary from 5,000 (5K), 10,000 (10K), 25,000 (25K), 50K, 100K, and 250K.

The samples in Tables 1 and 2 were analyzed using 5K burn-in and read-out cycles. Because this first set of data was composed of simple two contributor mixtures with known concentrations and mixture weights, the number of cycles was kept at 5K. The set of complex mixtures in Table 3 were analyzed using 50K cycles to account for the higher degree of variability in the data. All the requests were run once except for the following: D3, C4, D4, L3, J4, D3+D4, L3+L4, and F2+F3+F4. Initial run results for these samples deviated slightly in their inferred mixture weights compared to their original design and results from the previous studies and analyses performed on the same data in Perlin & Sinelnikov (2009). The averages of both runs were used, and the second run results were used for D3+D4.

Once the interpretation requests were done processing, the Review module was used to check the locus probability distributions for each set. The match logLR results for each interpretation request were obtained in the Report module. The inferred genotypes for each request were selected and compared to the references in the sample mixture relative to a random population. The NIST1036_2017_ALL was the population database chosen for this research which contains allele frequencies for the Caucasian, African American, Asian, and Hispanic population groups used to calculate match statistics (National Institute for Standards and Technology, 2014). Each comparison was broken down by sample, contributor, number of

contributors, weight of each contributor, the standard deviation, the KL (Kullback-Leibler) statistic, and the match logLR values for each reference. The evidence sample, designed mixture weight of each contributor, and the match logLR values are presented in tables below.

Data Analysis and Interpretation

The match logLRs obtained from each interpretation request were grouped by single, duplicate, and triplicate analysis for each variable group. Results from samples B-F and I-K showed a change in logLRs for single and joint analyses. The results for samples I-K are shown here to display the change in match information for 50/50 mixture weights, minor contributors, and low template concentrations. Tables 4-6 display mixture weight combinations, Tables 7-9 display LT-DNA results, and Table 10-11 contain results for complex mixtures.

Mixture Weight Comparison

Mixture weights (MW) are calculated by summing the peak height values of the minor peaks and dividing that value by the sum of all peaks at a locus. Manual calculations use only the loci where major and minor contributor peaks are distinguishable. TrueAllele and PG software can calculate the weights for all loci (Greenspoon et al., 2015; Perlin et al., 2013). Many studies state that equal weighted mixtures result in lower and more diffuse probabilities. Because each possible allele pair combination will be equally likely with similar sized peaks, the probability distribution will decrease in 50/50 mixtures. Ballantyne et al. (2013) claim that mixtures where two individuals have contributed an approximate equal amount of DNA are some of the most challenging because there are no distinct major and minor peaks. Differences in peak heights help attribute which contributor goes with which genotype thus increasing the identification information (Perlin et al., 2013, 2015; Perlin & Sinelnikov, 2009). Different mixture weight

combinations were analyzed to compare what mixture weight variations gained more identification information in the match logLR when interpreted jointly.

Table 4

Single Interpretation Results Comparing MW

Sample	Mixture Weight (H1/N1)	Reference H1 logLR	Reference N1 logLR
I1	0.9/0.1	17.7879	12.9775
J1	0.7/0.3	17.0208	18.1206
K1	0.5/0.5	9.2528	10.5971
L1	0.3/0.7	16.5517	18.3432
M1	0.1/0.9	11.7348	19.1745

Sample K1 contains 50/50 contributions of reference H1 and N1. It is seen in Table 4 above and Figure 4 below that both references in sample K1 have the lowest match logLR values in this set. This result was expected as were the lower logLRs for reference H1 in sample M1 and reference N1 in sample I1 because equal and low MWs (0.5 and 0.1) obtain lower probability distributions in their inferred genotypes.

Figure 4

MW - Histogram showing the match logLR values from single analysis

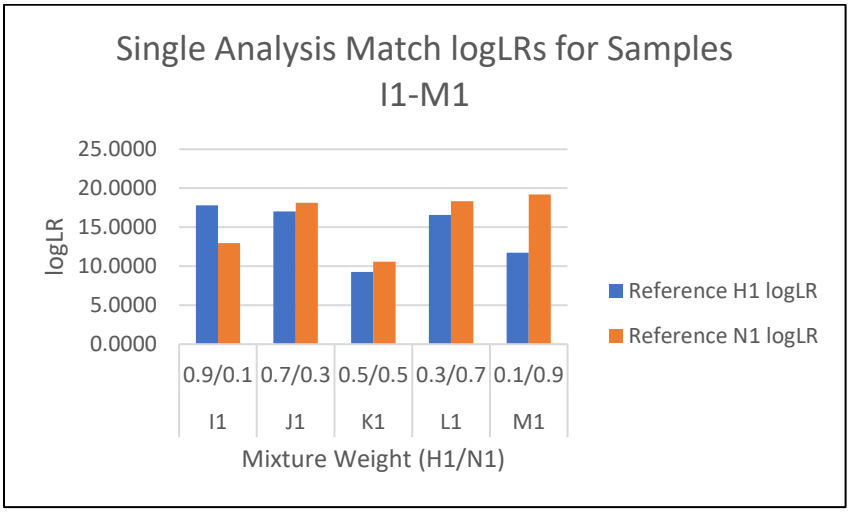


Table 5

Duplicate Interpretation Results Comparing MW

Sample	Mixture Weight (H1/N1)	Reference H1 logLR	Reference N1 logLR
I1+K1	0.9/0.1 + 0.5/0.5	17.7879	19.1727
J1+K1	0.7/0.3 + 0.5/0.5	17.6994	19.0926
I1+J1	0.9/0.1 + 0.7/0.3	17.7879	19.0615
K1+L1	0.5/0.5 + 0.3/0.7	17.7584	19.1468
K1+M1	0.5/0.5 + 0.1/0.9	17.7812	19.1745
L1+M1	0.3/0.7 + 0.1/0.9	17.4381	19.1745

Table 5 above displays the logLRs for the duplicate combinations. It can be noticed immediately that an information gain was observed for each reference in each duplicate as compared to the single analyses shown in table 4. The logLR values for I1+K1 and I1+J1 were equal at 17.7879 ban. This can occur when the probability is practically near or equal to 100% for evidence genotype allele pairs meaning that all the probability is concentrated on the reference allele pairs. When the same population database is used for the match logLR calculation, a maximum value is obtained because the LR calculation changes from uncertain probability divided by the population at the reference allele pair to one divided by the population genotype. Reference H1 in sample I1 has a weight of 0.9 or 90% as does reference N1 in sample M1indicating that probability is concentrated on the allele pairs of these references in the

duplicate and triplicate joint analyses. Duplicates and triplicates containing references whose weights are 90% have logLR values that are equal or close to equal indicating that high mixture weights whose probability is close to 100% "maxes out" a match logLR value.

Table 6

Triplicate Interpretation Results Comparing MW

Sample	Mixture Weight (H1/N1)	Reference H1 logLR	Reference N1 logLR
I1+J1+K1	0.9/0.1 + 0.7/0.3 + 0.5/0.5	17.7879	19.1732
K1+L1+M1	0.5/0.5 + 0.3/0.7 + 0.1/0.9	17.7879	19.1745

Triplicate analysis showed little to no change compared to duplicate analysis but a gain was demonstrated in triplicate analysis compared to single analysis. Some logLR values in Table 5 and 6 are equal or close to equal. When the quantity of DNA is high and the inferred genotype probability distribution is near or reaches 100% probability, the major contributor's logLR value ceases to increase and stays relatively constant at its maximum value (Bauer et al., 2020). Minor contributors can have their logLR values display this trend as well.

LT-DNA Comparison

After DNA extraction is performed, the quantity of DNA present in the evidence is determined. Quantities of 0.2 ng and lower are deemed low template (Butler, 2010, Chapter 14, 18). The stochastic effects of PCR are exacerbated as DNA quantities decrease leading to greater

occurrences of peak height imbalance, allele drop-out, stutter, etc. This results in heightened uncertainty, lower probability distributions, and lower match statistics for LT-DNA samples.

The proportional relationship between DNA weight contribution and logLR values dictates that as the amount of DNA contributed increases so does the logLR for major and minor contributors. The opposite is also observed. When the amount of DNA weight decreases so does the logLR (Bauer et al., 2020; Perlin & Sinelnikov, 2009). When a mixture sample is low template, minor contributors with exceptionally low mixture weights have even lower amounts of DNA present in the sample resulting in extremely low and uninformative logLRs. This trend is displayed in these results. The results of the single analyses for samples at the lowest template concentrations of 0.125 ng in the first data set are shown in Table 7. Though the concentrations are constant for samples I4-M4, each sample has a different mixture weight. It can be observed that as weight decreases, logLR values rapidly decrease as well at low template concentrations. Negative logLRs were even observed. Greater gains in identification information are seen for low template samples when analyzed jointly.

Table 7

Results from Single Interpretation of LT-DNA Samples

Sample (0.125 ng)	Target Weight (H1/N1)	Reference H1 logLR	Reference N1 logLR
I4	0.9/0.1	17.4153	1.9852
J4	0.7/0.3	8.6165	6.0900
K4	0.5/0.5	8.0504	8.4280
L4	0.3/0.7	-0.4628	13.7580
M4	0.1/0.9	-0.4874	18.2591

Table 8

Results from Duplicate Interpretation of LT-DNA Samples

Sample (0.25 ng + 0.125 ng)	Target Weight (H1/N1)	Reference H1 logLR	Reference N1 logLR
I3+I4	0.9/0.1	17.7828	9.8831
J3+J4	0.7/0.3	11.6383	10.6104
K3+K4	0.5/0.5	9.3065	10.7250
L3+L4	0.3/0.7	14.4494	17.1665
M3+M4	0.1/0.9	6.4534	19.1743

Since the joint amplifications were done within each sample group not across like in the MW group, sample K (50/50 MW) shows a small increase to the logLR across single, duplicate, and triplicate analyses for references H1 and N1 compared to the other samples whose weights were unequal. This was expected due to prior studies stating that equal weight ratios result in lower probability distributions compared to samples with unequal weight ratios. Though the LT-DNA sample group combinations used only varying template amounts, mixture weight influence was simultaneously observed. Samples where references H1 and N1 had weights of 90% obtained logLRs close to their maximum value when analyzed in duplicate and triplicate as seen with mixture weight combinations. Samples with 70%, 30% and 10% weight at 0.125 ng showed an increase in their logLR values from single, to duplicate, and to triplicate analyses. This was as expected due to the increasing constrains placed on each possible pair during MCMC sampling when more than one set of data are analyzed jointly. The probability distributions tighten and increase as more data is available especially for contributors with low weight contributions in LT-DNA samples.

Table 9

Results from Triplicate Interpretation of LT-DNA Samples

Sample (0.5 ng + 0.25 ng + 0.125 ng)	Target Weight (H1/N1)	Reference H1 logLR	Reference N1 logLR
I2+I3+I4	0.9/0.1	17.7879	13.7131
J2+J3+J4	0.7/0.3	15.7304	17.1075
K2+K3+K4	0.5/0.5	8.7466	9.8877
L2+L3+L4	0.3/0.7	14.4260	17.6315
M2+M3+M4	0.1/0.9	7.4670	19.1745

Complex Mixtures with up to 5 Contributors

The second data set used mixtures with two, three, four, and five contributors. Mixture weights varied for each reference contributor. References ASB and K01 were chosen based on predetermined mixture weights. Samples containing these two references were chosen and combined for joint analyses considering their weight differences. Sample 1.1 was chosen because ASB and K01 each had near 50/50 weight contributions. The remaining samples were chosen based on the unequal weight ratios of ASB compared to K01. Table 10 below shows the logLR results for references ASB and K01 along with their TrueAllele determined mixture weight. Like the first data set, logLR values for each reference decreased as mixture weight decreased.

Table 10

Results from Single Interpretation of Complex Samples

Mixture Sample	Mixture Weight (ASB/K01)	Reference ASB logLR	Reference K01 logLR
1.1	0.4965/0.5035	13.4193	11.1957
2.8	0.2552/0.6153	16.7984	16.3593
3.6	0.9006/0.0254	20.3219	3.3181
4.2	0.1372/0.5404	0.7290	17.3530

When these complex samples were analyzed jointly, the increase in information was greatest for the lowest weights but consistently greater as more samples were added to the joint likelihood. Tables 11 and 12 demonstrate this gain. The highest weights showed the same trend of little to no change in the maximum logLR.

Table 11

Results from Duplicate Interpretation of Complex Samples

Mixture Sample	Mixture Weight (ASB/K01)	Reference ASB logLR	Reference K01 logLR
1.1 + 2.8	0.4815 + 0.3146/ 0.5018 + 0.6354	19.1947	16.9255
1.1 + 3.6	0.1349 + 0.8729/ 0.5015 + 0.0612	20.3211	13.5960
1.1 + 4.2	0.4383 + 0.0846/ 0.2851 + 0.5493	16.6537	17.5757
3.6 + 4.2	0.9076 + 0.0538/ 0.0193 + 0.5782	20.3219	17.9900

Table 12

Results from Triplicate Interpretation of Complex Samples

Mixture Sample	Mixture Weight (ASB/K01)	Reference ASB logLR	Reference K01 logLR
1.1 + 3.6 + 4.2	0.4466 + 0.8616 + 0.0521/ 0.4656 + 0.0286 + 0.6233	20.3219	18.1666

Research Results and Discussion

The logLR results for single, duplicate, and triplicate analyses were used to calculate the difference between each analysis method. The differences were calculated as follows for references A1 and G1, H1 and N1, and ASB and K01:

Duplicate log LR - single log LR

Triplicate log LR - single log LR

Triplicate log LR - duplicate log LR

These calculations were made for all three variables of mixture weight, low template concentration, and complex mixtures. The resulting values indicate how much information was either gained or lost when performing joint analysis of multiple data samples using the JLF.

Mixture Weight

The difference calculations were performed for each sample group. The information gain or loss across each sample for mixture weight was consolidated for each reference shown numerically in Tables 14-17. Table 13 shows the log LR values across all three analysis methods for sample K1. Figures 5-8 visually represent this gain in histograms.

Table 13

Interpretation Results for Sample K1 Using Varied MW Combinations

Sample	Target Weight (H1/N1)	Reference H1 logLR	Reference N1 logLR	Information Gain/Loss H1	Information Gain/Loss N1
K1	0.5/0.5	9.2528	10.5971		
I1+K1	0.9/0.1 + 0.5/0.5	17.7879	19.1727	8.5351	8.5756
J1+K1	0.7/0.3 + 0.5/0.5	17.6994	19.0926	8.4466	8.4955
K1+L1	0.5/0.5 + 0.3/0.7	17.7584	19.1468	8.5056	8.5497
K1+M1	0.5/0.5 + 0.1/0.9	17.7812	19.1745	8.5284	8.5774
I1+J1+K1	0.9/0.1 + 0.7/0.3 +	17.7879	19.1732	8.5351	8.5761

Sample	Target Weight (H1/N1)	Reference H1 logLR	Reference N1 logLR	Information Gain/Loss H1	Information Gain/Loss N1
	0.5/0.5				
K1+L1+M1	0.5/0.5 + 0.3/0.7 + 0.1/0.9	17.7879	19.1745	8.5351	8.5774

Table 14

MW - Difference of Single and Joint Analysis for Reference A1

A1 Samples	Target Weight	Dup-single	Trip-single	Trip-Dup
B	0.9	0.0006	0.0006	0.0000
C	0.7	1.4820	1.4829	0.0009
D	0.5	15.7077	15.7084	0.0007
E	0.3	-0.2148	0.1107	0.3256
F	0.1	6.8666	7.1925	0.3259

Sample K1 gains identification information by approximately 8.5 ban using joint analysis shown in Table 13. As previously mentioned, the sample containing the higher mixture weight provided a higher logLR which eventually plateaued between duplicate and triplicate analysis methods. Tables 14 and 15 show that the greatest information gain for references A1 and G1 were seen when their weight contributions were 50% where information increased by 15.7 ban in A1 and close to 15 ban in G1. When references had a low weight contribution of 10%, gain in information was also seen. As mixture weight increased, the gain in information was negligible. The trend is clearly demonstrated in the histograms for each reference.

Table 15

MW - Difference of Single and Joint Analysis for Reference G1

G1 Samples	Target Weight	Dup-single	Trip-single	Trip-Dup
B	0.1	5.7571	5.9119	0.1549
C	0.3	1.0859	1.2416	0.1557
D	0.5	14.9558	14.9562	0.0004
E	0.7	0.0447	0.0447	0.0000
F	0.9	0.0000	0.0000	0.0000

Figure 5

MW - Histogram showing difference between single and joint analysis for A1

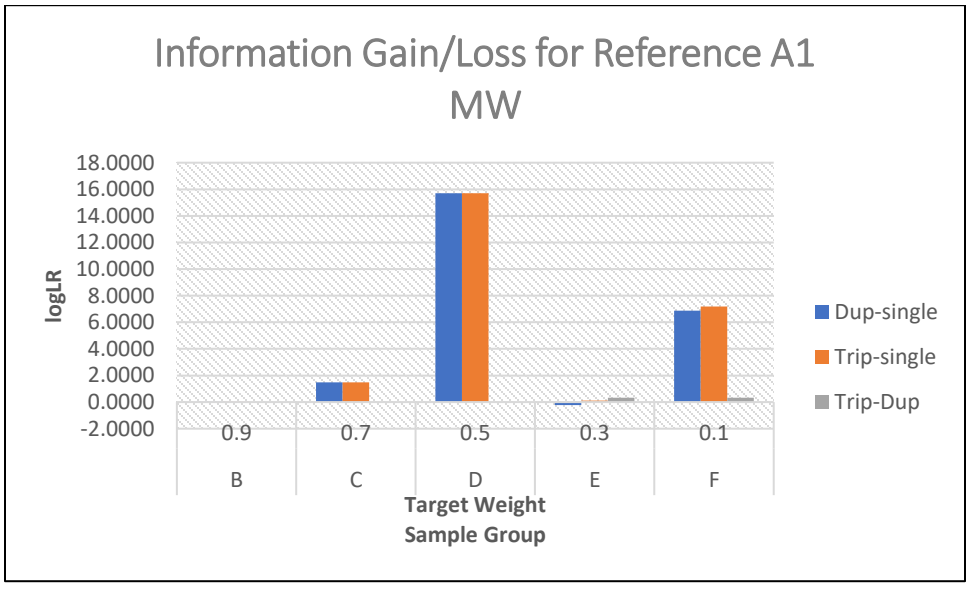


Figure 6

MW - Histogram showing difference between single and joint analysis for G1

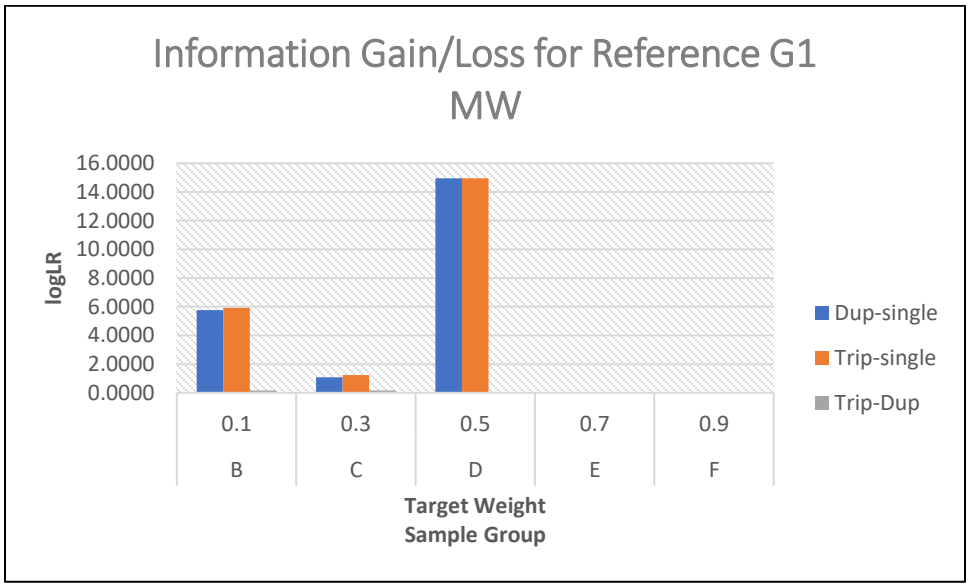


Table 16

MW - Difference of Single and Joint Analysis for Reference H1

H1 Samples	Target Weight	Dup-single	Trip-single	Trip-Dup
I	0.9	0.0000	0.0000	0.0000
J	0.7	0.7228	0.7671	0.0442
K	0.5	8.5039	8.5351	0.0312
L	0.3	1.0466	1.2362	0.1897
M	0.1	5.8749	6.0531	0.1783

Table 17

MW - Difference of Single and Joint Analysis for Reference N1

N1 Samples	Target Weight	Dup-single	Trip-single	Trip-Dup
I	0.1	6.1396	6.1957	0.0561
J	0.3	0.9565	1.0526	0.0962
K	0.5	8.5496	8.5768	0.0272
L	0.7	0.8174	0.8313	0.0139
M	0.9	0.0000	0.0000	0.0000

Similar information gain trends are seen for references for H1 and N1 where 50% and 10% weight contributions see an increase match logLR values. It is noted that the differences in information gain are greater for reference A1 and G1 at the 50% weight. This was because the single logLRs were lower for these references than those for reference H1 and N1.

The greatest gains in information are seen between single and duplicate and single and triplicate analysis when different mixture weight combinations are analyzed jointly. Adding an additional sample to the joint analysis showed small gains in identification information.

Figure 7

MW - Histogram for H1 showing the difference between single and joint analysis

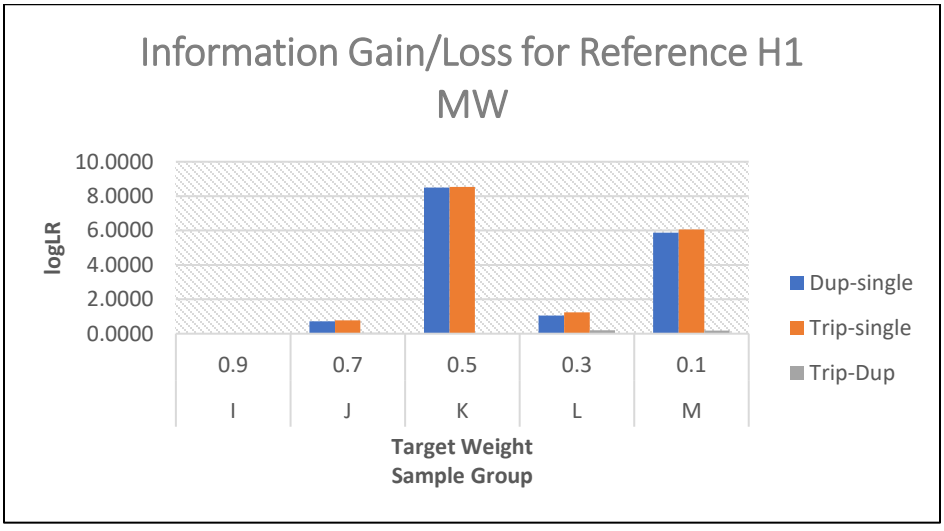
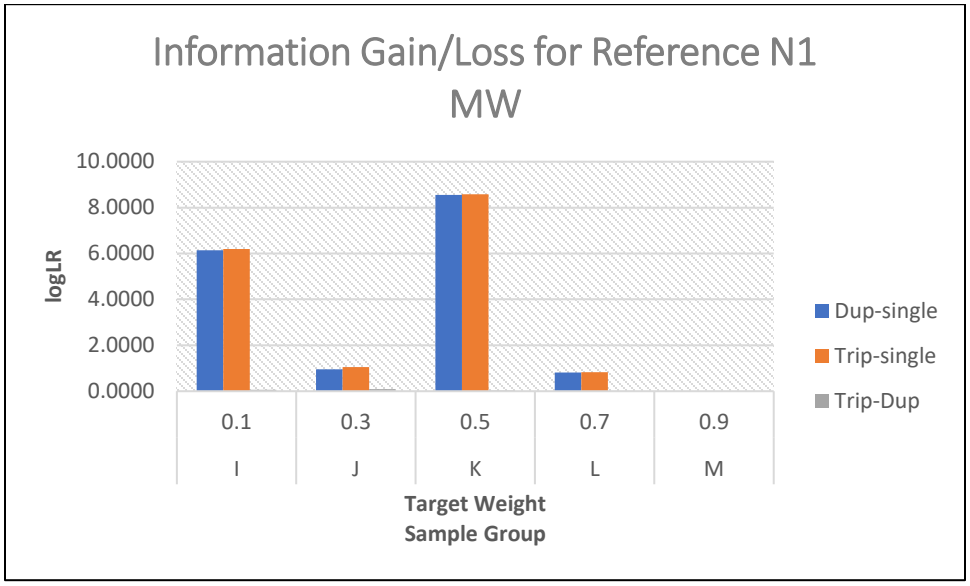


Figure 8

MW - Histogram for N1 showing the difference between single and joint analysis



LT-DNA

Information gain using joint analysis was consistently obtained for the minor contributor in low template samples for each sample group. The major contributor at the same template concentration also saw gains but not to the same extent. When using mixture weight as a variable, there was little difference between the duplicate and triplicate logLRs. Here, the gain is incremental when analyzing LT-DNA samples jointly as seen for reference N1 in sample I.

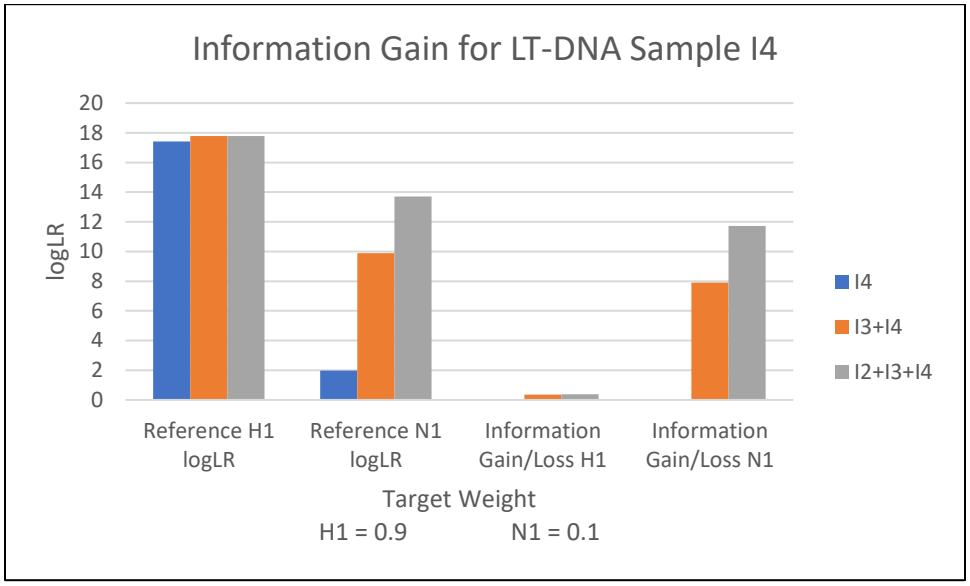
Table 18

Difference Between Single and Joint Analysis Results for LT-DNA Sample I4

Sample	Template Concentration (ng)	TA Weight (H1/N1)	Reference H1 logLR	Reference N1 logLR	Information Gain/Loss H1	Information Gain/Loss N1
I4	0.125	0.9132/0.0868	17.4153	1.9852		
I3+I4	0.25 + 0.125	0.8714 + 0.9087/ 0.1286 + 0.0913	17.7828	9.8831	0.3675	7.8979
I2+I3+I4	0.5 + 0.25+ 0.125	0.8872 + 0.8670 + 0.8618/ 0.1128 + 0.1330 + 0.1382	17.7879	13.7131	0.3726	11.7279

Figure 9

LT-DNA - Histogram showing increase in identification information for Sample I4



Negligible gains are seen for H1 in sample I as it was the major contributor at 90%. Table 18 and Figure 9 demonstrate how information increased for the minor contributor as more data was implemented in the JLF.

Table 19

LT-DNA - Difference Between Single and Joint Analysis Results for A1

A1 Samples	Target Weight	Avg TA Weight	Dup-single	Trip-single	Trip-Dup
B	0.9	0.8722	4.3333	6.6763	2.3430
C	0.7	0.6555	5.1950	7.0154	1.8204
D	0.5	0.4430	1.0953	1.9679	0.8726
E	0.3	0.2789	3.8418	3.3096	-0.5322
F	0.1	0.1064	5.7164	8.2118	2.4954

Table 20

LT-DNA - Difference Between Single and Joint Analysis Results for G1

G1 Samples	Target Weight	Avg TA Weight	Dup-single	Trip-single	Trip-Dup
B	0.1	0.1278	5.3227	17.1853	11.8626
C	0.3	0.3445	10.1584	11.8278	1.6694
D	0.5	0.5555	6.6943	6.2894	0.6687
E	0.7	0.7211	3.6723	2.7292	-0.9431
F	0.9	0.8942	0.0035	-0.0046	-0.0081

Tables 19 and 20 show the difference between single and joint analysis for references A1 and G1. Information gain is again greatest in the samples where the references have 10% mixture weight. This increase continues from duplicate to triplicate analysis. A minor decrease in identification information is seen for reference G1 when its weight is 90% between the triplicate and single sample.

Sample C also shows an increase of information for A1 and G1 that is not expected. This was due to differences in the target weight and the weights calculated by TrueAllele in the individual analysis data for C4. TrueAllele determined that C4 had a ratio close to 50/50 in the single analysis. Despite the TrueAllele average weight for sample C being close to the target weight, the difference between single and joint analysis shows a gain consistent with mixture weight combinations of 50/50 samples and unequal weighted samples. Figures 10 and 11 show the histograms for A1 and G1.

Figure 10

LT-DNA - Histogram showing change in identification information for A1

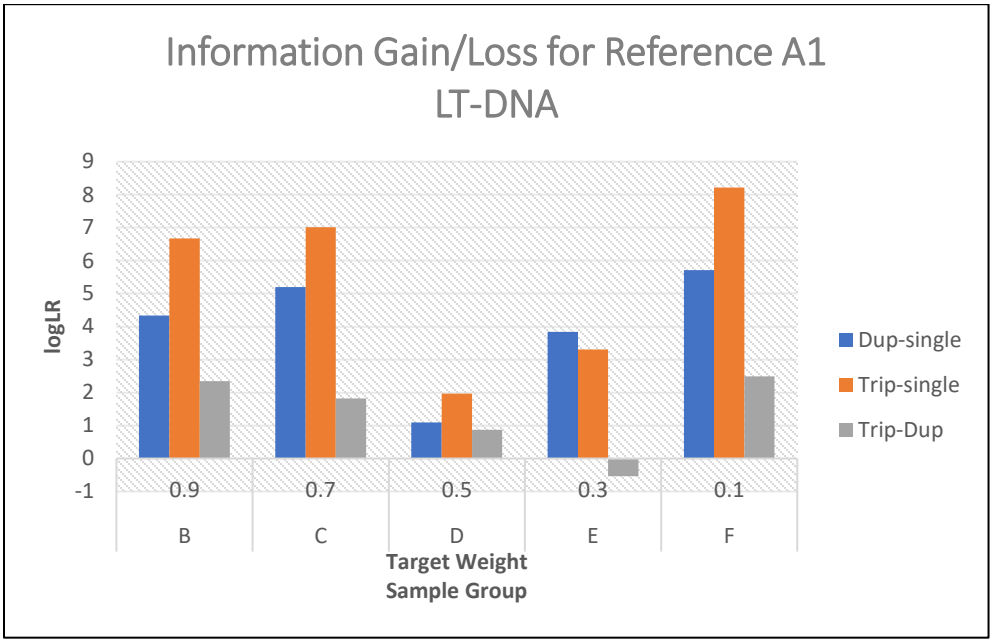
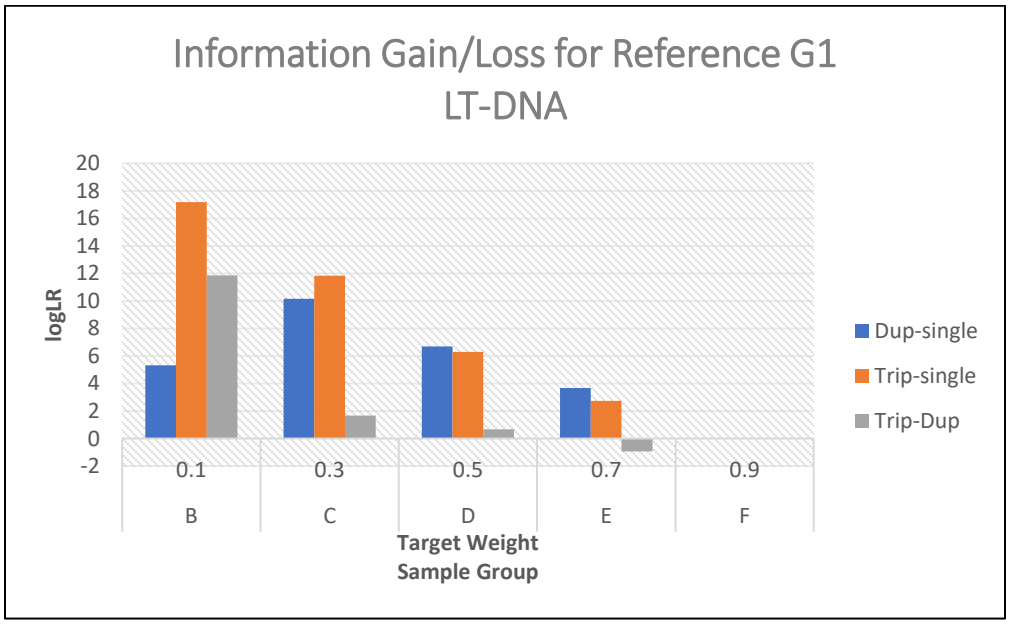


Figure 11

LT-DNA - Histogram showing change in identification information for G1



Tables 21 and 22 show the gain in identification information using joint analysis for references H1 and N1. Reference H1 showed the similar anomaly in sample L as was seen for references A1 and G1 in sample C. Both samples were a 70/30 mixture weight. Though unexpected, information gain was still the result. Reference N1 showed the expected trend as seen in sample I where gain in information increased when low template minor contributor samples were analyzed jointly with other samples. Figures 12 and 13 show the histograms for references H1 and N1. Sample K showed the least amount of information gain as expected for a 50/50 mixture, but a small gain was still observed.

Table 21

LT-DNA - Difference Between Single and Joint Analysis Results for H1

H1 Samples	Target Weight	Avg TA Weight	Dup-single	Trip-single	Trip-Dup
I	0.9	0.8849	0.3675	0.3726	0.0051
J	0.7	0.6708	3.0218	7.1139	4.0921
K	0.5	0.5014	1.2561	0.6962	-0.5599
L	0.3	0.3183	14.9122	14.8888	-0.0234
M	0.1	0.1777	6.9408	7.9544	1.0136

Table 22

LT-DNA - Difference Between Single and Joint Analysis Results for N1

N1 Samples	Target Weight	Avg TA Weight	Dup-single	Trip-single	Trip-Dup
I	0.1	0.1151	7.8979	11.7279	3.8300
J	0.3	0.3292	5.1346	11.6317	6.4971
K	0.5	0.4987	2.297	1.4597	-0.8373
L	0.7	0.6818	3.4085	3.8735	0.4650
M	0.9	0.8223	0.9152	0.9154	0.0002

Figure 12

LT-DNA - Histogram showing change in identification information for H1

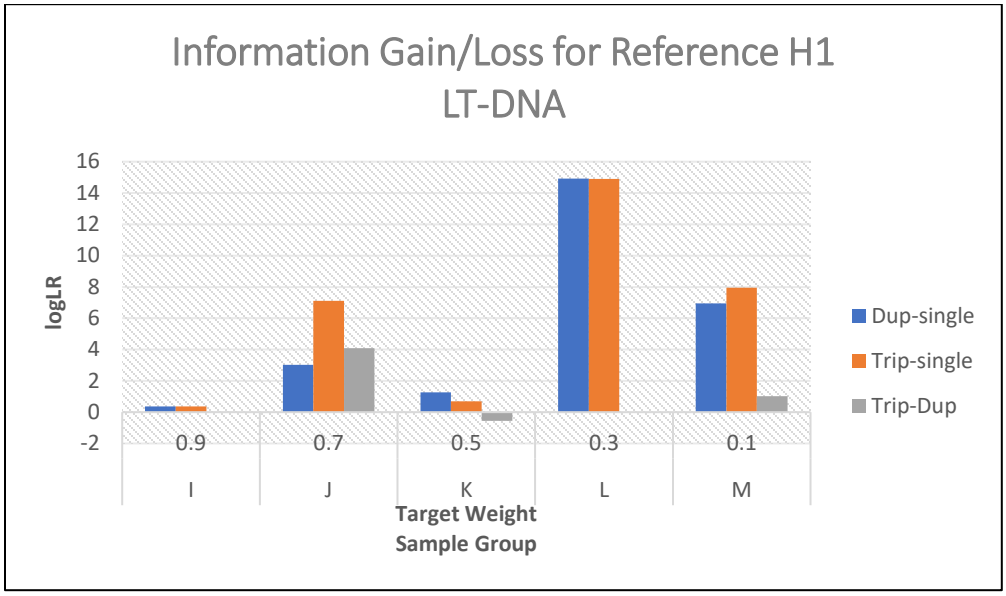
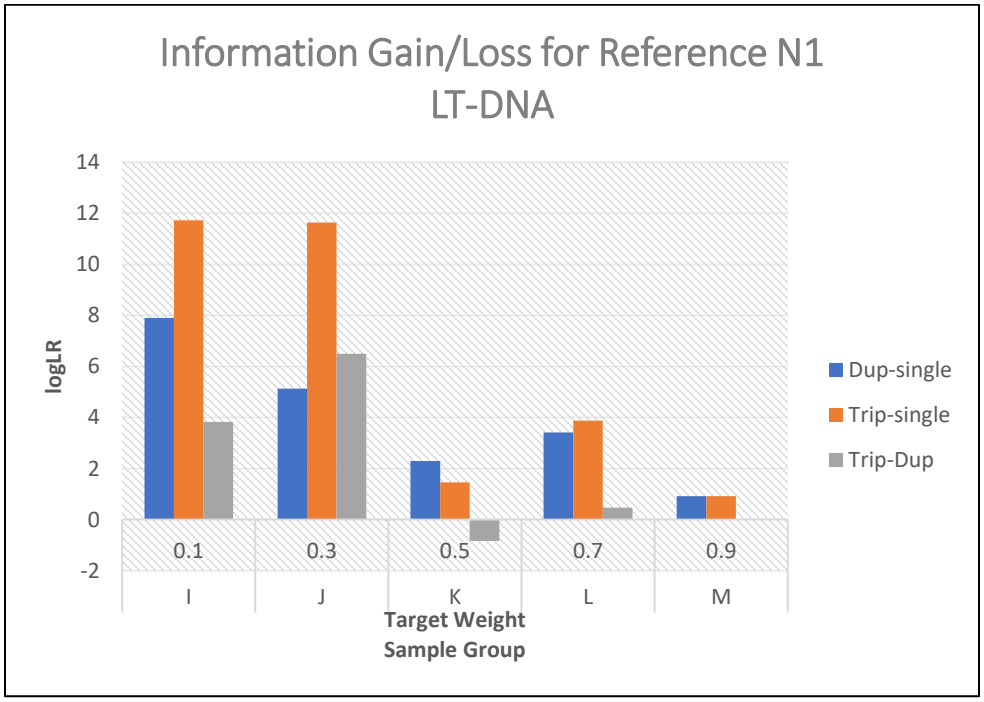


Figure 13

LT-DNA - Histogram showing change in identification information for N1



Complex Mixtures

This data set provided a close simulation to evidence data where mixture weight, template concentration, and number of contributors are random making mixture analysis difficult. The application of the JLF in this data set resulted in significant incremental gain in information for the contributor with the lowest MW. Reversing the roles of major and minor contributors was a consideration made when selecting these complex samples. The data complemented each other well when analyzed jointly. The information gain for reference K01 as the more minor contributor in sample 3.6 is shown in Table 23 and in Figure 14. Reference ASB is the major contributor in this sample and the logLR remains relatively unchanged with each analysis method.

Table 23

Difference Between Single and Joint Analysis Results for Complex Mixture 3.6

Sample	Mixture Weight (ASB/K01)	Reference ASB logLR	Reference K01 logLR	Information Gain/Loss ASB	Information Gain/Loss K01
3.6	0.9006/0.0254	20.3219	3.3181		
1.1 + 3.6	0.1349 + 0.8729/ 0.5015 + 0.0612	20.3211	13.5960	-0.0008	10.2779
1.1 + 3.6 + 4.2	0.4466 + 0.8616 + 0.0521/ 0.4656 + 0.0286 + 0.6233	20.3219	18.1666	0.0000	14.8485

Figure 14

Histogram showing change in identification information for Sample 3.6

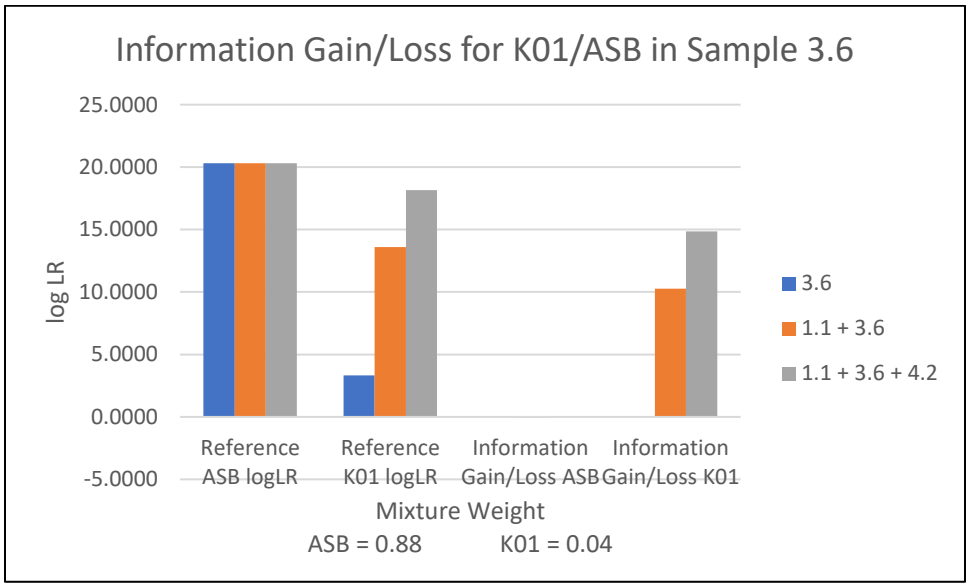


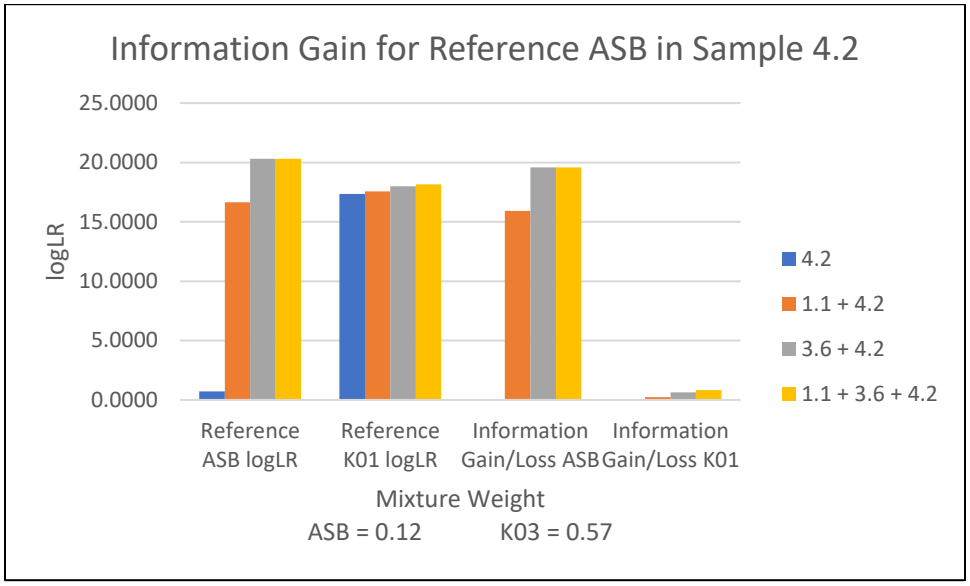
Table 24

Difference Between Single and Joint Analysis Results for Complex Mixture 4.2

Sample	Mixture Weight ASB/K01)	Reference ASB logLR	Reference K01 logLR	Information Gain/Loss ASB	Information Gain/Loss K01
4.2	0.1372/0.5404	0.7290	17.3530		
1.1 + 4.2	0.4383 + 0.0846/ 0.2851 + 0.5493	16.6537	17.5757	15.9247	0.2227
3.6 + 4.2	0.9076 + 0.0538/ 0.0193 + 0.5782	20.3219	17.9900	19.5929	0.6370
1.1 + 3.6 + 4.2	0.4466 + 0.8616 + 0.0521/ 0.4656 + 0.0286 + 0.6233	20.3219	18.1666	19.5929	0.8136

Figure 15

Histogram showing change in identification information for Sample 4.2



Sample 4.2 reversed the roles for references ASB and K01. ASB was the minor contributor and a significant information gain of 16 and 20 ban was obtained when sample 4.2 was analyzed jointly with samples 1.1 and 3.6 in duplicates and triplicate, respectively, as seen in Table 24 and Figure 15. As the major contributor, reference K01 had large logLRs regardless of analysis method but did have slight information gain unlike reference ASB when it was the major contributor in sample 3.6.

Table 25

Change in Identification Information for Reference ASB

ASB Samples	Avg Weight	Dup-single	Trip-single	Trip-Dup
1.1	0.4657	5.3039	6.9026	1.5987
2.8	0.3785	2.3963		
3.6	0.8784	-0.0008	0.0000	0.0008
4.2	0.1234	17.7588	19.5929	1.8341

Table 26

Change in Identification Information for Reference K01

K01 Samples	Avg Weight	Dup-single	Trip-single	Trip-Dup
1.1	0.4515	4.8367	6.9709	2.1342
2.8	0.6254	0.5662		
3.6	0.0384	10.2779	14.8485	4.5706
4.2	0.5728	0.4298	0.8136	0.3837

Change in identification information for references ASB and K01 in each sample between joint and single interpretation are shown in Tables 25 and 26. Information gain was the greatest when the references were the more minor contributors at average mixture weights of 0.1234 and 0.0384 for ASB and K01, respectively. Sample 2.8 was not included in the triplicate analysis because the pairings that better reversed major and minor contributors for each reference were samples 3.6 and 4.2. Sample 1.1 was used as the sample where the mixture weights for each reference were relatively equal. Figures 16 and 17 show how using joint analysis provides greater identification for those samples that usually result in diffuse probabilities and lower match logLRs when analyzed independently: mixtures with contributor weight ratios of 50/50 and low template minor contributors.

Figure 16

Histogram showing change in identification information for ASB

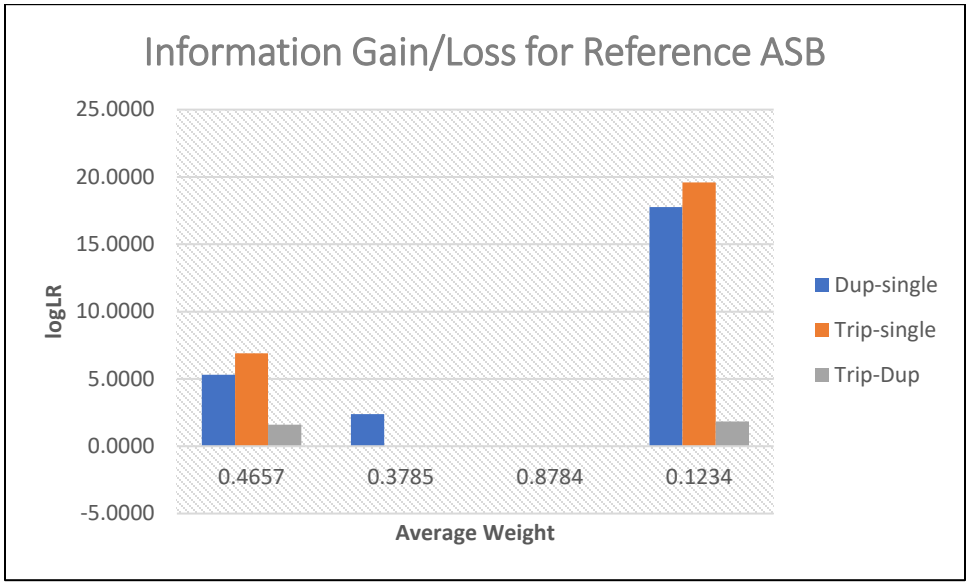
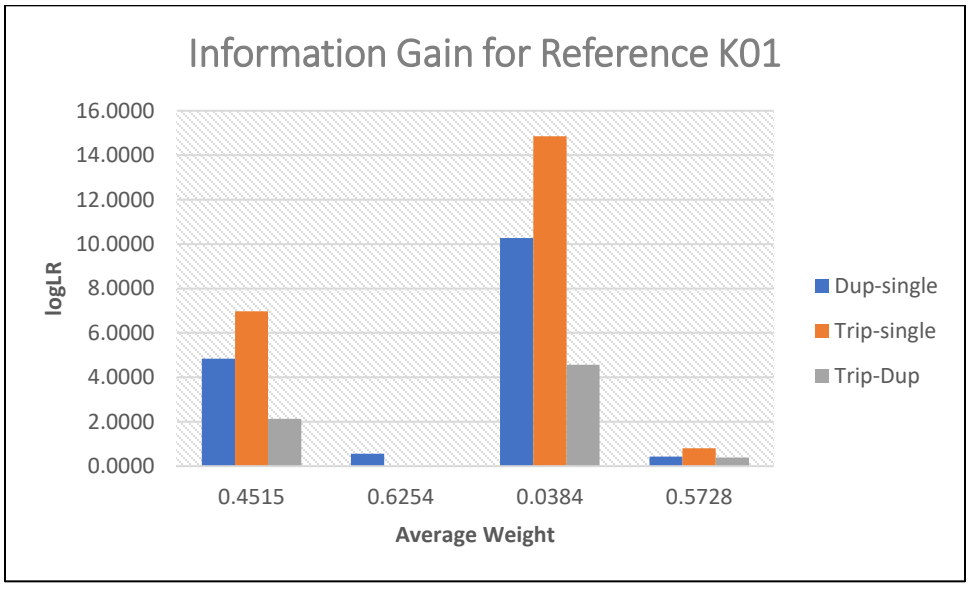


Figure 17

Histogram showing change in identification information for K01



Conclusion

Higher match statistics were consistently obtained when samples were analyzed jointly compared to the independent analysis of each sample. Mixture weight and template concentration played a significant role in how much identification information was elicited from the data when analyzed in duplicate and triplicate. Information gain was seen consistently in all the samples but in greater amounts for samples with a 50/50 mixture weight and for contributors whose weights were close to or lower than 10%. Overall, major contributors saw little to no change in their match logLR values but in several cases a slight increase was observed.

These research results impact the field of forensic biology analysis because joint likelihood functions can increase the probability distribution across all loci resulting in higher match statistics. This would support using PG as the standard for complex mixture interpretation. Another suggestion would be to modify DNA evidence collection and amplification procedures for LT-DNA and mixture samples so that more data is available for PG software programs like TrueAllele to analyze the data jointly using the JLF (Ballantyne et al., 2013). Certain limitations can exist with using TrueAllele which include cost of purchasing the software and understanding the mathematical and statistical concepts inherent with PG.

Future directions of this research would be to generate laboratory data and use TrueAllele mixture interpretation to perform the analysis. Touch DNA samples can be collected using multiple swabs to provide the independent sets of data needed for joint analysis. Different substrates such as glass, plastic, wood, metal, etc. can be used to assess how much touch DNA is retrieved from different surfaces and if probabilistic genotyping can preserve the identification information using joint likelihood functions.

References

Ballantyne, J., Hanson, E. K., & Perlin, M. W. (2013). DNA mixture genotyping by probabilistic computer interpretation of binomially-sampled laser captured cell populations: Combining quantitative data for greater identification information. *Science and Justice, 53*(2), 103-114. https://doi.org/10.1016/j.scijus.2012.04.004

Bauer, D. W., Butt, N., Hornyak, J. M., & Perlin, M. W. (2020). Validating TrueAllele® Interpretation of DNA Mixture Containing up to Ten Unknown Contributors. *Journal of Forensic Sciences, 65*(2), 380-398. https://doi.org/10.1111/1556-4029.14204

Butler, J. M. (2005). *Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers* (2nd ed.) Academic Press.

Butler, J. M. (2010). *Fundamentals of Forensic DNA Typing*. Academic Press.

Butler, J. M. (2015). *Advanced Topics in Forensic DNA Typing: Interpretation.* Academic Press.

Gill, P., Gusmão, L., Haned, H., Mayr, W. R., Morling, N., Parson, W., Prieto, L., Prinz, M., Schneider, H., Schneider, P. M., & Weir, B. S. (2012). DNA commission of the International Society of Forensic Genetics: Recommendations on the evaluation of STR typing results that may include drop-out and/or drop-in using probabilistic methods. *Forensic Science International: Genetics, 6*(6), 679-688. https://doi.org/10.1016/j.fsigen.2012.06.002

Greenspoon, S. A., Schiermeier-Wood, L., & Jenkins, B. C. (2015). Establishing the Limits of TrueAllele® Casework: A Validation Study. *Journal of Forensic Sciences, 60*(5), 1263-1276. https://doi.org/10.1111/1556-4029.12810

Hornyak, J. M., Herbert, T., Allan, W. P., & Perlin, M. W. (2015, August 4). *Baltimore Police Department TrueAllele® Validation.* Cybergenetics.

Kwong, K. (2017). The Algorithm Says You Did It: The Use of Black Box Algorithms to Analyze Complex DNA Evidence. *Harvard Journal of Law & Technology, 31*(1), 275-301. http://jolt.law.harvard.edu/assets/articlePDFs/v31/31HarvJLTech275.pdf

National Institute of Standards and Technology. (2014). *NIST 1036 U.S. Population Dataset – 29 autosomal STR loci and 23 Y-STR loci*. STRBase (SRD-130). Retrieved November 10, 2020, from https://strbase.nist.gov/NISTpop.htm

Perlin, M. W. (2013). When Good DNA Goes Bad. *Journal of Forensic Research, S11*(3). http://dx.doi.org/10.4172/2157-7145.S11-003

Perlin, M. W. (2015). Inclusion probability for DNA mixtures is a subjective one-sided match statistic unrelated to identification information. *J Pathol Inform, 6*(59). DOI: 10.4103/2153-3539.168525

Perlin, M. W., Belrose, J. L., Duceman, B. W. (2013). New York State TrueAllele® Casework Validation Study. *Journal of Forensic Sciences, 58*(6), 1458-1466. https://doi.org/10.1111/1556-4029.12223

Perlin, M. W., Dormer, K., Hornyak, J. M., Schiermeier-Wood, L., & Greenspoon, S. (2014). TrueAllele Casework on Virginia DNA Mixture Evidence: Computer and Manual Interpretation in 72 Reported Criminal Cases. *PLOS ONE, 9*(3), Article e92837. https://doi.org/10.1371/journal.pone.0092837

Perlin, M. W. & Galloway, J. (2012, May-June). Computer DNA Evidence Interpretation in the Real IRA Massereene Terrorist Attack. *Evidence Technology Magazine, 10*(3), 20-23. https://www.nxtbook.com/nxtbooks/evidencetechnology/20120506/index.php?startid=20#/p/20

Perlin, M. W., Hornyak, J. M., Sugimoto, G., & Miller, K. W. P. (2015). TrueAllele® Genotype

Identification on DNA Mixtures Containing up to Five Unknown Contributors. *Journal of Forensic Sciences, 60*(4), 857-868. https://doi.org/10.1111/1556-4029.12788

Perlin, M. W., Kadane, J. B., & Cotton, R. W. (2009). Match likelihood ratio for uncertain genotypes. *Law, Probability and Risk*, *8*(3), 289–302. https://doi.org/10.1093/lpr/mgp024

Perlin, M. W., Legler, M. M., Spencer, C.E., Smith, J. L., Allan, W. P., Belrose, J. L., & Duceman, B. W. (2011). Validating TrueAllele® DNA Mixture Interpretation. *Journal of Forensic Sciences*, *56*(6), 1430-1447. https://doi.org/10.1111/j.1556-4029.2011.01859.x

Perlin, M. W. & Sinelnikov, A. (2009). An Information Gap in DNA Evidence Interpretation. *PLoS One 4*(12): e8327. https://doi.org/10.1371/journal.pone.0008327

Perlin, M. W. & Szabady, B. (2001). Linear Mixture Analysis: A Mathematical Approach to Resolving Mixed DNA Samples. *Journal of Forensic Sciences*, *46*(6), 1372-1378. https://doi.org/10.1520/JFS15158J