TrueAllele® Casework Separates DNA Mixtures that Share Alleles

Meredith A. Clarke, MS; Jennifer Hornyak, MS;
William P. Allan, MS; and Mark W. Perlin, PhD, MD, PhD

Cybergenetics, Pittsburgh, PA

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Corresponding Author:

Mark W. Perlin, PhD, MD, PhD
Chief Scientific Officer
Cybergenetics
160 North Craig Street, Suite 210
Pittsburgh, PA 15213 USA
(412) 683-3004
(412) 683-3005 FAX
perlin@cybgen.com

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Introduction

A mixture is an item of biological evidence having DNA contributions from two or more individuals. Mixtures arise naturally in casework, but can be more difficult to interpret manually than single source samples. When the contributors to a mixture are related, or they share much genetic content, manual review becomes even more challenging.

Allele sharing in DNA mixtures can be classified by how many alleles appear at a locus. For example, a parent and a child will share half of their alleles, with one allele shared at every locus. This is a *high* level of allele sharing, seen amongst family members, and having relatively few alleles appearing at a locus. A *low* level of allele sharing is typically observed among unrelated individuals from different populations, where there are more distinct locus alleles. A *middle* level of allele sharing occurs among individuals from the same population who are more distantly related, and more alleles may be seen. Allele sharing can affect how well some interpretation methods are able separate a DNA mixture into its component genotypes.

Short tandem repeat (STR) testing of DNA evidence produces a quantitative pattern of peaks at a locus. The pattern of peaks and their heights roughly relates to the DNA amount of each contributor genotype or genetic type in the mixture. There is natural variation inherent in this STR pattern due to random polymerase chain reaction (PCR) amplification artifacts and effects. Other variation in the STR patterns can arise from degraded template DNA, contributor allele sharing, pipetting error, among other factors.

Computer interpretation of DNA mixtures can address PCR data variation and resolve allele sharing. TrueAllele® Casework (Cybergenetics, Pittsburgh, PA) is an established computer system for interpreting DNA mixtures. The system models DNA mixture patterns and their statistical variation using hierarchical Bayesian probability equations. Markov chain Monte Carlo (MCMC) methods solve these high-dimensional multivariate equations by statistical sampling from the posterior probability distribution. TrueAllele has been used in criminal casework since 2009. Several validation studies have been published that assess TrueAllele's reliability.

Computer interpretation of mixtures, regardless of the level of allele sharing, has potential sensitivity, specificity and reproducibility advantages over manual human review. The computer can extract more identification information from the data, reduce the number of false matches, quantify DNA exclusions, and provide reproducible results from independent analyses. Moreover, TrueAllele represents mixture allele sharing in a linear matrix model whose solution separates contributor genotypes.

This validation study assesses the TrueAllele system’s performance on laboratory synthesized DNA mixtures that have different levels of allele sharing between contributors. The two person mixtures were developed from known contributors in known ratios, and amplified using the Identifiler® STR panel. Sensitivity, specificity, and reproducibility of TrueAllele were assessed using the likelihood ratio, a match statistic that is a standard measure of identification information. Mixture weight reproducibility was also measured to help quantify the sensitivity information.

Materials

Design

The National Institute of Standards and Technology (NIST, Bethesda, MD) developed three DNA groups of DNA mixtures, each group formed from two designated contributors. Five known individuals had their genotypes combined in pairs over a range of mixing proportions (10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, and 90:10). Both 1000 picogram (pg) (high amount) and 100 pg (low amount) DNA templates were used. Table 1 gives the sample name, ethnicity and genotype of these five individuals.

The average number of alleles per locus was measured to describe the level of allele sharing for each pair of individuals as low, middle or high (Table 2). The low allele sharing genotype pair combined TT50722 and TT50916, the middle allele sharing paired TT50722 and GT37019, while the high allele sharing pair mixed UT57309 and MT95364. Each mixture sample was amplified at both 1000 pg (53 samples) and 100 pg (81 samples), for a total of 134 mixture samples.

The low allele sharing mixtures had four alleles at 10 of 16 loci (15 STR, plus Amelogenin). The middle allele sharing level had mainly three allele loci, along with some four allele loci. The high allele sharing mixtures had all the loci sharing at least one allele, and at one locus (vWA) only one allele (17) was present.

Data

The mixture samples were amplified by NIST using an Applied Biosystems (Foster City, CA) Identifiler® STR panel, and read out on an ABI 3100 series genetic analyzer. The 1000 pg samples were generally amplified twice, and the 100 pg samples amplified three times. One 1000 pg mixture (low sharing in a 80:20 ratio) was amplified only once. The STR electropherogram data were recorded in .fsa files that were sent to Cybergenetics.

Methods

Analysis

A trained TrueAllele analyst processed the mixture .fsa file data through the TrueAllele Casework system. The analyst had TrueAllele independently process the samples in quadruplicate, which facilitated determining the reproducibility of mixing proportions and match statistics. The 1000 pg samples were processed in TrueAllele using burn-in/read-out times 50,000/50,000 MCMC cycles. The 100 pg samples were run with burn-in/read-out times of 100,000/100,000 cycles.

TrueAllele processing objectively inferred contributor genotypes, without knowledge of comparison references. Afterwards, the inferred mixture evidence genotypes were compared to the known reference genotypes, relative to a population, in order to calculate DNA likelihood ratio (LR) match statistics. A contributor genotype's

corresponding reference was identified through its maximum match score. Each LR was recorded as its base ten logarithm; the log(LR) is a standard measure of information, kept in "ban" units. The match statistics were calculated using NIST's African-American, Caucasian and Hispanic population frequencies, with a co-ancestry coefficient (theta value) of 1%. For conservatism and consistency, the reported match score was the smallest of the three values.

Human and instrument variation can occur when creating mixtures designed in preset ratios. Therefore, TrueAllele assessed the reproducibility of mixture weights on the data set for the six mixture groups (three allele sharing levels, for two DNA amounts). The information sensitivity response to varying contributor amounts was determined. Match statistic specificity and reproducibility were also assessed.

Reproducibility

The reproducibility of a method describes how well it values can be replicated across independent measures on the same data. Once more than two values have been calculated on the same data, reproducibility can be quantified using a within-group standard deviation, measuring the variation around the average result within the data group. In this study, the within-group standard deviation is calculated for contributor mixture weight, and for log(LR) match statistics in each of the six mixture groups.

Results

Mixture weight

Table 3 shows the within-group standard deviation of the TrueAllele inferred mixture weights of the samples across four computer runs. With more DNA, the inferred mixture weight was less variable than with lower amounts of DNA. For the 1000 pg samples, the within-group standard deviation of the mixture weight was about 0.5% (or less) over all allele sharing levels. The within-group standard deviation of the mixture weight for the 100 pg samples was between 2.5-5% across all allele sharing levels. Thus, regardless of the level of allele sharing, TrueAllele inferred mixture weight consistently with little variation.

Sensitivity

With the reproducibility the mixture weights of each contributor proportion established, the method's information response and sensitivity was then examined. Sensitivity measures the extent to which a mixture interpretation method correctly includes a true contributor. TrueAllele's information sensitivity was assessed separately for each allele sharing level, for both 1000 pg and 100 pg mixtures.

The log(LR) information of each genotype match was compared with the log(w*[DNA]) DNA amount, as shown in Figure 1, where w is the average contributor mixture weight (percent), and [DNA] is the amount of template DNA (pg). The low template DNA data (Figure 1, right column) best shows the information response. As

the amount of contributor DNA increases (x-axis), there is a commensurate increase in the DNA match statistic (y-axis).

The high template DNA mixtures (Figure 1, left) partially obscure this linear relationship, since those mixtures are relatively easy for the computer to solve, resulting in the information saturation seen in the upper right portion of the scatterplots. This saturation is particularly evident with low allele sharing, since almost all the mixture genotypes (for both major and minor contributors) are highly informative. Note the information dip in linear progression with 50:50 mixtures, which are less informative since the contributors cannot be differentiated by their mixture weight.

The match information for each of the six mixture groups is shown (Table 4, average information column μ). Note that the 100 pg groups have similar means of 9.28 ban (low allele sharing), 7.93 ban (middle sharing) and 9.91 ban (high). The 1000 pg groups means are close, as well.

Pairwise t-tests (Table 5) showed that there was no statistical difference between the log(LR) means of the three low DNA template groups, since the p-values were all greater than 0.05 (column p). This result provides statistical support for TrueAllele's ability to preserve DNA identification information in the presence of high allele sharing. Moreover, with larger DNA amounts of 1000 pg, there was no statistical difference between the means of the middle (14.95 ban) and high (14.07) allele sharing groups ($p = 0.36 > 0.05$). The high template, low allele sharing mixture group was statistically different from the other two high template groups ($p < 0.01$), presumably due to the relative ease with which TrueAllele interpreted the more separated mixtures that had abundant DNA.

Specificity

Specificity measures the extent to which a mixture interpretation method correctly excludes a non-contributor. To evaluate specificity, each inferred genotype was compared with ten thousand randomly generated genotypes from each of the FBI African-American (BLK) and Caucasian (CAU) populations. There were around 700,000 genotype comparisons made for the 1000 pg samples across each allele sharing level, and 1,080,000 comparisons at each level for the 100 pg mixtures (Table 6).

Table 6 summarizes the specificity results for each population at each allele sharing level and DNA amount. With likelihood ratios, a negative log(LR) value indicates exclusion, just as a positive log(LR) value supports inclusion.

For the 1000 pg high template DNA mixture samples, the log(LR) non-contributor match statistics were highly exclusionary, averaging around -24 ban (one over a trillion trillion). There was one comparison in the low allele sharing level against the Caucasian population having a positive log(LR) value, for a false positive rate of 4.717×10^{-7}.

The low template 100 pg samples showed more positive log(LR) events, giving a false positive rate of 3.704×10^{-6} over all allele sharing levels and ethnic groups. Finding more false inclusions with smaller DNA amounts is expected, since these DNA quantities produce less certain genotypes. Regardless of allele sharing level, the inferred genotypes were highly specific.

Reproducibility

Over four replicate computer runs, TrueAllele reproducibility was measured across the three allele sharing levels for the DNA mixtures using within-group standard deviation (Table 4, column σ_w). In the 1000 pg mixture groups, the σ_w at each of the allele sharing levels was less than or equal to 0.5 ban, for a multiplicative factor of 3. In the 100 pg mixture groups, the within-group standard deviation was about 1 ban, or a factor of 10.

The level of allele sharing had little affect on TrueAllele reproducibility. If anything, higher allele sharing gave more reproducible results (Table 4, 100 pg groups), possibly due to the greater DNA quantities in stacked alleles that reduced peak variation.

Conclusions

Mixtures of related individuals can share more alleles at a locus than mixtures from unrelated individuals. Increased allele sharing between contributor genotypes can affect STR mixture interpretation, particularly human analysis methods based on STR peak height thresholds that discard allele quantity data. Since genotype modeling methods such as TrueAllele make better use of the quantitative data, and mathematically model the allele sharing of contributor genotypes, they can better separate the genotypes present in mixtures of relatives, and preserve DNA match information.

This study examined the computer's performance across low, middle, and high levels of allele sharing for both high and low template DNA amounts. Mixture weight

was found to be reproducible, regardless of allele sharing level and DNA amount. DNA match information, measured as log(LR) match statistics, was reproducible for each allele sharing level and DNA amount. TrueAllele was sensitive and specific in all these mixture groups. With the exception of one group (high template, low sharing), there was no significant difference in TrueAllele's average match information across the low, middle and high allele sharing mixture groups for high or low template amounts.

TrueAllele is a useful computational tool that performs accurately and reliably on DNA mixtures where there may be higher allele sharing, such as mixtures of relatives. This study provides empirical support for TrueAllele's ability to resolve DNA mixtures that contain related individuals.

Acknowledgements

The Applied Genetics Group at the National Institute of Standards and Technology (NIST) in Bethesda, MD provided the STR data files used in this study.

Tables

Table 1: Contributor genotypes. The names, ethnicities, and Identifiler locus genotypes are shown for the mixture references.

Locus	TT50722 Caucasian	TT50916 African-American	GT37019 African-American	UT57309 Caucasian	MT95364 African-American
AMELO	X,X	X,Y	X,Y	X,X	X,Y
CSF1PO	12,13	8,9	11,12	11,12	12,12
D13S317	8,9	11,13	11,12	11,12	11,12
D16S539	9,12	12,13	11,11	10,12	11,11
D18S51	12,15	15,16	17,18	12,14	12,14
D19S433	12,14	13,14.2	14,14	14,14	11,13
D21S11	30,31	27,28	28,30	28,28	29,29
D2S1338	16,24	20,25	16,17	16,17	16,17
D3S1358	15,16	14,18	16,16	16,17	16,16
D5S818	11,12	9,11	11,12	11,12	11,11
D7S820	9,11	8,10	10,11	12,12	10,10
D8S1179	11,14	13,15	14,15	12,14	12,13
FGA	23,24	22,23	23,24	20,25	24,24
TH01	8,10	7,9	6,7	9,9.3	8,9
TPOX	8,11	10,11	8,9	8,8	10,11
vWA	16,19	14,15	17,17	17,17	17,17

Table 2: Allele sharing. The number of loci containing different numbers of distinct alleles (1, 2, 3 or 4) is shown, along with the average number of alleles per locus.

Sharing level	Loci with 1 allele	Loci with 2 alleles	Loci with 3 alleles	Loci with 4 alleles	Average alleles per locus
low	0	1	5	10	3.56
middle	0	5	8	3	2.88
high	1	9	6	0	2.31

Table 3: Mixture weight reproducibility. The table measures contributor mixture weight reproducibility. Shown are the DNA amount (pg), allele sharing level, standard deviation (σ) and within-group standard deviation (σ_w). The mixture weight scale ranges from 0% to 100%. The mean value μ was 50% in every group, averaged over major and minor contributors.

DNA amount	Allele sharing level	σ	σ_w
100	low	25.53	2.55
100	middle	24.99	3.33
100	high	21.17	4.88
1000	low	26.61	0.52
1000	middle	26.83	0.28
1000	high	26.60	0.47

Table 4: Match information. Shown are information sensitivity and reproducibility over four TrueAllele computer runs for each mixture sample. At each DNA amount (pg) and allele sharing level, the average information (μ), total standard deviation (σ) and within-group standard deviation (σ_w) are shown in log(LR) ban units.

DNA Amount	Allele Sharing Level	μ	σ	σ_w
100	low	9.28	8.07	1.08
100	middle	7.93	6.61	0.89
100	high	9.91	5.05	0.71
1000	low	17.43	3.65	0.45
1000	middle	14.95	4.06	0.40
1000	high	14.07	3.95	0.50

Table 5: Statistical significance. Significance was measured using pairwise *t*-tests to compare log(LR) means different allele sharing groups for a fixed DNA amount. The table lists the DNA amount (pg), the two compared mixture groups, the difference of the group means (first – second), along with the *t*-statistic and *p*-value.

Amount	First	Second	Difference	*t*	*p*
100	low	middle	1.3568	0.9574	0.3405
100	low	high	-0.6213	-0.4806	0.6318
100	middle	high	-1.9781	-1.7505	0.0829
1000	low	middle	2.4818	2.6730	0.0094
1000	low	high	3.3551	3.6705	0.0005
1000	middle	high	0.8733	0.9210	0.3602

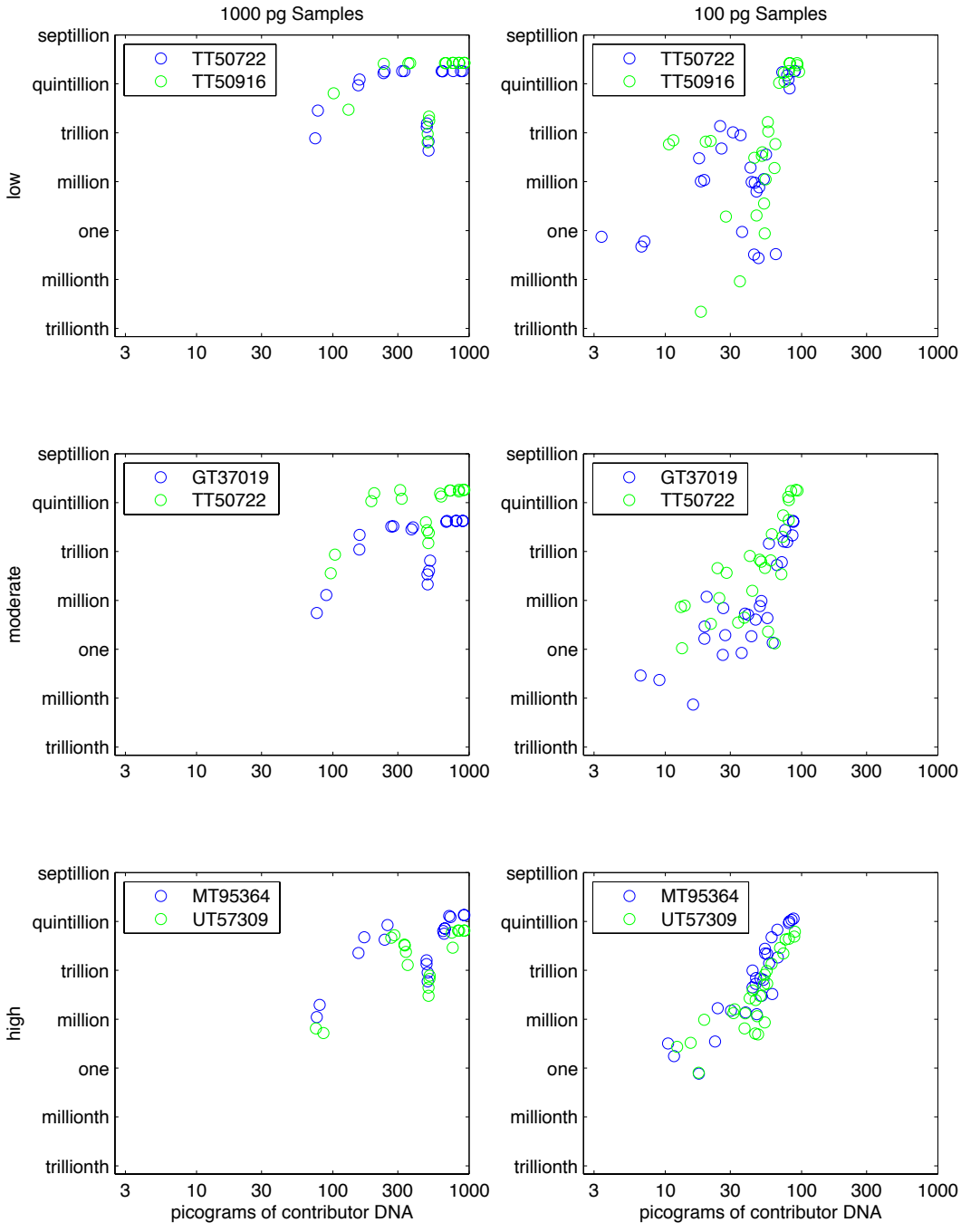
Table 6: Match specificity. Comparison was made between the inferred genotypes and ten thousand randomly generated non-contributors from FBI African-American (BLK) and Caucasian (CAU) populations. Shown are the number of comparisons and log(LR) statistics: minimum, mean, maximum and standard deviation. The number of times log(LR) was greater than zero indicates potential false inclusions.

		1000 pg						100 pg					
		count	min	mean	max	std	>0	count	min	mean	max	std	>0
low	BLK	340000	-30	-25.3	-6.1	4.1	0	540000	-30	-22.8	1.6	5.0	3
	CAU	340000	-30	-25.2	0.03	4.1	1	540000	-30	-22.8	0.7	5.0	2
middle	BLK	360000	-30	-24.0	-2.8	4.3	0	540000	-30	-22.4	0.04	4.6	1
	CAU	360000	-30	-23.8	-3.0	4.3	0	540000	-30	-22.3	1.3	4.6	3
high	BLK	360000	-30	-24.3	-0.4	4.0	0	540000	-30	-21.3	1.14	4.4	1
	CAU	360000	-30	-23.4	-3.4	4.3	0	540000	-30	-20	1.3	4.6	2

Figure Legend

Figure 1: DNA information vs. DNA amount. The figure shows the contributor DNA amount in picograms (average mixture weight times total amount of DNA) against the average LR for each level of allele sharing; low, middle, and high. The first and second columns are for the 1000 pg and 100 pg samples, respectively. The LR was calculated as the minimum value over the NIST ethnic populations. The matching known reference is indicated by color in each plot.

Figure


1000 pg Samples
100 pg Samples
TT50722
TT50916
GT37019
TT50722
MT95364
UT57309
low
moderate
high
septillion
quintillion
trillion
million
one
millionth
trillionth
3
10
30
100
300
1000
picograms of contributor DNA