Estimating Individual Contributions to Complex DNA SNP Mixtures

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Abstract

Mixture analysis and deconvolution methods can identify both known and unknown individuals contributing to DNA mixtures. These methods may not identify all DNA contributors with the remaining fraction of the mixture being contributed by one or more unknown individuals. The proportion of DNA contributed by individuals to a forensic sample can be estimated using their quantified mixture alleles. For short tandem repeats (STRs), methods to estimate individual contribution concentrations compare capillary electrophoresis peak heights and or peak areas within a mixture. For single nucleotide polymorphisms (SNPs), the major:minor allele ratios or counts, unique to each contributor, can be compared to estimate contributor proportion within the mixture. This article introduces three approaches (mean, median, and slope methods) for estimating individual DNA contributions to forensic mixtures for SNP panels and high throughput sequencing (HTS)/massively parallel sequencing (MPS) technology.

Introduction

The amount of DNA contributed by individuals to forensic DNA mixture samples is known to vary by individual[1, 2]. Estimates of how much DNA an individual contributed to a mixture can provide useful inferences such as subject approximate touch order, relative time handling an object, and the quantity of DNA that can be explained by known reference profiles[1]. Traditional STR forensics analysis leverages peak height or peak area information to estimate individual contribution amounts, deconvolute mixtures, and enhance likelihood ratio calculations[3-7]. Recent advances are pushing this upper boundary to characterize STR mixtures with more contributors[8-15].

An alternative or complimentary approach for analyzing DNA mixtures is the characterization of SNPs using HTS/MPS technology[16]. SNP panels have been designed to identify individuals in DNA mixtures with more contributors[17, 18]. Additional benefits that forensic SNP panels enable are enhanced mixture deconvolution capabilities[19], expanded kinship identification[20, 21] due to the larger number of loci characterized, biogeographic ancestry prediction[22, 23], and phenotype predictions[24]. GrigoraSNPs[25], ForenSeq[26], ExactID[27], and STRaitRazor[28] are programs capable of characterizing MPS forensic SNP panels.

These SNP panels have the potential to make significant impacts on forensic investigations. Similar to STR allele quantification, the ratios of major:minor alleles reflect DNA contributor concentrations. We introduce three methods (mean, median, and slope) for estimating contributor concentrations in mixture samples characterized by SNP panels[29]. These methods can quantify DNA contributions from multiple contributors, as well as estimate the proportion of DNA contributed by unknown contributors.

Methods

Ion Torrent MPS Sample Prep and Sequencing

For defined mixture experiments, swabs (Bode cat# P13D04) were used to collect buccal cells from the inside of cheeks of volunteers, rubbing up and down for at least 10 seconds, with pressure similar to that used while brushing teeth. For touch mixture experiments, swabs (Bode cat# P13D04) were used to collect DNA from objects of different surface types that had touch history logs. DNA was isolated from swabs using the QIAamp DNA Investigator Kit (QIAGEN cat#56504), using the "Isolation of Total DNA from Surface and Buccal Swabs" protocol, and eluted in 100uL of low TE (carrier RNA not used; low TE has 0.1mM of EDTA). Quantitation was done using Quantifilier HP kit (Thermo Fisher Scientific cat#4482911) according to manufacturer, with the exception that human genomic DNA from Aviva Systems Biology (cat#AVAHG0001) was used for the standard. Purified DNA from individuals were combined to produce defined two to ten person mixtures. Primers for the 2,655 targets in the MITLL SNP mixture panel were previously designed and vetted . Libraries were prepared using the AmpliSeq 2.0 library kit protocol according to the manufacturer, with the exception that 19 cycles were performed (no secondary amplification) and the library was eluted in 25uL low TE. Library quantitation was performed using the Ion Library Quantitation Kit (Thermo Fisher cat#4468802), according to the manufacturer. Template preparation and sequencing were performed using the Ion Chef and Ion Proton according to the manufacturer (Thermo Fisher Ion Chef and Proton cat#A27198 and Proton chips cat#A26771).

MPS SNP Data Analysis

Ion Torrent BAM files were converted to FASTQ format using Samtools fastq[30]. The GrigoraSNPs[25] program was used to call SNP alleles from multiplexed HTS FASTQ sequences. Mixture analysis was performed using the MIT Lincoln Laboratory IdPrism HTS DNA Forensics system. IdPrism uses the FastID[31] program to compare mixtures to reference samples. The IdPrism Plateau[19] method can also identify individual SNP profile signatures by mixture deconvolution. IdPrism uses the Fast P(RMNE)[32] program to automatically calculate the probability of a random person (man) not excluded P(RMNE)[17, 18]. For saturated (i.e., > 70% of loci detected with minor alleles) SNP mixtures, IdPrism uses the TranslucentID[33] program to identify individuals in saturated mixtures that are not detected using standard mixture analysis techniques. The TranslucentID method is a novel approach to the identification of individuals in a saturated mixture that creates a derivative desaturated mixture by treating the SNPs with the lowest mAR values as MM alleles[33]. This method was used on the equimolar ten person defined mixture (URK5V:IX-25).

EuroForMix(v1.11.4)[3, 4] was used to calculate continuous maximum likelihood ratios for each subject identified for mixtures with five or fewer contributions. The R program, Grigora_to_euroformix. R, was used to parse GrigoraSNPs output files into EuroForMix input format. EuroForMix settings were either tailored to the MITLL mixture panel or set at levels described in Bleka et al.[3] For each likelihood ratio the following hypotheses were compared:

H1: The person of interest and N-1 unknown individuals contributed to the mixture
H2: N unknown individuals contributed to the mixture

Mean and Median Minor Allele Ratios Methods

The most common SNP allele in a population is referred to as the major (M) allele. The other SNP alleles are referred to as the minor (m) allele(s). The majority of SNP loci have predominantly two alleles. The minor allele ratio (mAR) at a locus is defined as the ratio of minor allele sequence counts divided by the total sequence counts. The minor allele ratio (mAR) for alleles in a SNP reference profile are approximately 0.0 for MM alleles (genotypes), a normal distribution centered on 0.5 for mM alleles, and at or near 1.0 for mm alleles. The loci with mM SNP alleles for an individual within a mixture reflect the relative concentration of the DNA contributed – i.e., the mAR of minor alleles contributed by only one individual will be represented in relative proportion to the contributor's DNA in the sample compared to all other major alleles at each of these loci. When two or more minor alleles are present at a locus, the mAR approximate the additive combination of the contributors' individual loci mAR values. When reference profiles or Plateau[19] method profiles are available, the average or median of the unique mM alleles contributed to a mixture can be used to estimate the relative contributions of the individual's DNA to the mixture. SNP loci with minor alleles shared by two or more individuals are excluded from the calculations for known contributors with reference profiles. The concentration of that individual's DNA is then estimated as $2 * \mu$, where μ is either the mean or the median mAR of the SNP loci where the given individual uniquely contributes a minor allele to the mixture.

Figure 1 illustrates three two-person dilution mixtures of individual 19 and 94. A subset of the unique mM alleles for individuals 19 and 94 are highlighted in the blue boxes in Figure 1. The mean or median of the contributor unique mM SNPs are used to estimate the amount contributed by each individual. Unassigned mM alleles are leveraged to estimate the concentration for remaining unknowns in the mixture. The computed mean or median values are divided by the sum of the corresponding mean or median values computed to determine DNA concentrations.

Figure 1. Two-person dilution series (5Z1MO) illustrating individuals 19 and 94 unique mM SNP loci used by the mean, median, and slope methods to estimate contributor concentrations.

Slope Intercept Method

Unique mM SNPs for each individual in a mixture are determined by FastID[31] or the Plateau method[19]. A linear regression (R language lm function) is performed with mAR as the dependent variable and each SNP as an independent variable. The slope intercept of each mAR regression is summed. Each individual slope intercept is divided by the sum of slope intercepts to determine individual DNA concentrations.

Results

Defined Mixtures

Analysis results of defined mixtures of two to five individuals are illustrated in Table 1, where the estimated contributions from all three methods are compared to the intended contributions (the planned concentrations). The difference between the estimated concentration and the planned concentration is typically within 5%, with the exception of experiment UYLML:IX-31. For this four-person mixture, the methods consistently estimate more DNA from individual 93 and less DNA from individual 94 than was intended. Experimental error was identified after analysis of mixtures 5Z1MO:IX-01, IX-02, and IX-03. Table 1 reflects the revised planned concentration values for these three experiments.

Example Saturated Equimolar Mixture

An equimolar mixture was created with DNA from ten individuals. This number of DNA contributors saturated the MITLL SNP mixture panel. By default, IdPrism does not perform

standard mixture analysis on saturated mixtures. The MITLL TranslucentID method was applied to desaturate the mixture. FastID[31] identifies nine of the ten individuals in the desaturated mixture, see Table 2.

Table 1. Estimation of contributions compared with intended percent contribution

Sample Name	Reference	P(RMNE)	Planned Concentration	Mean Method	Median Method	Slope Method	Planned minus Mean	Planned minus Mediam	Planned minus Slope	EuroForMix log LR
5Z1M0:IX-01	19	1.44E-123	94.44%[1]	93.9%	94.2%	95.1%	0.6%	0.3%	-0.7%	278.5
5Z1M0:IX-01	94	5.34E-122	5.56%[1]	5.5%	5.8%	4.9%	0.1%	-0.3%	0.7%	120.6
5Z1M0:IX-02	19	3.98E-106	97.22%[1]	97.1%	96.9%	97.1%	0.1%	0.3%	0.1%	324.9
5Z1M0:IX-02	94	1.14E-58	2.78%[1]	2.9%	3.1%	2.9%	-0.1%	-0.3%	-0.1%	78.8
5Z1M0:IX-03	19	6.10E-141	98.6%[1]	98.5%	98.3%	98.4%	0.1%	0.3%	0.2%	381.7
5Z1M0:IX-03	94	1.00E-80	1.39%[1]	1.5%	1.7%	1.6%	-0.1%	-0.3%	-0.2%	71.1
VL8Y9:IX-16	4	3.85E-144	75.0%	70.0%	70.2%	65.3%	5.0%	4.8%	9.7%	418.2
VL8Y9:IX-16	93	1.39E-149	25.0%	30.0%	29.8%	34.7%	-5.0%	-4.8%	-9.7%	307.9
VL8Y9:IX-18	4	1.64E-144	90.0%	87.3%	87.8%	90.6%	2.7%	2.2%	-0.6%	356.1
VL8Y9:IX-18	93	9.15E-145	10.0%	12.7%	12.2%	9.4%	-2.7%	-2.2%	0.6%	144.8
TQTPV:IX-28	4	1.72E-144	99.0%	98.3%	99.3%	99.3%	0.7%	-0.3%	-0.3%	545.5
TQTPV:IX-28	93	1.68E-137	1.0%	1.7%	0.7%	0.7%	-0.7%	0.3%	0.3%	77.2
TQTPV:IX-30	4	4.43E-141	99.5%	99.0%	99.3%	99.3%	0.5%	0.2%	0.2%	545.5
TQTPV:IX-30	93	9.01E-115	0.5%	1.0%	0.7%	0.7%	-0.5%	-0.2%	-0.2%	35.7
TQTPV:IX-31	4	2.70E-155	99.75%	99.2%	99.6%	99.6%	0.6%	0.2%	0.1%	545.5
TQTPV:IX-31	93	7.35E-37	0.25%	0.8%	0.4%	0.4%	-0.6%	-0.2%	-0.1%	4.3
HRXEL:IX-11	93	2.28E-95	75.0%	74.5%	73.6%	72.1%	0.5%	1.4%	2.9%	173.8
HRXEL:IX-11	24	1.24E-96	20.0%	21.0%	21.5%	22.2%	-0.9%	-1.5%	-2.2%	98.1
HRXEL:IX-11	56	1.24E-96	5.0%	4.6%	4.9%	5.7%	0.5%	0.1%	-0.7%	54.1
UYLML:IX-31	93	2.95E-88	47.0%	58.7%	62.5%	63.5%	-11.7%	-15.5%	-16.5%	341.4
UYLML:IX-31	78	8.20E-83	32.0%	30.7%	30.9%	30.4%	1.3%	1.1%	1.6%	142.8
UYLML:IX-31	94	4.24E-90	17.0%	5.7%	5.6%	4.8%	11.3%	11.4%	12.2%	150.1
UYLML:IX-31	4	1.89E-73	2.0%	1.1%	1.0%	1.3%	0.9%	1.0%	0.7%	-6.8
TUG7L:IX-22	57	4.32E-78	40.0%	43.3%	44.1%	44.8%	-3.3%	-4.1%	-4.8%	127.3
TUG7L:IX-22	94	4.32E-78	30.0%	26.2%	26.3%	27.2%	3.8%	3.7%	2.8%	84.3
TUG7L:IX-22	93	5.08E-77	20.0%	24.5%	19.7%	18.2%	-4.5%	0.3%	1.8%	68.1
TUG7L:IX-22	56	4.32E-78	10.0%	9.3%	9.9%	9.7%	0.7%	0.1%	0.3%	41.8
FHSIJ:IX-11	56	4.80E-78	40.0%	40.8%	42.3%	41.1%	-0.8%	-2.3%	-1.1%	127.5
FHSIJ:IX-11	86	4.80E-78	30.0%	32.4%	27.9%	30.3%	-2.4%	2.1%	-0.3%	94.7
FHSIJ:IX-11	83	5.89E-76	20.0%	18.7%	20.4%	22.0%	1.3%	-0.4%	-2.0%	72.9
FHSIJ:IX-11	94	4.80E-78	10.0%	8.1%	9.4%	6.6%	1.9%	0.6%	3.4%	59.3
NJ0KF:IX-12	24	1.24E-65	39.5%	35.0%	36.2%	36.7%	4.5%	3.3%	2.8%	157.7
NJ0KF:IX-12	57	1.24E-65	30.0%	32.7%	30.3%	30.8%	-2.7%	-0.3%	-0.8%	126.8
NJ0KF:IX-12	78	6.96E-59	20.0%	21.8%	24.7%	24.2%	-1.8%	-4.7%	-4.2%	77.9
NJ0KF:IX-12	83	8.51E-62	10.0%	7.8%	7.4%	7.4%	2.3%	2.6%	2.6%	74.6
NJ0KF:IX-12	4	2.56E-39	0.5%	2.8%	0.5%	0.8%	-2.3%	0.0%	-0.3%	67.8

[1]Revised planned concentration values after experimental error identified

Table 2. Estimation of contributions for desaturated 10 individuals equimolar mixture (URK5V:IX-25)

Reference	P(RMNE)	Target Concentration	Mean Method	Median Method	Slope Method	Planned minus Mean	Planned minus Mediam	Planned minus Slope
4	1.30E-25	10%	10.27%	10.03%	13.15%	-0.3%	0.0%	-3.2%
24	3.05E-28	10%	10.31%	10.56%	7.99%	-0.3%	-0.6%	2.0%
41	ND	10%	-	-	-	-	-	-
57	3.05E-28	10%	9.37%	9.34%	9.46%	0.6%	0.7%	0.5%
68	3.05E-28	10%	10.30%	10.39%	9.05%	-0.3%	-0.4%	0.9%
69	3.05E-28	10%	10.01%	10.19%	12.96%	0.0%	-0.2%	-3.0%
76	3.05E-28	10%	11.35%	11.70%	8.70%	-1.4%	-1.7%	1.3%
78	1.30E-24	10%	9.48%	9.39%	13.53%	0.5%	0.6%	-3.5%
83	1.30E-24	10%	9.81%	9.60%	9.07%	0.2%	0.4%	0.9%
93	1.15E-27	10%	12.60%	12.13%	12.57%	-2.6%	-2.1%	-2.6%

Example Visualizations

Figures 2 and 3 illustrate the mean method applied to a defined mixture and a mixture created from individuals touching an object. While there are no known concentrations for the touch mixture, the number of people and touch order was recorded. Mixture analysis with FastID[31] and reference profiles identifies five of the six individuals who touched this object (wood). Individual 83 was not detected in the mixture, but may be partially included in the signature marked "unknowns". Table 3 estimates the relative contributions to the mixture by the individuals using the mean method.

Figure 2. IdPrism screenshot of Five person mixture (NJ0KF:IX-12) with reference 4 selected in the Pie chart and displayed on the companion P(RMNE) [32] graph. Individual 4 has 17 dropped SNP minor alleles not detected above the analytical threshold[19]; individuals 24, 57, 78, and 83 have no dropped minor alleles.

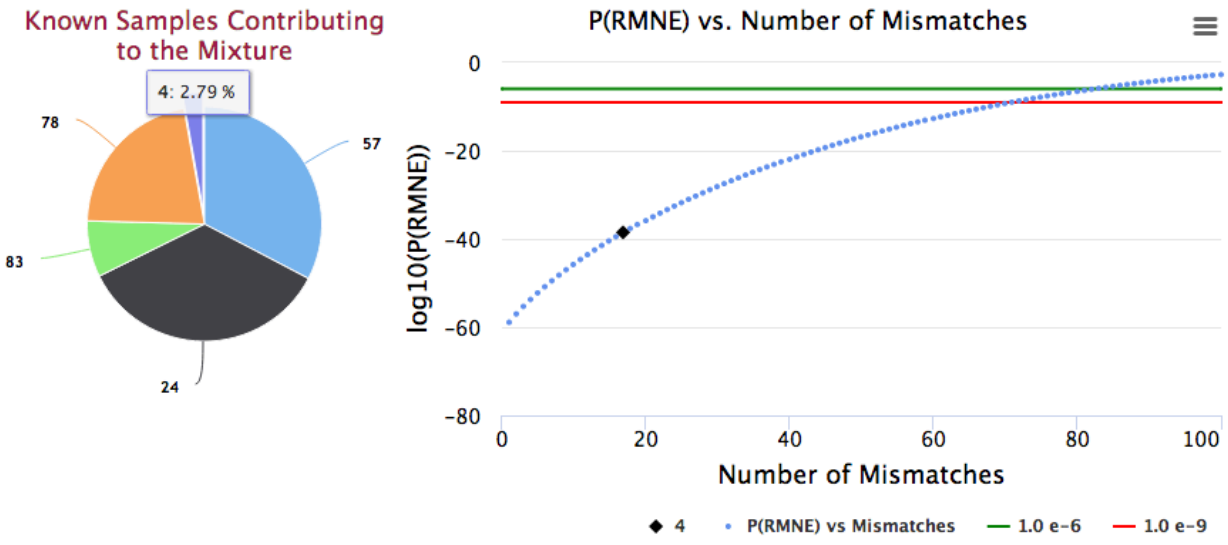

Figure 3. IdPrism screenshot of a touch mixture (09N7W:IX-29) with reference 94 selected (yellow slice) in the Pie chart and displayed on the companion P(RMNE) graph. This touch mixture had six known DNA contributors: 4, 57, 69, 83, 93, and 94 of which five contributors are detected with high confidence. Highlighted individual 94 has a concentration of 4.58% and a P(RMNE) of 1.05e-18, with 56 dropped mM loci not detected above the mixture analytical threshold.

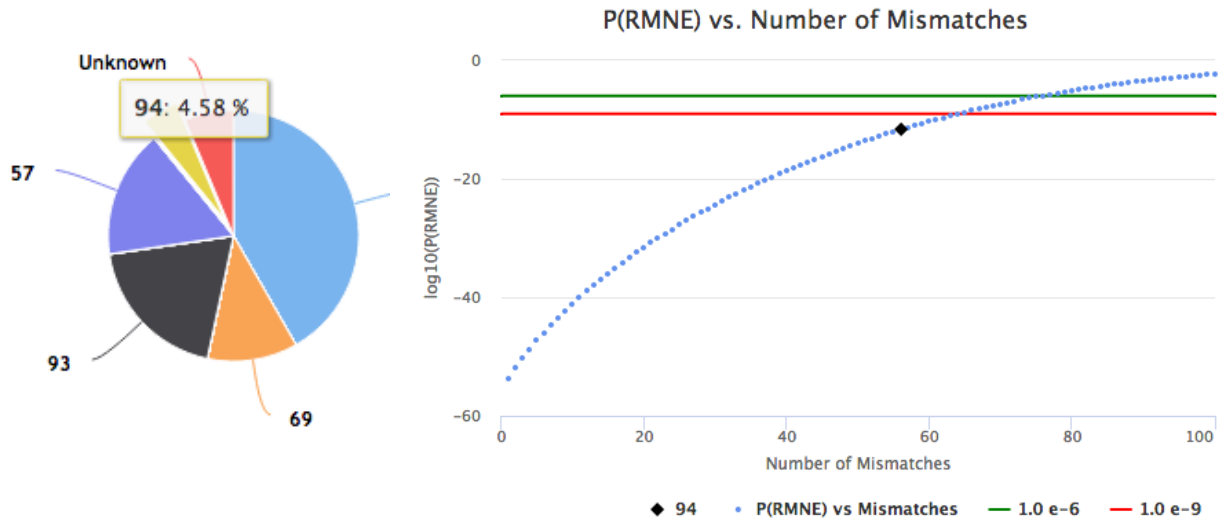

Table 3. Estimated contributor concentrations for touch mixture mix160

	Ref 4	Ref 69	Ref 94	Ref 57	Ref 93	Unknown(s)
Average Estimate	41.7%	11.6%	4.6%	16.4%	19.3%	6.4%

Discussion

The variance from intended experimental planned concentrations in defined mixtures is typically less than 5% for all three methods, see Table 1. These methods work for DNA contributor concentrations as low as 1:400 (TQTPV:IX-31 in Table 1), and mixtures with large numbers of contributors, (Table 2). The combined contributions for unknown individuals can also be estimated (Figure 3 and Table 3). Observed variation may reflect minor inaccuracy of source DNA concentration assessment, pipetting variability, or other mitigating factors. The IdPrism Pie chart visualization provide immediate insights into mixture contributors and unknowns. The inclusion of the mean method into MITLL IdPrism HTS Forensic System enabled the immediate identification of discrepancies in the intended concentrations of 5Z1MO:IX-01 of 1:200 at 1:18, 5Z1MO:IX-02 of 1:400 as closer to 1:34, and 5Z1MO:IX-03 of 1:800 as closer to 1:67. Detection of an experimental error enabled the correction of the planned concentrations of these experiments to 1:18, 1:36, and 1:72 as shown in Table 1. These updated concentrations are in close agreement with the values estimated by these methods.

These methods are also useful for DNA mixtures with no *a priori* known concentrations. For the touch example (09N7W:IX-29), individual 4 contributed more DNA to the object even though individual 93 was the last documented individual to touch the object. It has been previously established that the amount of DNA contributed by touch varies by individual[34]. Figure 2 illustrates the estimated concentrations for the 5 detected individuals and the amount contributed by unknown individuals estimated at 6.3%.

Conclusion

Three methods were introduced for estimating DNA contribution in mixtures characterized by HTS SNP panels. The values estimated by these methods are typically within 5% for defined DNA mixtures. These methods can also estimate the remaining amount of DNA contributed from unidentified individuals (unknowns).

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