# Development of Kinship Mixtures and Subsequent Analysis
## Using TrueAllele® Casework

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## Introduction

According to the FBIs 2013 crime clock statistics, one rape occurs every 6.6 minutes. In 2013 alone, U.S residents age 12 or older experienced 300,170 rapes/sexual assaults, according to the Bureau of Justice Statistics' (BJS) National Crime Victimization Survey (NCVS)[1]. A total of 173,610 individual victims were involved in these 300,170 rapes/sexual assaults. While rape/sexual assault is clearly prevalent in todays society, a mere 34.8% of victimizations were reported to the police[1]. A report entitled "Female Victims of Sexual Violence, 1994-2010" presented data from 2005-2010, which suggested most rape or sexual assault victims (78%) knew the offender[2]. The report goes on to say that 6% of all rape of sexual assault victimizations were committed by a relative or family member[2]. This means there are approximately 16,189 individuals per year are victimized by a relative or family member.

In the field of Forensic DNA investigation, mixture evidence – including samples collected from a rape case – can prove problematic, due to the complexity of the data. When an analyst is processing mixtures involving kinship, which is defined as a blood relationship, the analyst must take into account the possibility of encountering alleles that are identical by descent (IBD). If an analyst obtains mixture evidence from a rape case that involved relatives or family members, s(he) will probably attempt to utilize Combined Likelihood Ratio (CLR) or Combined Probability of Inclusion (CPI) in an effort to deconvolute the mixture. More information on CLR and CPI can be found in the TrueAllele Mixture Analysis section. Both CLR and CPI look at the

data as a whole, which can be problematic if there are many shared alleles. Furthermore, CLR and CPI utilize thresholds, which can result in the loss of alleles. Therefore, it can be asserted mixtures that involve kinship have an increased complexity and are more difficult to analyze than mixtures, which lack a kinship component. Previous studies have failed to establish a method of kinship mixture analysis that can accurately analyze, and subsequently preserve the most DNA identification information. This research strives to develop kinship mixtures for analysis on the TrueAllele® casework system.

A quantitative analysis method, such as TrueAllele, may be used to obtain genetic profiles and matches if a profile contains DNA from multiple contributors[3]. This newer method of quantitative analysis makes use of the same evidence as widely used qualitative analysis methods, but is able to yield greater identification information[4]. When analyzing DNA mixture evidence, it is important for an analyst to be able to reproduce results, while maintaining a high level of objectivity. TrueAlelle allows analysts to reproduce results and it can also be deemed objective. This objectivity exists because, during analysis of the DNA evidence, the computer first infers an unknown genotype from the evidence and then matches this inferred genotype to a suspect genotype[5].

## Buccal Swabs

Originally, when one was attempting to collect genetic material for analysis with multiplex PCR-based genotyping assays, one would simply collect blood from the individual[6]. The problem with collecting blood for genetic testing is that it is quite invasive. Blood sampling is also time-consuming and expensive. As a result, samples are now collected from the mouth because it is convenient and it provides several potential sources for DNA isolation[7,8]. Buccal

swabs have become the preferred method of DNA collection from the oral cavity[9]. The swabs

are preferred because they are easy to use, much less invasive than blood collection, and the

buccal swabs pose a lower risk for both the subject and the laboratory personel[6,10]. Furthermore,

buccal cells have been shown by many in the scientific community to be a cost effective method

to isolate DNA[11,12]. As far as storage is concerned, room temperature storage has proven to be

convenient because it reduces transportation, storage cost, and space[13]. Additionally, a study that

appeared in the Journal of Applied Oral Science, looked at the effect of storage conditions on

buccal swab DNA yield and quality[14]. This study observed no significant difference for the

DNA yields when the buccal swabs were extracted immediately, when the buccal swabs were

stored at room temperature prior to extraction, and when the buccal swabs were stored at 4° C

prior to extraction[14].


## Extraction of DNA from Buccal Swabs

Extracting DNA is an important component in the forensic analysis of biological

samples. It is utilized to break down the nuclear and cellular membranes, and release the DNA.

Once the DNA is extracted from a sample – a buccal swab – the DNA can be amplified using

Polymerase Chain Reaction (PCR). Data obtained from the PCR can be used to construct DNA

profiles, and these can be used to help convict or exonerate a suspect in a criminal case. The

important thing to keep in mind about the extraction method, is that it is not only required to

efficiently extract DNA, it is also responsible for removing inhibitors that can interfere with

downstream processes such as DNA amplification etc[15].

A fast and simple extraction method is the Chelex reaction. Despite the ease of this

method, it is not ideal for casework samples, since it often fails to eliminate amplification

inhibitors[16].  Another extraction method is the organic extraction involving phenol and chloroform[17].  The problem with organic extraction is the multiple centrifugation and transfer steps in the procedure increase the chance for DNA loss and contamination.  Taking these two methods and their limitations into account, it was determined the DNA IQ™ System would be pursued as the extraction method for this research.

The DNA IQ™ System produced by Promega has become a common extraction and clean up method in the forensic community because of its capacity to produce clean DNA from both small and large casework samples[18–22].  The DNA IQ™ System contains magnetic beads – composed of silicon dioxide (SiO2)-magnetite (Fe3O4) with an average diameter of $12 \pm 4$ μm and low-macroporosity – to maximize DNA binding and minimize binding from potential contaminants (competition of DNA Binding sites using Promega DNA IQ paramagnetic beads)[23].  Furthermore, the DNA IQ™ System utilizes a magnetic stand, which serves to separate the paramagnetic resin from washes and lysates[24].  In this way, the contaminants bound to the beads are washed away during the wash steps and a clean DNA extract is obtained[19,20,22].

## Quantification of DNA

All sources of DNA are extracted when a biological evidence sample is processed.  For this reason, the FBI standards require human-specific quantitation to be performed for all evidence samples.  Quantification makes it possible for the analyst to adjust the concentration of the template DNA – used for STR analysis – in order to obtain the optimal amount for PCR reactions.  It is important to have the optimal concentration of DNA, because multiplex STR typing works best with a fairly narrow range of human DNA, typically 1 ng.  If the concentration of Human DNA does not fall within the narrow range, PCR artifacts may be amplified and the

detection format results range may be exceeded due to an oversaturation of signal.  If too much

DNA is present it can result in split peaks, increased stutter, amplification by-products, and

secondary structures.  If not enough DNA is present, allelic drop-out and stochastic fluctuations

can occur.  However, if an analyst is able to obtain the optimal DNA concentration through

normalization – achieved by diluting or concentrating the sample – higher quality data will result

and the data will be easier to interpret.

For this research, the Quantifiler™ Human DNA Quantification kit, from Applied

Biosystems, will be utilized.  This kit is human specific, so it complies with the FBI Standards.

The Quantifiler™ Human DNA Quantification kit is based on a single copy gene hTERT –

human telomerase reverse transcriptase[25].  Most importantly, in the developmental validation, the

accuracy and precision of the Quantifiler Kit method was comparable or superior to that of other

quantification methods[26].  An internal validation study also demonstrated that the Quantifiler Kit

is a reliable system with high sensitivity, which gives both accurate and reproducible results[27].

## Genotyping

Short Tandem Repeat (STR) analysis of forensic samples relies on the presence of large

population databases to help estimate the probability of identity by chance[28,29].  Two kits that are

widely-used for genotyping are AmpFlSTR Identifiler® Plus PCR Amplification Kit available

from Applied Biosystems and PowerPlex® 16 available from Promega[30].

An internal validation of the AmpFlSTR Identifiler® Plus PCR™ amplification kit on the

ABI Prism® 3100 Genetic Analyzer was done by the Department of Chemistry Malaysia[31].  The

AmpFlSTR Identifiler® Plus PCR™ co-amplifies fifteen STR loci: D8S1179, D21S11, D7S820,

CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51,

FGA, and the amelogenin (gene marker).  They studied sensitivity, precision, reproducibility, non-probative casework, stutter, heterozygous peak height ratio, and mixture interpretations. DNA was mainly extracted using the chelex extraction method, and samples were purified and amplified directly using punched blood disks from FTA® cards[31].

For this project, the AmpFLSTR® Identifiler® Plus PCR Amplification Kit from Applied Biosystems will be utilized.  This kit was chosen over PowerPlex® 16 due to the resources available.

## TrueAllele Mixture Analysis

Human review of DNA mixtures is a common practice in forensic science laboratories, and it can often provide identification information to aid in a criminal investigation[32].  Two of the most commonly employed methods in United States crime laboratories are combined probability of exclusion (CPI) and combined likelihood ratio (CLR)[33].  Human DNA review methods tend to take a qualitative approach by applying thresholds to the DNA data.  The CLR method takes the victims genotype into consideration, while the CPI method does not take any of the case information into account.  It can be argued this kind of qualitative review can produce a biased genotype, since the inference utilizes a known suspect genotype[34].

For this research, the mixture interpretation software TrueAllele® was utilized to obtain genetic profiles and matches from kinship mixture samples.  The TrueAllele® genetic calculator is a statistical computer system capable of solving multiple DNA casework problems at the same time[5].  The TrueAllele calculator utilizes all of the available quantitative DNA data to infer an informative genotype from the DNA evidence.  If the genotype is uncertain, it will determine the probability of each allele pair.  After the calculator commits to this genotype, it can then be

matched to a suspect genotype.  In this way, the computer can guarantee objectivity.  When the inferred genotype is compared with a suspect genotype, a likelihood ratio is formed.  This likelihood ratio represents the weight of the evidence statistic relative to a reference population[5].

Overall, use of a computational analysis method can improve the review of DNA mixtures in several ways.  First, computational analysis can enable analysts to review more cases in a shorter amount of time, thereby boosting productivity and eliminating backlog[5].  Second, computers can often infer a stronger match statistic from weak DNA signals, since thresholds are not applied.  Finally, the analysis can be deemed objective by first inferring an unknown genotype from the DNA evidence and then matching this inferred genotype to a suspect genotype[5].

Now for an overview of how the TrueAllele® interface operates.  In the first interface, Analyze, the .fsa files from the lab are quality checked to insure all of the necessary peaks are present.  After the electronic data has been quality checked, the data is uploaded to the TrueAllele® Server.  Uploading the data to the server is very important because once the data is uploaded it is accessible for interpretation.  In the Request interface, the analyst is able to upload questions, regarding evidence items, to the system.  An example of a question the analyst could pose to the system is as follows: if there was an evidence item, that after reviewing the loci, the analyst deemed to be a possible two person mixture, the analyst could set up a request asking the computer to analyze it as a mixture with two unknown individuals.  It is important to note, if an item is uploaded as an evidence item, it will automatically be matched against all of the available Reference, Victim, Suspect, or Elimination profiles.  Once it is free, the system will begin to solve the questions posed to it by performing a Markov Chain Monte Carlo statistical search[35].  The inferred probabilistic genotypes that are found are recorded onto the TrueAllele® database.

In the fourth interface, Report, the analyst is able to view the results inferred by the computer. These results include both the genotyping answers and the LR match statistics. In forensic science, one of the preferred ways to formulate a LR is to balance the (conditional probability) likelihoods[35].

$$LR = \frac{Pr\{evidence|suspect\ is\ a\ contributor\}}{Pr\{evidence|suspect\ is\ not\ a\ contributor\}}$$

Also, the LR can be determined by comparing the inferred evidence genotype with a known suspect genotype, relative to a reference population[35].

$$LR = \frac{Pr\{match\ between\ suspect\ and\ evidence\ genotypes\}}{Pr\{coincidental\ genotype\ match\}}$$

Once an LR is obtained it can be stated as: "a match between the suspect and the evidence is (some number) times more probable than coincidence"[35].

## Materials and Methods

*Buccal Swab Collection*

Four volunteers were each given a collection kit containing: buccal swabs and 1.5mL microcentrifuge tube – each filled with 400μl of 1X TE Buffer. Each of the volunteers were asked to obtain a buccal swab from approximately ten of the following individuals (if applicable): grandmothers(s), grandfather(s), mother, father, siblings(s), aunt(s), uncle(s), and cousin(s).

The collection protocol was as follows: family members were asked to abstain from eating or drinking for at least an hour prior to collection. The buccal swab was removed from the wrapper, without touching the swab end. The individual was asked to swab along the inside

of both cheeks and along the gum line.  The cotton swabs were then allowed to air dry for 15

min.  The end of each swab was broken into a separate tube with TE, and the tubes were labeled

($A_1$, $A_2$, $A_3$,…, $A_n$).  A master list was created to record whose swab was placed in each tube.

All tubes were stored at room temperature prior to extraction.

Due to the collection method and storage method, profiles were not obtained from the

first round of samples collected.  Therefore, a revised protocol for collection and storage was

implemented and a second round of samples were collected.  Three volunteers were each given a

collection kit containing: buccal swabs and white envelopes.  Each of the volunteers were asked

to obtain a buccal swab from approximately ten of the following individuals (if applicable):

grandmothers(s), grandfather(s), mother, father, siblings(s), aunt(s), uncle(s), and cousin(s).

The collection protocol was as follows: the buccal swab was removed from the wrapper,

without touching the swab end.  The individual was asked to swab along the inside of both

cheeks and along the gum line.  The cotton swabs were then allowed to air dry for 30 min.  Each

swab was then placed into a separate labeled envelope ($M_1$, $M_2$, $M_3$,…, $M_n$) and the envelope

was sealed with a piece of tape.  A master list was created to record whose swab was placed in

each envelope.  All envelopes were stored at room temperature prior to extraction.  The

pedigrees, for all of the collected samples, can be seen in figures 1-3.
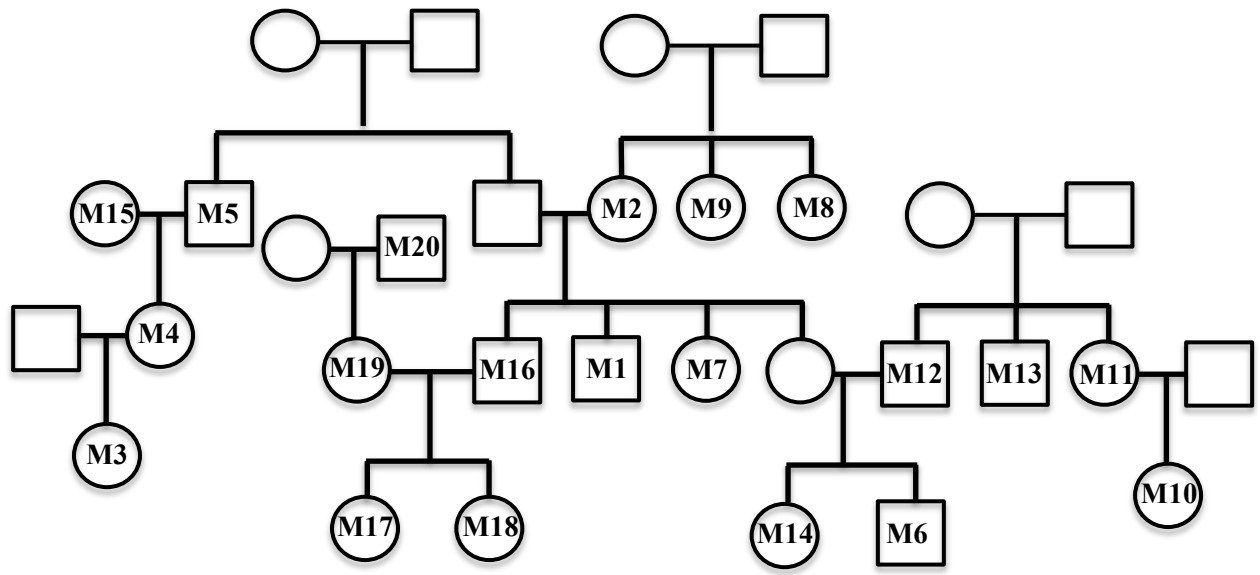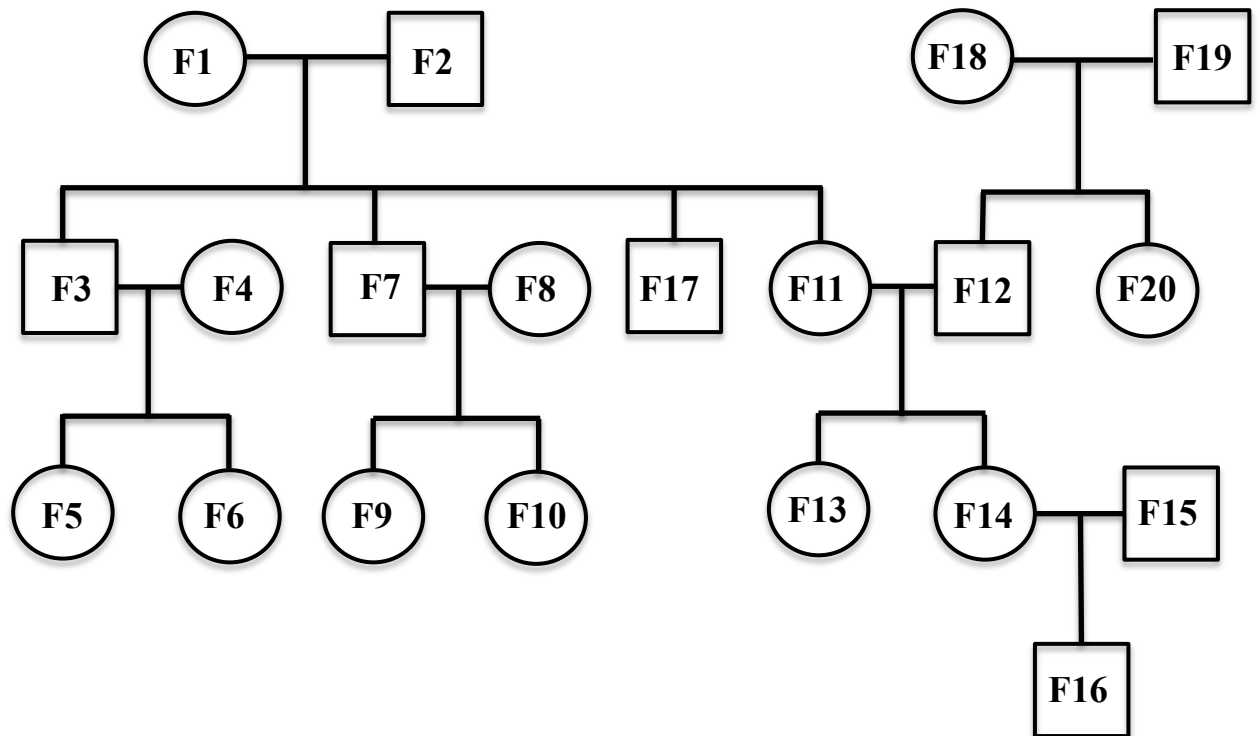
Figure 1: Pedigree of Family M
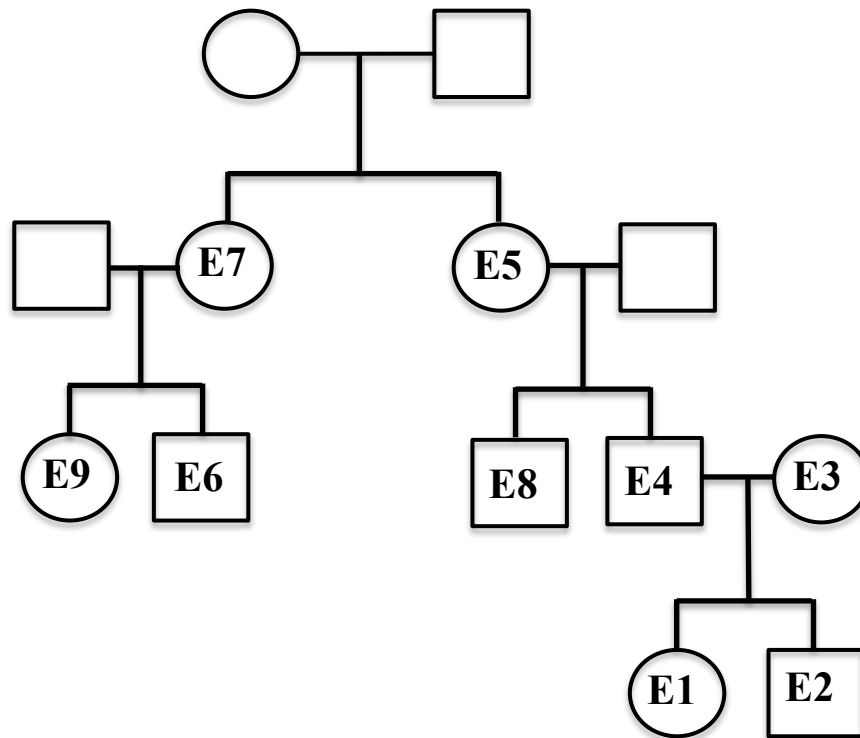


Figure 2: Pedigree of Family F

Figure 3: Pedigree of Family E

*Buccal Swab Extraction*

Forty-seven buccal swabs were collected in total during the first collection: $A_1$-$A_{13}$, $B_1$-$B_{17}$, $C_1$-$C_{11}$, and $D_1$-$D_6$. Forty-nine buccal swabs were collected in total during the second collection: $E_1$-$E_9$, $F_1$-$F_{20}$, and $M_1$-$M_{20}$. Genomic DNA was extracted by the DNA IQ™ System[36], following the protocol for DNA Isolation from Stains and Buccal Swabs (Fig. 4). Each swab was placed in an individual labeled 1.5mL microcentrifuge tube. 250µL of Lysis Buffer was added to each tube, and the tubes were placed in a water bath to incubate at 70°C for 30 minutes. After incubation, the cotton swab was removed from the 1.5mL tube using forceps – that were cleaned with alcohol wipes between each sample – and a spin basket was placed into the 1.5mL tube. The cotton swab was then returned to the spin basket. The tubes were centrifuged at room temperature for 2 minutes at maximum speed. After centrifuging, the spin

basket and cotton swab were removed.  The stock resin bottle was vortexed on high speed for 10 seconds, and 7μl of the resin was added to each sample – the resin was resuspended after every third sample.  The sample/Lysis Buffer/ resin mixture was vortexed for 3 seconds at high speed and incubated at room temperature for 5 minutes.  The mixtures were vortexed for 3 seconds every minute during this 5 minute incubation period.  After incubation, the tubes were vortexed at high speed for 2 seconds and then placed on the magnetic stand.  The supernatant was removed and discarded, and 100μl of Lysis Buffer was added to each tube.  The tubes were removed from the magnetic stand, vortexed on high for 2 seconds, and returned to the magnetic stand.  The Lysis Buffer was discarded and 100μl of 1X Wash Buffer was added to each tube. The tubes were removed from the magnetic stand, vortexed on high for 2 seconds, and returned to the magnetic stand.  The Wash Buffer was discarded and the samples were washed two more times with the Wash Buffer.  Following the third wash, the tubes were placed in the magnetic stand and all of the lids were opened.  The resin was allowed to air-dry for 5 minutes.  100μL of elution buffer was added to each tube, the lids were closed, and the tubes were vortexed for 2 seconds at high speed.  The tubes were incubated at 65°C for 5 minutes.  After incubation, the tubes were immediately vortexed for 2 seconds on high speed, and placed in the magnetic stands. The solution was transferred into labeled twist-top storage tubes, and placed at 4°C.
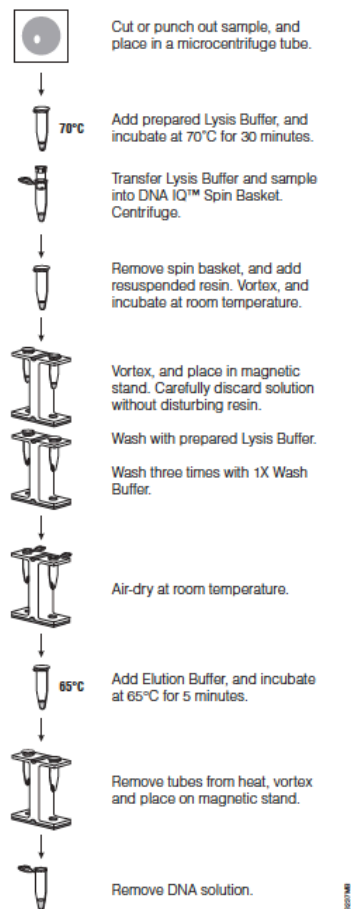
Figure 4: Schematic of DNA isolation from stains on solid material using the DNA IQ™ System[36]

*DNA Quantitation*

The concentrations of all extracted DNA samples were measured using the Quantifiler® Human DNA Quantification Kit and the Applied Biosystems® 7500 Real-Time PCR System.  A total of 2µl of DNA was used in a 25µl reaction, according to the manufacturers instructions.

*STR Profile Development*

Amplification reactions were prepared using the AmpFLSTR® Identifiler® Plus PCR Amplification Kit, using 0.7ng of DNA in a 25µl reaction.  The samples were then amplified

using the GeneAmp® PCR System 9700 Thermal Cycler. The PCR products were separated and

visualized on an Applied Biosystems® 3130 Genetic Analyzer, and were analyzed using

GeneMapper ID software version 3.2.

*Mixture Creation*

*Father – Daughter Mixtures*

After single-source profiles were obtained, mixtures were created. Since father –

daughter mixtures are the most prevalent in casework, ten father – daughter mixtures were

created. In these mixtures, the daughter was the major contributor and the father was the minor

contributor. Five of the father – daughter mixtures contained the same two contributors at

differing mixture ratios: 90:10, 80:20, 70:30, 60:40, and 50:50 (Table 1).

| Daughter | Father | Mixture Ratio |
|----------|--------|---------------|
| E1 | E4 | 90:10 |
| E1 | E4 | 80:20 |
| E1 | E4 | 70:30 |
| E1 | E4 | 60:40 |
| E1 | E4 | 50:50 |

Table 1: Father – Daughter E1 and E4 mixtures. Daughter (E1) is the major contributor in each mixture. Father (E4) is the minor contributor in each mixture. The Mixture Ratio is the target mixture ratio for each mixture.

The remaining five father – daughter mixtures were created with different contributors at

a consistent mixture ratio of 70:30 (Table 2).

| Daughter | Father | Mixture Ratio |
|----------|--------|---------------|
| M4 | M5 | 70:30 |
| M17 | M16 | 70:30 |
| M18 | M16 | 70:30 |
| M19 | M20 | 70:30 |
| F5 | F3 | 70:30 |

Table 2: Father – Daughter different contributor mixtures.  Daughter is the major contributor (70%) in each mixture.  Father is the minor contributor (30%) in each mixture.  The individuals vary for each mixture.  The Mixture Ratio is the target mixture ratio and it is kept constant – 70:30 – for all five mixtures.

*Sibling Mixtures*

Four sibling mixtures were also created with different contributors at a consistent mixture ratio of 70:30.  The major and minor contributors were randomly chosen for each mixture.  Three sister – sister mixtures were created: E7:E5, F14:F13, and M17:M18 and one brother – brother mixture was created: E8:E4 (Table 3).

| Contributor 1 | Contributor 2 | Mixture Ratio |
|---------------|---------------|---------------|
| E7 | E5 | 70:30 |
| F14 | F13 | 70:30 |
| M17 | M18 | 70:30 |
| E8 | E4 | 70:30 |

Table 3: Sibling mixtures.  Contributor 1 is the major contributor (70%) in each mixture. Contributor 2 is the minor contributor (30%) in each mixture.  The individuals vary for each mixture.  The Mixture Ratio is the target mixture ratio and it is kept constant – 70:30 – for both mixtures.

*Uncle – Niece Mixtures*

Two uncle – niece mixtures were created.  In these mixtures, the niece was the major

contributor and the uncle was the minor contributor.  The uncle – niece mixtures were created

with different contributors at a consistent mixture ratio of 70:30 (Table 4).

| Uncle | Niece | Mixture Ratio |
|:-----:|:-----:|:-------------:|
| E1 | E8 | 70:30 |
| M13 | M10 | 70:30 |

Table 4: Uncle – Niece mixtures. Niece is the major contributor (70%) in each mixture.  Uncle is the minor contributor (30%) in each mixture.  The individuals vary for each mixture.  The Mixture Ratio is the target mixture ratio and it is kept constant – 70:30 – for both mixtures.

*Grandfather – Granddaughter Mixtures*

Two grandfather – granddaughter mixtures were created; the granddaughter was the

major contributor and the grandfather was the minor contributor.  The grandfather –

granddaughter mixtures were created with different contributors at a consistent mixture ratio of

70:30 (Table 5).

| Granddaughter | Grandfather | Mixture Ratio |
|:-------------:|:-----------:|:-------------:|
| M18 | M20 | 70:30 |
| M17 | M20 | 70:30 |

Table 5: Grandfather – Granddaughter mixtures. Granddaughter is the major contributor (70%) in each mixture.  Grandfather is the minor contributor (30%) in each mixture.  The individuals vary for each mixture.  The Mixture Ratio is the target mixture ratio and it is kept constant – 70:30 – for both mixtures.

Next, a total of eight mixtures were created, these included: three-person, four-person,

and five-person mixtures.  Two father – son – mother mixtures were created.  The father was

present in the mixture at 60%, the son was present in the mixture at 30%, and the mother was

present in the mixture at 10% (Table 6).  These mixture ratios were chosen to simulate a case

analyzed using TrueAllele®.

| Father | Son | Mother | Mixture Ratio |
|--------|-----|--------|---------------|
| E4 | E2 | E3 | 60:30:10 |
| F15 | F16 | F14 | 60:30:10 |

Table 6: Three-person mixtures. Father is the major contributor (60%) in each mixture.  Son is the next highest contributor by mixture weight (30%).  Mother is the minor contributor (10%) in each mixture.  The individuals vary for each mixture.  The Mixture Ratio is the target mixture ratio and it is kept constant – 60:30:10 – for both mixtures.

Four four-person mixtures were created (Table 7).  Three of the four contributors

remained consistent for each mixture, while the 20% contributor varied.  In the first mixture, the

daughter (E1) was present at 40%, the son (E2) was present at 30%, the father (E4) was present

at 20%, and the mother (E3) was present at 10% (Figure 5).

| Mixture Number | Contributor 1 | Contributor 2 | Contributor 3 | Contributor 4 | Mixture Ratio |
|----------------|---------------|---------------|---------------|---------------|---------------|
| 1 | E1 | E2 | E4 | E3 | 40:30:20:10 |
| 2 | E1 | E2 | E8 | E3 | 40:30:20:10 |
| 3 | E1 | E2 | E5 | E3 | 40:30:20:10 |
| 4 | E1 | E2 | E7 | E3 | 40:30:20:10 |

Table 7: Four-person mixtures.  Contributor 1 is the Daughter (E1) and the major contributor (40%) in each mixture.  Contributor 2, Son (E2), is the next highest contributor by mixture weight (30%).  Followed by Contributor 3, the individual who varies for each mixture with a mixture weight of (20%).  Contributor 4 is the Mother (E3) and minor contributor (10%) in each mixture.  The individuals vary for each mixture.  The Mixture Ratio is the target mixture ratio and it is kept constant – 40:30:20:10 – for all four mixtures.

Figure 5: Four-person mixture E4.  Pedigree displaying the individuals included in mixture number 1.  Daughter (E1), brother (E2), father (E4), and mother (E3).

In the second mixture, the daughter (E1) was present at 40%, the son (E2) was present at

30%, the uncle (E8) was present at 20%, and the mother (E3) was present at 10% (Figure 6).



Figure 6: Four-person mixture E8.  Pedigree displaying the individuals included in mixture number 2.  Daughter (E1), brother (E2), uncle (E8), and mother (E3).

In the third mixture, the daughter (E1) was present at 40%, the son (E2) was present at

30%, the paternal grandmother (E5) was present at 20%, and the mother (E3) was present at 10%
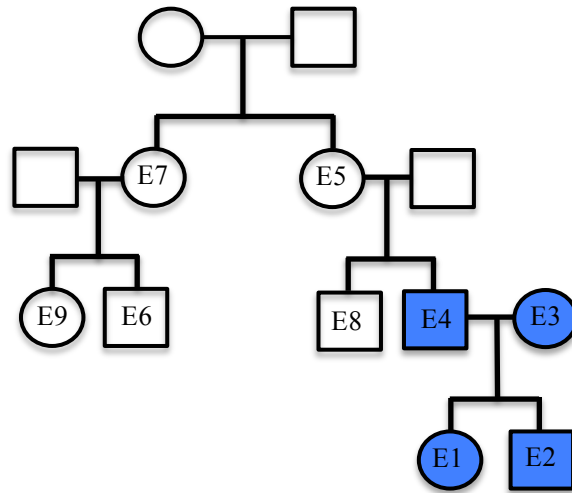
(Figure 7).
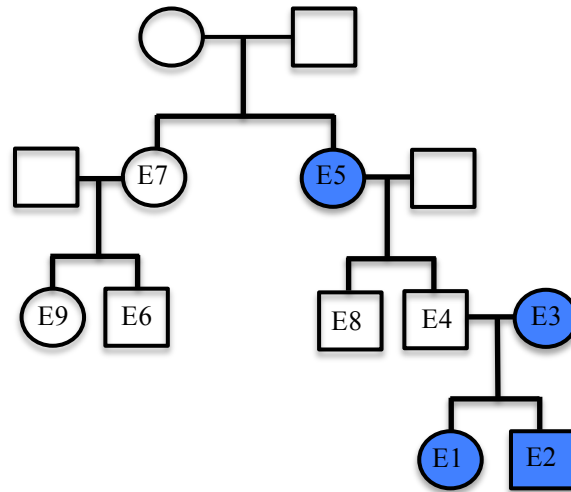


Figure 7: Four-person mixture E5.  Pedigree displaying the individuals included in mixture
number 3.  Daughter (E1), brother (E2), grandmother (E5), and mother (E3).

In the fourth mixture, the daughter (E1) was present at 40%, the son (E2) was present at

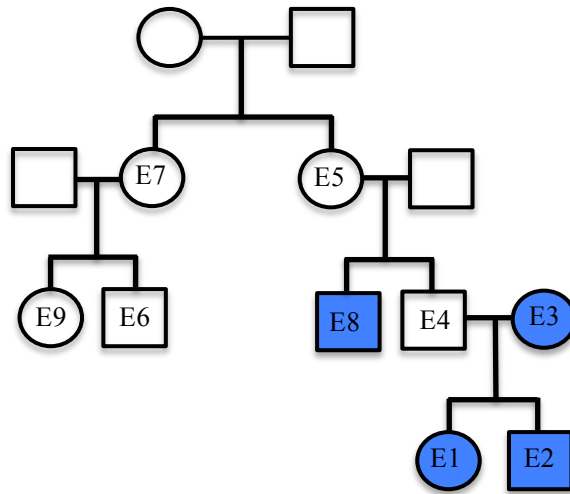30%, the paternal grandmother's sister (E7) was present at 20%, and the mother (E3) was present

at 10% (Figure 8).
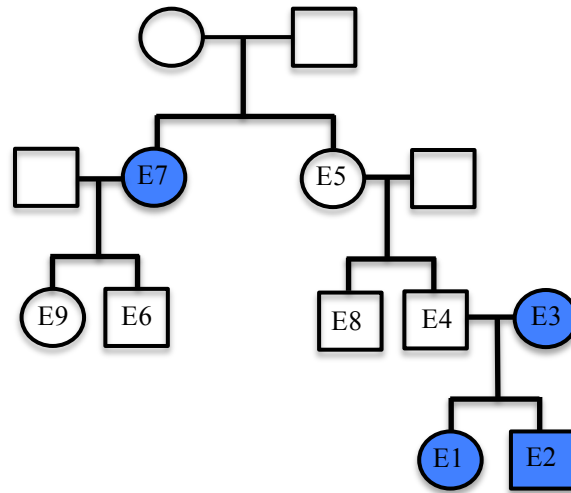
Figure 8: Four-person mixture E7. Pedigree displaying the individuals included in mixture number 4. Daughter (E1), brother (E2), grandmother's sister (E7), and mother (E3).

Two five-person mixtures were created. In the first mixture, daughter (M17) was present at 35%, sister (M18) was present at 30%, the mother (M19) was present at 20%, the father (M16) was present at 10%, and the uncle (M1) was present at 5% (Figure 9).
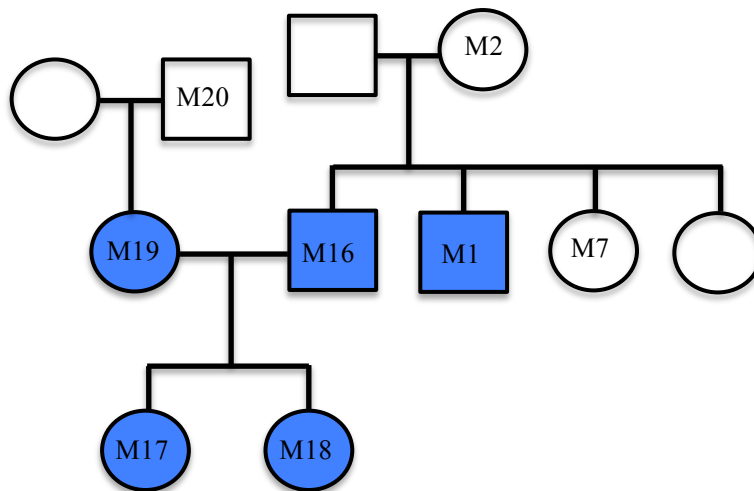


Figure 9: Five-person mixture family M. Pedigree displaying the individuals included in the five-person family M mixture. Daughter (M17), sister (M18), mother (M19), father (M16), and uncle (M1).

In the second mixture, the daughter (E1) was present at 35%, the son (E2) was present at 30%, the uncle (E8) was present at 20%, the father (E4) was present at 10%, and the mother (E3) was present at 5% (Figure 10).  These mixture ratios were chosen to simulate a case analyzed using TrueAllele®.
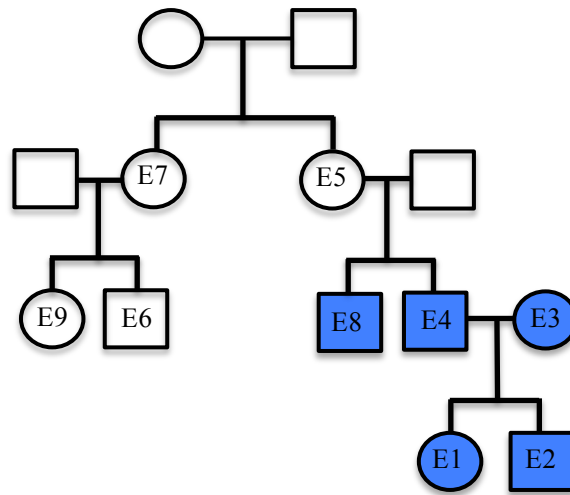


Figure 10: Five-person mixture family E.  Pedigree displaying the individuals included in the five-person family M mixture.  Daughter (E1), son (E2), uncle (E8), father (E4), and mother (E3).

Amplification reactions for the mixtures were prepared using the AmpFLSTR® Identifiler® Plus PCR Amplification Kit, and the samples were amplified using the GeneAmp® PCR System 9700 Thermal Cycler.  After amplification, the samples were genotyped using the Applied Biosystems® 3130 Genetic Analyzer.  The obtained sample data, for both the single-source samples and the mixture samples, was collected and the mixtures were analyzed using the expert system TrueAllele®.

## Results and Discussion

| Evidence | Ratio | Contributor | Known | Weight | Daughter (E1) | Father (E4) |
|----------|-------|-------------|-------|--------|---------------|-------------|
| E1E4 | 50:50 | 1 | | 50% | 15.60 | |
| E1E4 | 50:50 | 2 | | 50% | | 13.98 |
| E1E4+E1 | 50:50 | 2 | Daughter (E1) | 52% | | 18.23 |
| E1E4 | 60:40 | 1 | | 54% | 16.97 | |
| E1E4 | 60:40 | 2 | | 46% | | 15.25 |
| E1E4+E1 | 60:40 | 2 | Daughter (E1) | 38% | | 18.04 |
| E1E4 | 70:30 | 1 | | 72% | 19.32 | |
| E1E4 | 70:30 | 2 | | 28% | | 15.55 |
| E1E4+E1 | 70:30 | 2 | Daughter (E1) | 28% | | 16.23 |
| E1E4 | 80:20 | 1 | | 91% | 20.03 | |
| E1E4 | 80:20 | 2 | | 9% | | 4.29 |
| E1E4+E1 | 80:20 | 2 | Daughter (E1) | 8% | | 4.40 |
| E1E4 | 90:10 | 1 | | 93% | 20.03 | |
| E1E4 | 90:10 | 2 | | 7% | | - |
| E1E4+E1 | 90:10 | 2 | Daughter (E1) | 7% | | - |

Table 8: Father – Daughter E1 and E4 mixture table.  Evidence is the evidence item name.  Ratio is the target ratio for the mixture, it varies from mixture to mixture.  Known specifies if any individual was assumed to be in that evidence item.  Weight is the inferred mixture weight obtained from TrueAllele®.  Daughter (E1) is the major contributor in each mixture.  Father (E4) is the minor contributor in each mixture.  The obtained match statistics are in log(LR) units.

*Father – Daughter Mixtures*

The Father (E4) – Daughter (E1) mixtures utilized the same two contributors and five different mixture ratios: 50:50, 60:40, 70:30, 80:20, and 90:10 (Table 8).  In each of the mixtures, the daughter (E1) was the major contributor.  The log(LR) match statistic for the major contributor (Daughter E1) improved – from 15.6 to 20.03 – as the mixture ratio moved away from 50:50 toward 90:10.  The log(LR) for the minor contributor (Father E4) increased to a log(LR) match statistic of 15.25, at the inferred mixture ratio of 54:46.  The log(LR) match statistic for the minor contributor (Father E4) then increased to 15.55 at the inferred mixture ratio of 72:28, and it finally decreased to a log(LR) match statistic of 4.29 at the inferred mixture ratio

of 91:9.  No log(LR) for the minor contributor (Father E4) was obtained for the evidence sample

with a target mixture ratio of 90:10 and an inferred mixture ratio of 93:7 (Table 8).

The mixture with a target ratio of 50:50 obtained a log(LR) match statistic for the major

contributor (Daughter E1) of 15.60 and a  log(LR) match statistic for the minor contributor

(Father E4) of 13.98 (Table 8).  The log(LR) match statistic for the minor contributor (Father E4)

of 13.98 improved to 18.23 when the major contributor (Daughter E1) was assumed.  The

mixture with a target ratio of 60:40 obtained a log(LR) match statistic for the major contributor

(Daughter E1) of 16.97 and a  log(LR) match statistic for the minor contributor (Father E4) of

15.25.  The log(LR) match statistic for the minor contributor (Father E4) of 15.25 improved to

18.04 when the major contributor (Daughter E1) was assumed.  The mixture with a target ratio of

70:30 obtained a log(LR) match statistic for the major contributor (Daughter E1) of 19.32 and a

log(LR) match statistic for the minor contributor (Father E4) of 15.55.  The log(LR) match

statistic for the minor contributor (Father E4) of 15.55 improved to 16.23 when the major

contributor (Daughter E1) was assumed.  The mixture with a target ratio of 80:20 obtained a

log(LR) match statistic for the major contributor (Daughter E1) of 20.03 and a  log(LR) match

statistic for the minor contributor (Father E4) of 4.29.  The log(LR) match statistic for the minor

contributor (Father E4) of 4.29 improved to 4.40 when the major contributor (Daughter E1) was

assumed.  Finally, the mixture with a target ratio of 90:10 obtained a log(LR) match statistic for

the major contributor (Daughter E1) of 20.03 while no match statistic for the minor contributor

(Father E4) was obtained (Table 8).

| Evidence | Contributor | Weight | Daughter | Father |
|:---:|:---:|:---:|:---:|:---:|
| F5F3 | 1 | 72% | 19.68 | |
| F5F3 | 2 | 28% | | 19.00 |
| M4M5 | 1 | 83% | 16.89 | |
| M4M5 | 2 | 17% | | 14.04 |
| M17M16 | 1 | 86% | 20.25 | |
| M17M16 | 2 | 14% | | 14.83 |
| M18M16 | 1 | 76% | 17.65 | |
| M18M16 | 2 | 24% | | 19.31 |
| M19M20 | 1 | 74% | 18.38 | |
| M19M20 | 2 | 26% | | 19.71 |

Table 9: Father – Daughter different contributors mixture table. Evidence is the evidence item name. Weight is the inferred mixture weight obtained from TrueAllele®. Daughter is the major contributor in each mixture. Father is the minor contributor in each mixture. The obtained match statistics are in log(LR) units.

The mixtures – F5:F3, M18:M16, M19:M20 – whose inferred mixture weights were closest to the target weights of 70% and 30%, obtained similar log(LR) match statistics for both the major contributor (Daughter) and the minor contributor (Father) (Table 9). While the mixtures that obtained inferred mixture weights closer to 80% and 20%, – M4:M5 and M17:M16 – obtained lower log(LR) match statistics for the minor contributor (Father) (Table 9).

The mixture F5:F3 obtained a log(LR) match statistic for the major contributor (Daughter F5) of 19.68 and a log(LR) match statistic for the minor contributor (Father F3) of 19.00 (Table 9). The mixture M4:M5 obtained a log(LR) match statistic for the major contributor (Daughter M4) of 16.89 and a log(LR) match statistic for the minor contributor (Father M5) of 14.04. The mixture M17:M16 obtained a log(LR) match statistic for the major contributor (Daughter M17) of 20.25 and a log(LR) match statistic for the minor contributor (Father M16) of 14.83. The mixture M18:M16 obtained a log(LR) match statistic for the major contributor (Daughter M18) of 17.65 and a log(LR) match statistic for the minor contributor (Father M16) of 19.31. Finally,

the mixture M19:M20 obtained a log(LR) match statistic for the major contributor (Daughter

M19) of 18.38 and a  log(LR) match statistic for the minor contributor (Father M20) of 19.71

(Table 9).

The AmpFLSTR® Identifiler® Plus PCR Amplification Kit amplifies 16 loci.  If

Amelogenin is not taken into consideration, because it is not used when calculating statistics,

there are 15 loci remaining.  The contributors can share a maximum of two alleles per locus,

resulting in 30 possible shared alleles total.  A father and daughter are expected to share ½ of

their alleles, since the father and mother each pass one allele to their offspring.  Therefore, in a

father – daughter mixture one would expect there to be 15 shared alleles.  The number of shared

alleles was obtained for each mixture by looking at the genotypes of the father and the daughter

and assessing the number of shared alleles at each locus.  If the father and daughter were both

heterozygotes, and they possessed the same two alleles at a specific locus, it was counted as a

two (2).  If the father and daughter were both homozygotes, at the same allele at a specific locus,

it was counted as a two (2).  If the father and daughter shared one allele at a specific locus, it was

counted as a one (1).  The value – 1 or 2 – obtained from each locus was then added together to

determine the number of shared alleles for that specific mixture.

For these six father – daughter mixtures, the F5:F3 mixture was found to have 16 shared

alleles, the M18:M16 and M19:M20 mixtures were found to have 18 shared alleles, and the

E1:E4, M4:M5, and M17:M16 mixtures were found to have 21 shared alleles (Figure 11).  All of

the obtained shared allele values were higher than the expected value of 15 alleles.  When the

log(LR) was plotted against the number of shared alleles, the number of shared alleles in the

mixture did not seem to impact the log(LR) obtained for the major contributor (Daughter).

However, the number of shared alleles did seem to impact the log(LR) obtained for the minor

contributor (Father).  When the number of shared alleles climbed to 21, the log(LR) for the

minor contributor (Father) dropped by almost 5 log(LR) units.  While this could be attributed to

the increase in the number of shared alleles, it is important to note, two of the three father –

daughter mixtures – M4:M5 and M17:M16 – obtained inferred mixture weights closer to 80:20

than 70:30.  Therefore, it is possible that the mixture weight led to the decreased minor log(LR)

match statistics obtained for these two mixtures (Figure 11).



Figure 11: Father – Daughter 70:30 mixtures log(LR) vs. number of shared alleles.  The log(LR)
reflects the match statistic obtained for each major and minor contributor.  The number of shared
alleles refers to the total number of alleles shared between the two individuals present in the
mixture.  The obtained match statistics for the major contributor (Daughter) are shown using red
diamonds.  The obtained match statistics for the minor contributor (Father) are shown using blue
squares.

| Evidence | Contributor | Weight | Major | Minor |
|----------|-------------|--------|-------|-------|
| E7:E5 | 1 | 73% | 18.73 | |
| E7:E5 | 2 | 27% | | 17.11 |
| E8:E4 | 1 | 68% | 17.35 | |
| E8:E4 | 2 | 32% | | 16.13 |
| F14:F13 | 1 | 81% | 21.94 | |
| F14:F13 | 2 | 19% | | 18.48 |
| M17:M18 | 1 | 72% | 20.22 | |
| M17:M18 | 2 | 28% | | 17.58 |

Table 10: Siblings 70:30 mixture table. Evidence is the evidence item name. Weight is the inferred mixture weight obtained from TrueAllele®. Major is the major contributor in each mixture. Minor is the minor contributor in each mixture. The obtained match statistics are in log(LR) units.

*Sibling Mixtures*

The three mixtures – E7:E5, E8:E4, and M17:M18 – whose inferred mixture weights were closest to the target weights of 70% and 30%, obtained similar log(LR) match statistics for both the major contributor and the minor contributor (Table 10). The mixture that obtained inferred mixture weights of 81% and 19% – F14:F13 – obtained the highest log(LR) match statistic for both the major contributor and the minor contributor (Table 10).

The mixture E7:E5 obtained a log(LR) match statistic for the major contributor (Sister E7) of 18.73 and a log(LR) match statistic for the minor contributor (Sister E5) of 17.11 (Table 10). The mixture E8:E4 obtained a log(LR) match statistic for the major contributor (Brother E8) of 17.35 and a log(LR) match statistic for the minor contributor (Brother E5) of 16.13. The mixture F14:F13 obtained a log(LR) match statistic for the major contributor (Sister F14) of 21.94 and a log(LR) match statistic for the minor contributor (Sister F13) of 18.48. Finally, the mixture M17:M18 obtained a log(LR) match statistic for the major contributor (Sister M17) of 20.22 and a log(LR) match statistic for the minor contributor (Sister M18) of 17.58 (Table 10).
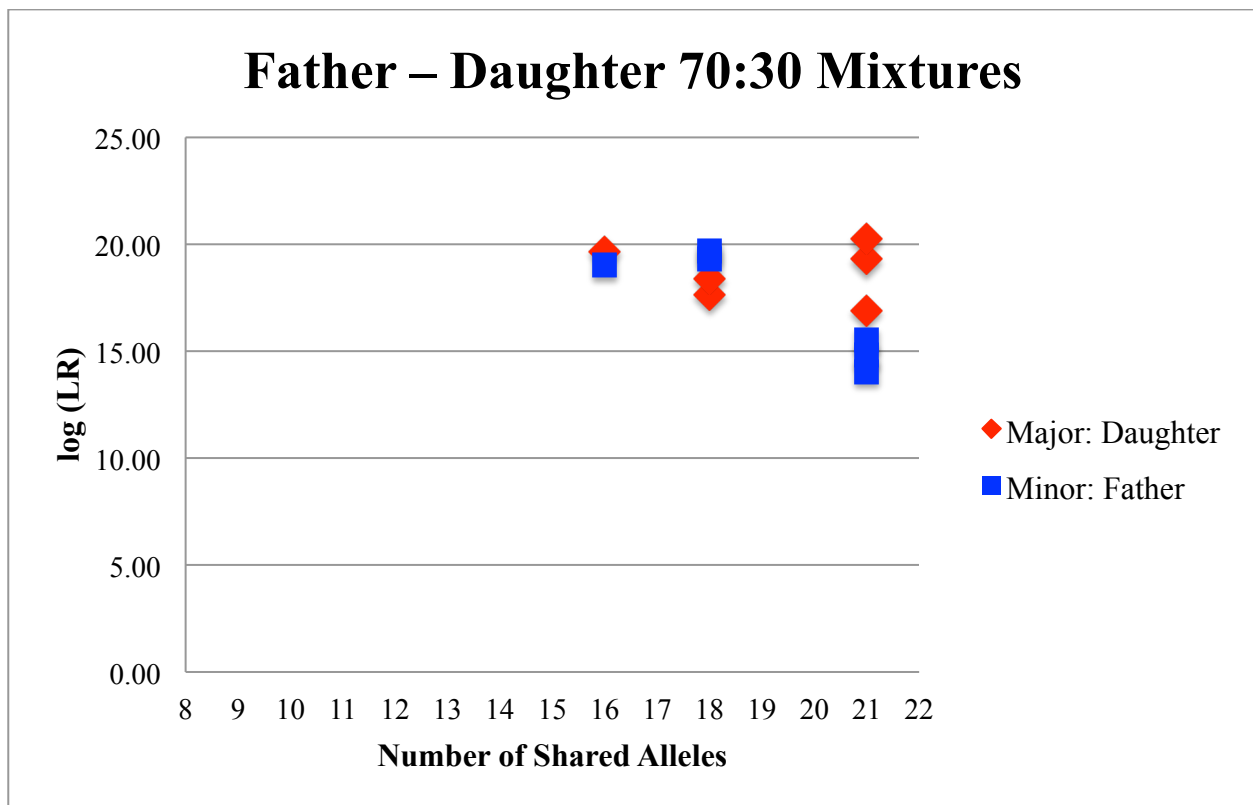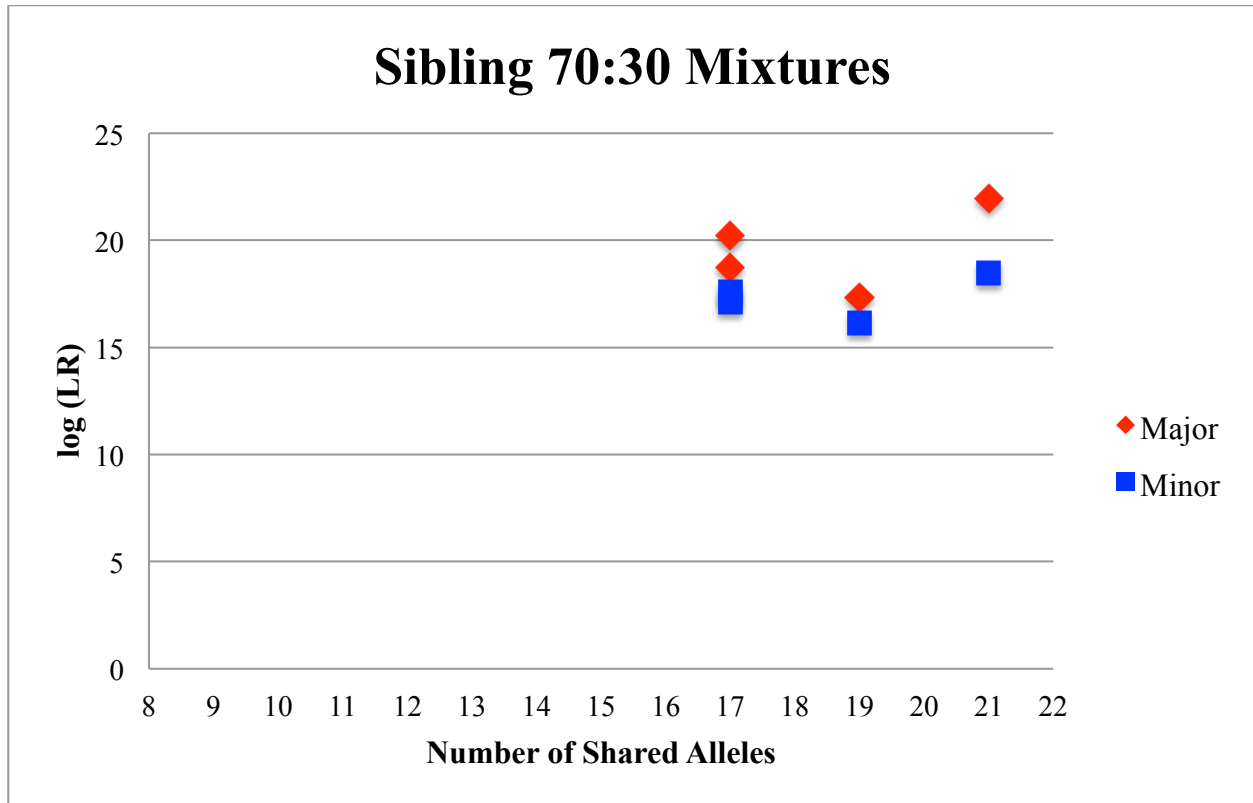
Figure 12: Sibling 70:30 mixtures log(LR) vs. number of shared alleles.  The log(LR) reflects the match statistic obtained for each major and minor contributor.  The number of shared alleles refers to the total number of alleles shared between the two individuals present in the mixture. The obtained match statistics for the major contributor are shown using red diamonds.  The obtained match statistics for the minor contributor are shown using blue squares.

Similar to a parent child relationship, siblings are also expected to share ½ of their alleles, or 15 alleles.  Once again, the number of shared alleles was obtained for each mixture by looking at the genotypes of the major and minor contributors and assessing the number of shared alleles at each locus.  If the major and minor contributors were both heterozygotes, and they possessed the same two alleles at a specific locus, it was counted as a two (2).  If the major and minor contributors were both homozygotes, at the same allele at a specific locus, it was counted as a two (2).  If the major and minor contributors shared one allele at a specific locus, it was counted as a one (1).  Finally, if the major and minor contributors did not share any alleles at a specific

locus, it was counted as zero (0).  The value – 0, 1, or 2 – obtained from each locus was then added together to determine the number of shared alleles for that specific mixture.

In these four sibling mixtures, the E7:E5 and M17:M18 mixtures were found to have 17 shared alleles, the E8:E4 mixture was found to have 19 shared alleles, and the F13:F14 mixture was found to have 21 shared alleles (Figure 12).  All of the obtained shared allele values were found to be higher than the expected value of 15 alleles.  When the log(LR) was plotted against the number of shared alleles, the number of shared alleles in the mixture did not seem to impact the log(LR) obtained for either the major or the minor contributor.  This could be partially due to the fact that three out of the four mixtures – E7:E5, E8:E4, and M17:M18 – obtained inferred mixture weights close to the target mixture ratio of 70:30.  The remaining mixture – F13:F14 – obtained an inferred mixture weight closer to 80:20 and it also obtained the highest log(LR) match statistic for both the major and the minor contributor.  Therefore, this inferred mixture weight of 80:20 could explain why the log(LR) match statistics, for both the major and the minor contributor, did not decrease as expected, when the number of shared alleles increased (Figure 12).

| Evidence | Contributor | Weight | Niece | Uncle |
|----------|-------------|--------|-------|-------|
| E1:E8 | 1 | 67% | 18.79 | |
| E1:E8 | 2 | 33% | | 15.40 |
| M10:M13 | 1 | 77% | 19.39 | |
| M10:M13 | 2 | 23% | | 20.50 |

Table 11: Uncle – Niece 70:30 mixture table.  Evidence is the evidence item name. Weight is the inferred mixture weight obtained from TrueAllele®.  Niece is the major contributor in each mixture.  Uncle is the minor contributor in each mixture.  The obtained match statistics are in log(LR) units.

*Uncle – Niece Mixtures*

Both of the mixtures – E1:E8 and M10M13 – obtained inferred mixture weights that were close to the target weights of 70% and 30% (Table 11).  The obtained log(LR) match statistics for the major contributor (Niece) were similar – 18.79 and 19.39 – in both of the mixtures. While the obtained log(LR) match statistics for the minor contributor (Uncle) were found to be 15.40 and 20.50 (Table 11).

## Uncle - Niece 70:30 Mixtures
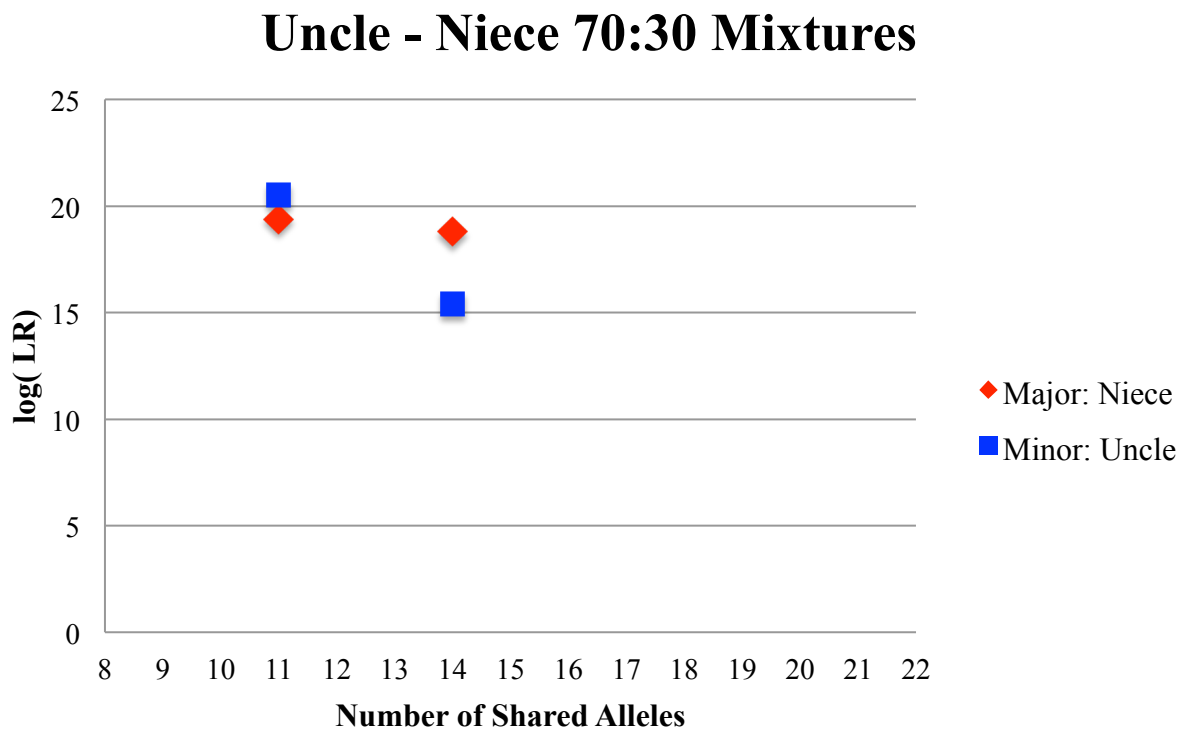


Figure 13: Uncle – Niece 70:30 mixtures log(LR) vs. number of shared alleles.  The log(LR) reflects the match statistic obtained for each major and minor contributor.  The number of shared alleles refers to the total number of alleles shared between the two individuals present in the mixture.  The obtained match statistics for the major contributor (Niece) are shown using red diamonds.  The obtained match statistics for the minor contributor (Uncle) are shown using blue squares.

Unlike the previous two relationships, uncles and nieces are second degree relatives, which means they are expected to share ¼ of there alleles, or 7.5 alleles. Since half of an allele is not seen, for purposes of comparison, we expected to see 7 or 8 shared alleles.

In these two uncle – niece mixtures, the M10:M13 mixture was found to have 11 shared alleles and the E1:E8 mixture was found to have 14 shared alleles (Figure 13). All of the obtained shared allele values were found to be higher than the expected value of 7-8 alleles. When the log(LR) was plotted against the number of shared alleles, the number of shared alleles in the mixture appeared to affect the log(LR) obtained for the minor contributor. When the number of shared alleles increased from 11 to 14, the log(LR) for the minor contributor (Uncle) dropped by almost 5 log(LR) units. Furthermore, both mixtures obtained an inferred mixture weight close to the target mixture ratio of 70:30. Therefore, it is unlikely this decrease in log(LR) units is due to mixture weight (Figure 13).

| Evidence | Contributor | Known | Weight | Granddaughter | Grandfather |
|---|---|---|---|---|---|
| M17:M20 | 1 | | 72% | 20.22 | |
| M17:M20 | 2 | | 28% | | 19.54 |
| M17:M20+M17 | 2 | Granddaughter (M17) | 28% | | 19.68 |
| M18:M20 | 1 | | 51% | 11.38 | |
| M18:M20 | 2 | | 49% | | 12.74 |
| M18:M20+M18 | 2 | Granddaughter (M18) | 51% | | 20.13 |

Table 12: Grandfather - Granddaughter 70:30 mixture table. Evidence is the evidence item name. Weight is the inferred mixture weight obtained from TrueAllele®. Granddaughter is the major contributor in each mixture. Grandfather is the minor contributor in each mixture. The obtained match statistics are in log(LR) units.

*Grandfather – Granddaughter Mixtures*

      The M17:M20 mixture obtained inferred mixture weights of 72% and 28%, which were close to the target weights of 70% and 30% (Table 12).  The remaining mixture M18:M20 obtained inferred mixture weights of 51% and 49%.  The M17:M20 mixture obtained a log(LR) match statistic for the major contributor (Granddaughter) of 20.22 and a log(LR) match statistic for the minor contributor (Grandfather) of 19.54.  The log(LR) match statistic for the minor contributor (Grandfather) of 19.54 improved to 19.68 when the major contributor (Granddaughter) was assumed.  The M18:M20 mixture obtained a log(LR) match statistic for the major contributor (Granddaughter) of 11.38 and a  log(LR) match statistic for the minor contributor (Grandfather) of 12.74.  The log(LR) match statistic for the minor contributor (Grandfather) of 12.74 improved to 20.13 when the major contributor (Granddaughter) was assumed (Table 12).
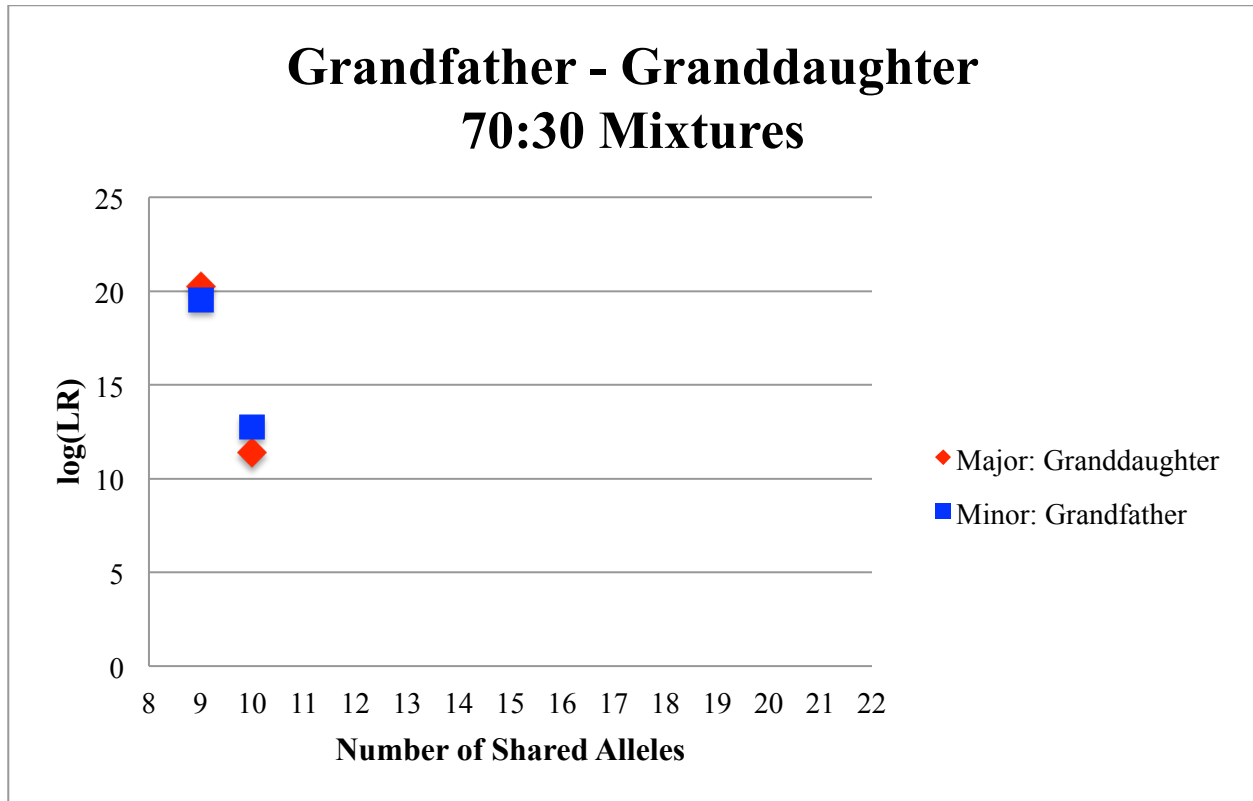
Figure 14: Granddaughter – Grandfather 70:30 mixtures log(LR) vs. number of shared alleles. The log(LR) reflects the match statistic obtained for each major and minor contributor. The number of shared alleles refers to the total number of alleles shared between the two individuals present in the mixture. The obtained match statistics for the major contributor (Granddaughter) are shown using red diamonds. The obtained match statistics for the minor contributor (Grandfather) are shown using blue squares.

Similar to a uncle - niece relationship, grandfather - granddaughters are also expected to share ¼ of there alleles, or 7.5 alleles. Since half of an allele is not seen, for purposes of comparison, we expected to see 7 or 8 shared alleles.

In these two grandfather – granddaughter mixtures, the M17:M20 mixture was found to have 9 shared alleles and the M18:M20 mixture was found to have 10 shared alleles (Figure 14). All of the obtained shared allele values were found to be higher than the expected value of 7-8 alleles. However, these obtained values were not as high as the shared allele values of 11 and 14, found with the uncle-niece mixtures. When the log(LR) was plotted against the number of

shared alleles, the number of shared alleles in the mixture appeared to affect the log(LR)

obtained for both the major (niece) and the minor (uncle) contributors.  When the number of

shared alleles increased from 9 to 10, the log(LR) for the major contributor (niece) dropped by

almost 9 log(LR) units and the log(LR) for the minor contributor (uncle) dropped by almost 7

log(LR) units.  It is important to note, the mixture (M18:M20) that displayed the much lower

log(LR) statistics for both the major and the minor contributor, was found to have an inferred

mixture weight close to 50:50.  Therefore, it is possible that mixture weight, and not the number

of shared alleles, led to the decreased major log(LR) match statistic and minor log(LR) match

statistic obtained for this particular mixture (Figure 14).

Figure 15: Major contributors 70% and 80% mixture weight vs. number of shared alleles. The log(LR) reflects the match statistic obtained for each major contributor. The number of shared alleles refers to the total number of alleles shared between the two individuals present each of the mixtures. The obtained match statistics for the major contributor (Daughter) in the father – daughter mixtures are shown using red diamonds. The obtained match statistics for the major contributor in the sibling mixtures are shown using blue squares. The obtained match statistics for the major contributor (Niece) in the niece - uncle mixtures are shown using purple triangles. The obtained match statistics for the major contributor (Granddaughter) in the grandfather - granddaughter mixtures are shown using green *x*'s.

The obtained match statistics for the major contributors at 70%-80% mixture weight, across all of the tested relationships, ranged from 16.89 to 20.25. Therefore, a match statistics can be reported, for each major contributor, in all of the tested mixtures.
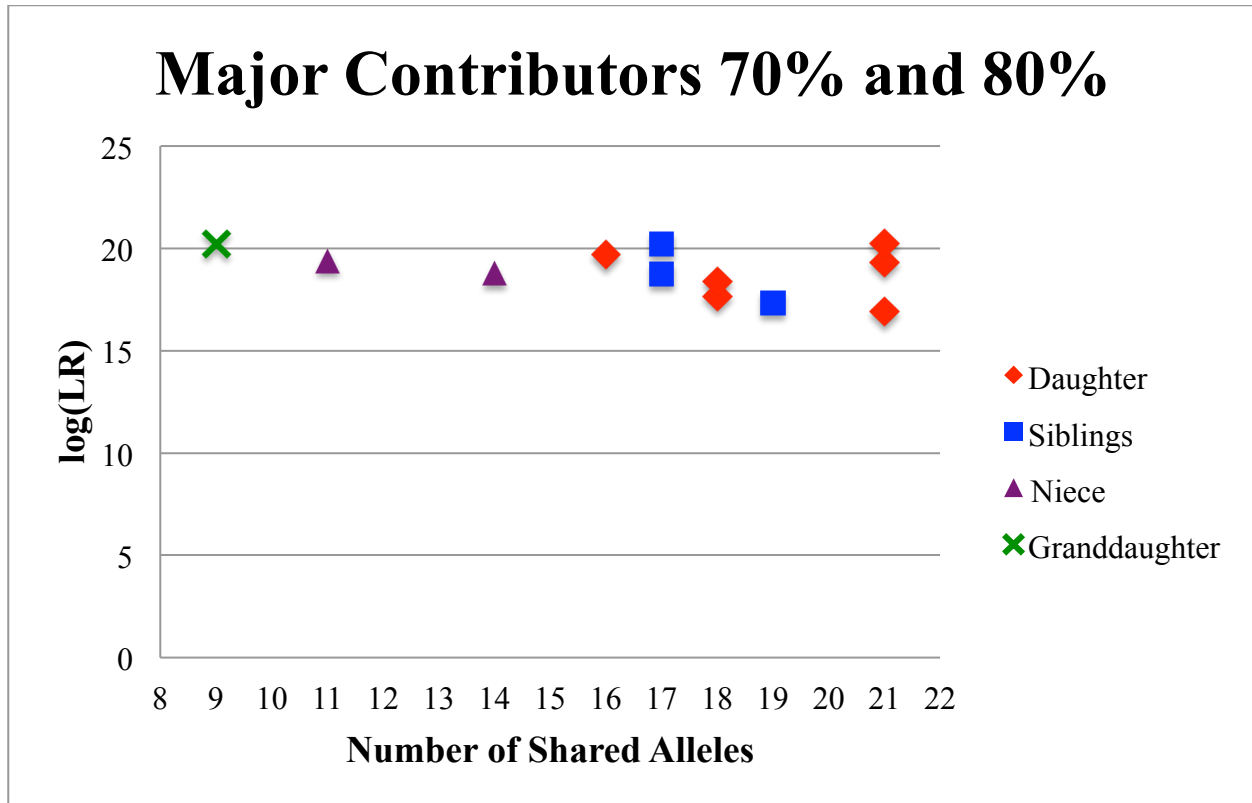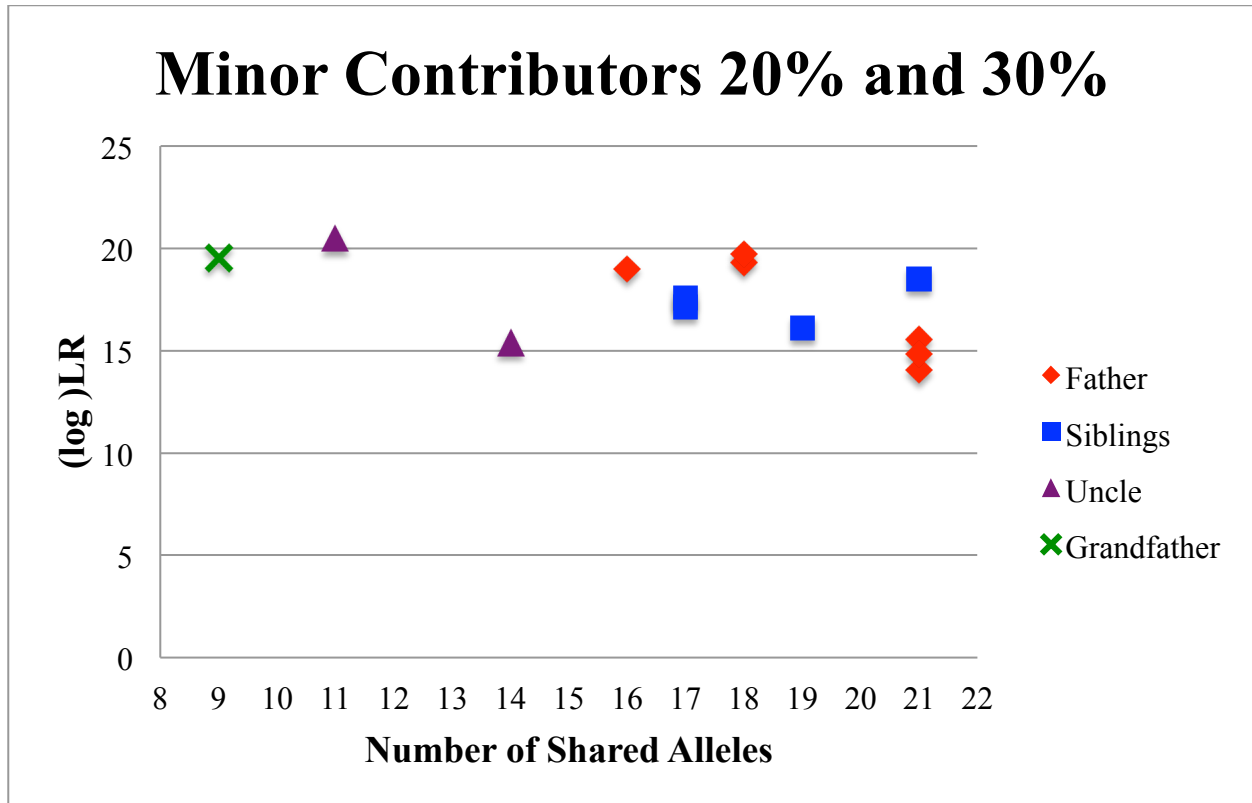
Figure 16: Minor contributors 20% and 30% log(LR) vs. number of shared alleles.  The log(LR) reflects the match statistic obtained for each major contributor.  The number of shared alleles refers to the total number of alleles shared between the two individuals present each of the mixtures.  The obtained match statistics for the minor contributor (Father) in the father – daughter mixtures are shown using red diamonds.  The obtained match statistics for the minor contributor in the sibling mixtures are shown using blue squares.  The obtained match statistics for the minor contributor (Uncle) in the niece - uncle mixtures are shown using purple triangles. The obtained match statistics for the minor contributor (Grandfather) in the grandfather - granddaughter mixtures are shown using green x's.

The obtained match statistics for the minor contributors at 20%-30% mixture weight, across all of the tested relationships, ranged from 14.04 to 20.50.  Therefore, a match statistics can be reported, for each minor contributor, in all of the tested mixtures.

| Evidence | Contributor | Known(s) | Father (E4) | Son (E2) | Mother (E3) |
|----------|-------------|----------|-------------|----------|-------------|
| E4:E2:E3 | 1 | - | 12.92 | | |
| E4:E2:E3 | 2 | - | | 13.55 | |
| E4:E2:E3 | 3 | - | | | 4.29 |
| E4:E2:E3 | 1 | E4 | | 15.42 | |
| E4:E2:E3 | 2 | E4 | | | 7.58 |
| E4:E2:E3 | 1 | E4, E2 | | | 2.53 |

Table 13: Father – Son – Mother Family E mixture table.  Evidence is the evidence item name. Contributor specifies the number of genotypes TrueAllele® was asked to search for in the mixture.  Known(s) are the genotypes that were fixed in the mixture.  Father (E4) is the major contributor in the mixture at 60%.  The son (E2) is the middle contributor in the mixture at 30%. The mother (E3) is the minor contributor in the mixture at 10%.  The obtained match statistics are in log(LR) units.

*Three-person Mixtures*

The evidence item was first run assuming three unknowns, and a match statistic was obtained for each of the contributors in the mixture (Table 13).  Next, a technique known as peeling was utilized.  The genotype for the major contributor by mixture weight – the father (E4) in the mixture at 60% – was fixed, and the computer was asked to solve for the remaining two genotypes in the mixture.  Assuming the father (E4) increased the match statistics obtained for both the son (E2) and the mother (E3).  Peeling was continued, and the next highest contributor by mixture weight – the son (E2) in the mixture at 30% – was also assumed.  With both the father's (E4) and the son's (E2) genotypes fixed, the computer was asked to solve for the remaining genotype in the mixture.  Assuming both the father (E4) and the son (E2) decreased the match statistic obtained for the mother (E3) from 7.58 to 2.53 (Table 13).  The mother's match statistic decreased because she shares half of her alleles with the son.  Therefore, when the son's genotype was fixed it took away allele possibilities from the mother, which led to a decrease in her match statistic.

| Evidence | Contributor | Known(s) | Father (F15) | Son (F16) | Mother (F14) |
|---|---|---|---|---|---|
| F15:F16:F14 | 1 | - | 17.53 | | |
| F15:F16:F14 | 2 | - | | 17.98 | |
| F15:F16:F14 | 3 | - | | | 7.12 |
| F15:F16:F14 | 2 | F15 | | 20.23 | |
| F15:F16:F14 | 3 | F15 | | | 5.33 |
| F15:F16:F14 | 3 | F15, F16 | | | 7.24 |

Table 14: Father – Son – Mother Family F mixture table. Evidence is the evidence item name. Contributor specifies the number of genotypes TrueAllele® was asked to search for in the mixture. Known(s) are the genotypes that were fixed in the mixture. Father (F15) is the major contributor in the mixture at 60%. The son (F16) is the middle contributor in the mixture at 30%. The mother (F14) is the minor contributor in the mixture at 10%. The obtained match statistics are in log(LR) units.

Following the same procedure utilized for the other three-person mixture, the evidence item was first run assuming three unknowns, and a match statistic was obtained for each of the contributors in the mixture (Table 14). Assuming the father (F15) increased the match statistic obtained for the son (F16) from 17.98 to 20.03 and decreased the match statistic obtained for the mother (F14) from 7.12 to 5.33. When both the father (F15) and the son (F16) were fixed, the match statistic obtained for the mother (F14) increased from 5.33 to 7.24 (Table 14). Assuming both the father and the son, allowed any alleles that were not part of the son's or father's genotypes to be attributed to the mother, which caused her match statistic to increase.

| Evidence | Contributor | Known | Daughter (E1) | Brother (E2) | Father (E4) | Mother (E3) |
|---|---|---|---|---|---|---|
| 4PE4 | 1 | - | 14.44 | | | |
| 4PE4 | 2 | - | | 13.61 | | |
| 4PE4 | 3 | - | | | 13.07 | |
| 4PE4 | 4 | - | | | | 7.62 |
| 4PE4 | 2 | E1 | | 15.79 | | |
| 4PE4 | 3 | E1 | | | 6.20 | |
| 4PE4 | 4 | E1 | | | | 4.15 |
| 4PE4 | 3 | E1, E2 | | | 4.95 | |
| 4PE4 | 4 | E1, E2 | | | | 0.57 |
| 4PE4 | 4 | E1, E2, E4 | | | | 1.65 |

Table 15: Four-person mixture containing the father (E4) mixture table. Evidence is the evidence item name. Contributor specifies the number of genotypes TrueAllele® was asked to search for in the mixture. Known(s) are the genotypes that were fixed in the mixture. Daughter (E1) is the major contributor in the mixture at 40%. The brother (E2) is the next highest contributor in the mixture at 30%. The father (E4) is the next highest contributor in the mixture at 20%. The mother (E3) is the minor contributor in the mixture at 10%. The obtained match statistics are in log(LR) units.

*Four – Person Mixtures*

The evidence item was initially run as a four unknown, and a match statistic was obtained to each of the contributors in the mixture (Table 15). Next, peeling commenced and the major contributor by mixture weight – the daughter (E1) at 40% – was assumed. When the daughter's genotype was fixed the computer was asked to solve for the remaining three genotypes in the mixture. Assuming the daughter: increased the match statistic obtained for the brother (E2) from 13.61 to 15.79, decreased the match statistic obtained for the father (E4) from 13.07 to 6.20, and decreased the match statistic obtained for the mother (E3) from 7.62 to 4.15. Peeling continued, and the next major contributor by mixture weight – the brother (E2) at 30% – was also assumed. When both the daughter's (E1) and the brother's (E2) genotypes were fixed, the computer was asked to search for the remaining two genotypes. Assuming both the daughter and the brother decreased the match statistic obtained to the father (E4) from 6.20 to 4.95 and decreased the

match statistic obtained from the mother (E3) from 4.15 to 0.57. The final step in peeling was to assume the father (E4) who contributed to the mixture at 20%. Fixing the daughter's (E1), brother's (E2), and father's (E4) genotypes, left the computer only one genotype to search for. Assuming all three genotypes increased the match statistic obtained for the mother (E3) from 0.57 to 1.65 (Table 15).

Assuming the daughter (E1) led to a decrease in the match statistic obtained for the father (E4) and mother (E3) because both the father and the mother pass on one allele to their daughter. When the daughter was assumed, the allele possibilities for both the mother and the father decreased, which was reflected in both match statistics. This also explains why the match statistic for both the mother and the father decreased when the brother's genotype was fixed. When the daughter's (E1), brother's (E2), and father's (E4) genotypes were fixed, the match statistic obtained for the mother (E3) improved. This is because any alleles that were not attributed to the daughter, brother, or father could then be attributed to the mother, which caused her match statistic to increase.

| Evidence | Contributor | Known | Daughter (E1) | Brother (E2) | Uncle (E8) | Mother (E3) |
|----------|-------------|-------|---------------|--------------|------------|-------------|
| 4PE8 | 1 | - | 13.33 | | | |
| 4PE8 | 2 | - | | 11.95 | | |
| 4PE8 | 3 | - | | | 8.47 | |
| 4PE8 | 4 | - | | | | 9.25 |
| 4PE8 | 1 | E1 | | 12.45 | | |
| 4PE8 | 2 | E1 | | | 9.47 | |
| 4PE8 | 3 | E1 | | | | 8.26 |
| 4PE8 | 1 | E1, E2 | | | 10.16 | |
| 4PE8 | 2 | E1, E2 | | | | 2.99 |
| 4PE8 | 1 | E1, E2, E8 | | | | 2.70 |

Table 16: Four-person mixture containing the uncle (E8) mixture table. Evidence is the evidence item name. Contributor specifies the number of genotypes TrueAllele® was asked to search for in the mixture. Known(s) are the genotypes that were fixed in the mixture. Daughter (E1) is the major contributor in the mixture at 40%. The brother (E2) is the next highest contributor in the mixture at 30%. The uncle (E8) is the next highest contributor in the mixture at 20%. The mother (E3) is the minor contributor in the mixture at 10%. The obtained match statistics are in log(LR) units.

The evidence item was initially run as a four unknown, and a match statistic was obtained to each of the contributors in the mixture (Table 16). When the daughter's (E1) genotype was fixed: the match statistic obtained for the brother (E2) increased from 11.95 to 12.45, the match statistic obtained for the uncle (E8) increased from 8.47 to 9.47, and the match statistic obtained for the mother (E3) decreased from 9.25 to 8.26. Assuming both the daughter's (E1) and the brother's (E2) genotypes increased the match statistic obtained to the uncle (E8) from 9.47 to 10.16 and decreased the match statistic obtained from the mother (E3) from 8.26 to 2.99. Fixing the daughter's (E1), brother's (E2), and uncle's (E8) genotypes decreased the match statistic obtained for the mother (E3) from 2.99 to 2.70 (Table 16).

Assuming the daughter (E1) led to a decrease in the match statistic obtained for the mother (E3) because the mother passes on one allele to her daughter. When the daughter was assumed, the allele possibilities for the mother decreased, which was reflected in the match

statistic.  This also explains why the match statistic obtained for the mother decreased when the

brother's genotype was fixed.  On the other hand, the match statistic obtained for the uncle (E8)

first increased when the daughter (E1) was assumed, and then continued to increase when the

brother (E2) was assumed.  This increase can be attributed to the uncle being a second degree

relative.  As a second-degree relative, the uncle contributes alleles to the mixture that are not

shared by the daughter, brother, or mother thus affording the uncle a wider range of alleles

possibilities.

| Evidence | Contributor | Known | Daughter (E1) | Brother (E2) | Grandmother (E5) | Mother (E3) |
|---|---|---|---|---|---|---|
| 4PE5 | 1 | - | 12.14 | | | |
| 4PE5 | 2 | - | | 11.08 | | |
| 4PE5 | 3 | - | | | 9.47 | |
| 4PE5 | 4 | - | | | | 8.95 |
| 4PE5 | 2 | E1 | | 12.22 | | |
| 4PE5 | 3 | E1 | | | 9.83 | |
| 4PE5 | 4 | E1 | | | | 6.05 |
| 4PE5 | 3 | E1, E2 | | | 11.38 | |
| 4PE5 | 4 | E1, E2 | | | | 2.24 |
| 4PE5 | 4 | E1, E2, E5 | | | | 4.77 |

Table 17: Four-person mixture containing the grandmother (E5) mixture table.  Evidence is the evidence item name.  Contributor specifies the number of genotypes TrueAllele® was asked to search for in the mixture.  Known(s) are the genotypes that were fixed in the mixture.  Daughter (E1) is the major contributor in the mixture at 40%.  The brother (E2) is the next highest contributor in the mixture at 30%.  The grandmother (E5) is the next highest contributor in the mixture at 20%.  The mother (E3) is the minor contributor in the mixture at 10%.  The obtained match statistics are in log(LR) units.

Utilizing the same method, the evidence item was first run as a four unknown, and a

match statistic was obtained to each of the contributors in the mixture (Table 17).  Fixing the

daughter's (E1) genotype: increased the match statistic obtained for the brother (E2) from 11.08

to 12.22, increased the match statistic obtained for the grandmother (E5) from 9.47 to 9.83, and decreased the match statistic obtained for the mother (E3) from 8.95 to 6.05.  When both the daughter's (E1) and the brother's (E2) genotypes were fixed, the match statistic obtained to the grandmother (E5) increased from 9.83 to 11.38 and the match statistic obtained from the mother (E3) decreased from 6.05 to 2.24.  Fixing the daughter's (E1), brother's (E2), and grandmother's (E5) genotypes, increased the match statistic obtained for the mother (E3) from 2.24 to 4.77 (Table 17).

Assuming the daughter (E1) led to a decrease in the match statistic obtained for the mother (E3) because the mother passes on one allele to her daughter.  When the daughter was assumed, the allele possibilities for the mother decreased, which was reflected in the match statistic.  This also explains why the match statistic obtained for the mother decreased when the brother's genotype was fixed.  On the other hand, the match statistic obtained for the grandmother (E5) first increased when the daughter (E1) was assumed, and then continued to increase when the brother (E2) was assumed.  This increase can be attributed to the grandmother being a second degree relative.  As a second-degree relative, the grandmother contributes alleles to the mixture that are not shared by the daughter, son, or mother thus affording the grandmother a wider range of alleles possibilities.  When the daughter's (E1), brother's (E2), and grandmother's (E5) genotypes were fixed, the match statistic obtained for the mother (E3) improved.  This is because any alleles that were not attributed to the daughter, brother, or grandmother could then be attributed to the mother, which caused her match statistic to increase.

| Evidence | Contributor | Known | Daughter (E1) | Brother (E2) | Grandmothers Sister (E7) | Mother (E3) |
|---|---|---|---|---|---|---|
| 4PE7 | 1 | - | 11.25 | | | |
| 4PE7 | 2 | - | | 11.42 | | |
| 4PE7 | 3 | - | | | 9.63 | |
| 4PE7 | 4 | - | | | | 9.60 |
| 4PE7 | 2 | E1 | | 10.88 | | |
| 4PE7 | 3 | E1 | | | 10.34 | |
| 4PE7 | 4 | E1 | | | | 7.48 |
| 4PE7 | 3 | E1, E2 | | | 14.83 | |
| 4PE7 | 4 | E1, E2 | | | | 3.74 |
| 4PE7 | 4 | E1, E2, E7 | | | | 5.37 |

Table 18: Four-person mixture containing the grandmother's sister (E7) mixture table. Evidence is the evidence item name. Contributor specifies the number of genotypes TrueAllele® was asked to search for in the mixture. Known(s) are the genotypes that were fixed in the mixture. Daughter (E1) is the major contributor in the mixture at 40%. The brother (E2) is the next highest contributor in the mixture at 30%. The grandmother's sister (E7) is the next highest contributor in the mixture at 20%. The mother (E3) is the minor contributor in the mixture at 10%. The obtained match statistics are in log(LR) units.

The evidence item was first run as a four unknown, and a match statistic was obtained to each of the contributors in the mixture (Table 18). Fixing the daughter's (E1) genotype: decreased the match statistic obtained for the brother (E2) from 11.42 to 10.88, decreased the match statistic obtained for the mother (E3) from 9.60 to 7.48, and increased the match statistic obtained for the grandmother's sister (E5) from 9.63 to 10.34. When both the daughter's (E1) and the brother's (E2) genotypes were fixed, the match statistic obtained to the grandmother's sister (E7) increased from 10.34 to 14.83 and the match statistic obtained from the mother (E3) decreased from 7.48 to 3.74. Fixing the daughter's (E1), brother's (E2), and grandmother's sister's (E7) genotypes increased the match statistic obtained for the mother (E3) from 3.74 to 5.37 (Table 18).

Assuming the daughter (E1) led to a decrease in the match statistic obtained for the mother (E3) because the mother passes on one allele to her daughter.  When the daughter was assumed, the allele possibilities for the mother decreased, which was reflected in the match statistic.  This also explains why the match statistic obtained for the mother decreased when the brother's genotype was fixed.  On the other hand, the match statistic obtained for the grandmother's sister (E7) first increased when the daughter (E1) was assumed, and then continued to increase when the brother (E2) was assumed.  This increase can be attributed to the grandmother's sister contributing alleles to the mixture that are not shared by the daughter, son, or mother, thus affording the grandmother's sister a wider range of alleles possibilities.  When the daughter's (E1), brother's (E2), and grandmother's sister's (E7) genotypes were fixed, the match statistic obtained for the mother (E3) improved.  This is because any alleles that were not attributed to the daughter, brother, or grandmother's sister could then be attributed to the mother, which caused her match statistic to increase.

| Evidence | Contributor | Known | Daughter (E1) | Brother (E2) | Uncle (E8) | Father (E4) | Mother (E3) |
|---|---|---|---|---|---|---|---|
| 5PE | 1 | - | 11.80 | | | | |
| 5PE | 2 | - | | 10.68 | | | |
| 5PE | 3 | - | | | 8.73 | | |
| 5PE | 4 | - | | | | 10.41 | |
| 5PE | 5 | - | | | | | 9.08 |
| 5PE | 2 | E1 | | 12.57 | | | |
| 5PE | 3 | E1 | | | 9.07 | | |
| 5PE | 4 | E1 | | | | 8.06 | |
| 5PE | 5 | E1 | | | | | 6.87 |
| 5PE | 3 | E1, E2 | | | 11.09 | | |
| 5PE | 4 | E1, E2 | | | | 5.99 | |
| 5PE | 5 | E1, E2 | | | | | 1.71 |
| 5PE | 4 | E1, E2, E8 | | | | 2.64 | |
| 5PE | 5 | E1, E2, E8 | | | | | 2.31 |
| 5PE | 5 | E1, E2, E8, E4 | | | | | - |

Table 19: Five-person mixture Family E mixture table. Evidence is the evidence item name. Contributor specifies the number of genotypes TrueAllele® was asked to search for in the mixture. Known(s) are the genotypes that were fixed in the mixture. Daughter (E1) is the major contributor in the mixture at 35%. The brother (E2) is the next highest contributor in the mixture at 30%. The uncle (E8) is in the mixture at 20%. The father (E4) is in the mixture at 10%. The mother (E3) is the minor contributor in the mixture at 5%. The obtained match statistics are in log(LR) units.

*Five – Person Mixtures*

The evidence mixture was initially run as a five unknown, and a match statistic was obtained to each of the contributors in the mixture (Table 19). Next peeling commenced, the major contributor by mixture weight – the daughter (E1) at 35% – was assumed first. When the daughter's genotype was fixed the computer was asked to solve for the remaining four genotypes in the mixture. Assuming the daughter: increased the match statistic obtained for the brother (E2) from 10.68 to 12.57, increased the match statistic obtained for the uncle (E8) from 8.73 to 9.07, decreased the match statistic obtained from the father from 10.41 to 8.06, and decreased the match statistic obtained

for the mother (E3) from 9.08 to 6.87.  Peeling continued, and the next major contributor

by mixture weight – the brother (E2) at 30% – was also assumed.  When both the

daughter's (E1) and the brother's (E2) genotypes were fixed, the computer was asked to

search for the remaining three genotypes.  Assuming both the daughter and the brother

increased the match statistic obtained to the uncle (E8) from 9.07 to 11.09, decreased the

match statistic obtained to the father (E4) from 8.06 to 5.99, and decreased the match

statistic obtained from the mother (E3) from 6.87 to 1.71.  The next step in peeling was to

assume the uncle (E8) who contributed to the mixture at 20%.  Fixing the daughter's

(E1), brother's (E2), and uncle's (E8) genotypes, left the computer two genotypes to

search for.  Assuming all three genotypes decreased the match statistic obtained for the

father from 5.99 to 2.64 and increased the match statistic obtained for the mother (E3)

from 1.71 to 2.31.  The final step in the peeling process was to assume the father (E4)

who contributed to the mixture at 10%.  Fixing the daughter's (E1), brother's (E2), and

uncle's (E8), and father's (E4) genotypes, left the computer only one genotype to search

for.  Assuming all four genotypes decreased the match statistic obtained for the mother

from 2.31 to a negative value (Table 19).

    Assuming the daughter (E1) led to a decrease in the match statistic obtained for

the father (E4) and mother (E3) because both the father and the mother pass on one allele

to their daughter.  When the daughter was assumed, the allele possibilities for both the

mother and the father decreased, which was reflected in both match statistics.  This also

explains why the match statistic for both the mother and the father decreased farther

when the brother's genotype was fixed.  On the other hand, the match statistic obtained

for the uncle (E8) first increased when the daughter (E1) was assumed, and then

continued to increase when the brother (E2) was assumed. This increase can be attributed to the uncle being a second degree relative to both the daughter and the brother. As a second-degree relative, the uncle contributes alleles to the mixture that are not shared by the daughter or the brother, thus affording the uncle a wider range of alleles possibilities when both the daughter's and the brother's genotypes are fixed. When the daughter's (E1), brother's (E2), and uncle's (E8) genotypes were fixed the match statistic obtained to the father (E4) decreased and the match statistic obtained to the mother (E3) increased. The match statistic obtained to the father decreased because the father and the uncle are brothers; thus, the uncle shares an expected half of his alleles with the father. When the uncle's genotype is fixed, it removes allele possibilities from the father, which leads to a decreased match statistic. Since the mother is not a blood relative of the uncle, when the uncles's genotype is fixed, the mother's match statistic increases. This increase is because any alleles that were not fixed to the daughter, brother, or uncle could then be attributed to the mother. When the daughter's (E1), brother's (E2), uncles's (E8), and father's (E4) genotypes were fixed, no match statistic was obtained to the mother (E3). Since the mother was the minor contributor in the mixture at 5%, when all of the other contributors to the mixture were assumed, there were not enough alleles left to attribute to her. In other words, due to her relatedness to the individuals in the mixture, the mother's alleles were masked and a match statistic could not be obtained.

| Evidence | Contributor | Known | Daughter (M17) | Sister (M18) | Mother (M19) | Father (M16) | Uncle (M1) |
|---|---|---|---|---|---|---|---|
| 5PM | 1 | - | 16.55 | | | | |
| 5PM | 2 | - | | 3.90 | | | |
| 5PM | 3 | - | | | 6.15 | | |
| 5PM | 4 | - | | | | 19.29 | |
| 5PM | 5 | - | | | | | 1.94 |
| 5PM | 2 | M17 | | 6.62 | | | |
| 5PM | 3 | M17 | | | 7.71 | | |
| 5PM | 4 | M17 | | | | 19.75 | |
| 5PM | 5 | M17 | | | | | 5.25 |
| 5PM | 3 | M17, M18 | | | - | | |
| 5PM | 4 | M17, M18 | | | | 20.00 | |
| 5PM | 5 | M17, M18 | | | | | - |
| 5PM | 4 | M17, M18, M19 | | | | 20.02 | |
| 5PM | 5 | M17, M18, M19 | | | | | - |
| 5PM | 5 | M17, M18, M19, M16 | | | | | - |

Table 20: Five-person mixture Family M mixture table.  Evidence is the evidence item name.  Contributor specifies the number of genotypes TrueAllele® was asked to search for in the mixture.  Known(s) are the genotypes that were fixed in the mixture.  Daughter (M17) is the major contributor in the mixture at 35%.  The sister (M18) is the next highest contributor in the mixture at 30%.  The mother (M19) is in the mixture at 20%.  The father (M16) is in the mixture at 10%.  The uncle (M1) is the minor contributor in the mixture at 5%.  The obtained match statistics are in log(LR) units.

        The evidence mixture was initially run as a five unknown, and a match statistic

was obtained to each of the contributors in the mixture (Table 20).  Assuming the

daughter's (M17) genotype: increased the match statistic obtained for the sister (M18)

from 3.90 to 6.62, increased the match statistic obtained for the mother (M19) from 6.15

to 7.71, increased the match statistic obtained for the father (M16) from 19.29 to 19.75,

and increased the match statistic obtained for the uncle (M1) from 1.94 to 5.25. When

both the daughter's (M17) and the sister's (M18) genotypes were fixed, the match

statistic obtained for the mother (M19) decreased from 7.71 to a negative value, the

match statistic obtained for the father (M16) increased from 19.75 to 20.00, and the

match statistic obtained for the uncle (M1) decreased from 5.25 to a negative value.

Fixing the daughter's (M17), sister's (M18), and mother's (M19) genotypes, increased

the match statistic obtained for the father (M16) from 20.00 to 20.02, and a positive

match statistic for the uncle (M1) could still not be reported. Fixing the daughter's

(M17), sister's (M18), mother's (M19), and father's (M16) genotypes, showed no change

in the match statistic obtained for the uncle (M1) (Table 20).

This mixture appears unique because it exhibits trends not seen in the other tested

mixtures. However, upon farther analysis it was discovered M1 (uncle) is not a blood

relative to the other four people in the mixture. Assuming the daughter (M17) led to an

increase in the match statistic obtained for the father (M16), the mother (M19), and the

uncle (M1). In the other tested mixtures, the match statistic to the mother and father

decreased when the daughter's genotype was fixed. In this mixture, the match statistics

increased only slightly and this can be attributed to the random nature of MCMC. On the

other hand, the match statistic obtained for the uncle (M1) increased when the daughter

(E1) was assumed. This increase can be attributed to the uncle (M1) not being a blood

relative of the other individuals in the mixture. When the daughter's genotype was fixed,

it resulted in more allelic possibilities for the uncle (M1), which led to an increase in his

match statistic. When both the daughter (M17) and the sister (M18) were assumed, the

match statistic decreased for the mother (M19), slightly increased for the father (M16),

and decreased for the uncle (M1). The slight increase in the match statistic obtained for

the father can once again be attributed to the random nature of MCMC. The decrease in

the match statistic obtained for the mother is a result of the mother passing on one allele

to each of her daughter's. When both the daughter and the sister were assumed, the allele

possibilities for the mother decreased, which was reflected in her match statistic. Also,

the match statistic to the uncle decreased to a negative value. This decrease in match

statistic suggests the uncle (M1) possessed alleles that were shared by the daughter and/or

sister. When the daughter's and sister's genotypes were fixed, it removed allele

possibilities from the uncle, which led to a decrease in his match statistic. The uncle

(M1) was also the minor contributor in the mixture at 5%; therefore, it is possible that

when contributors were fixed in the mixture, there were not enough alleles left to

attribute to him. In other words, the uncle's alleles were masked and a match statistic

could not be obtained. As seen, with this mixture in particular, the main source of error

in this research is the random nature of Markov Chain Monte Carlo.


## Conclusion

In summary, a computational based analysis system like TrueAllele$^{®}$ can be a

useful tool for DNA analysts. When two-person mixtures were created and analyzed, a

match statistic was obtained for both the major and the minor contributor in each mixture.

The only exception applied to one minor contributor in a father – daughter mixture who

had an obtained mixture weight of 7%. It was also found that when the major contributor

– by mixture weight – was assumed in a two-person mixture, the obtained match statistic

improved for the minor contributor. Furthermore, both the obtained mixture weight and

the number of shared alleles seemed to affect the obtained match statistic.  Finally, for the two-person mixtures, the number of shared alleles (between individuals) were found to be greater than expected for all of the tested mixtures.

In regards to the four-person mixtures, the match statistic obtained to the minor contributor – the mother at 10% – decreased for all of the tested mixture when peeling was utilized.  When peeling continued and the two highest contributors – by mixture weight – the daughter (E1) and the brother (E2) were assumed, the match statistics for all 1$^{st}$ degree relatives decreased and the match statistics for all non 1$^{st}$ degree relatives increased.  These results suggest that while peeling can be effective for mixtures that contain multiple contributors, if kinship mixtures are being analyzed, the obtained match statistics may decreased due to the high number of shared alleles.

In conclusion, it was found that match statistics could be obtained for two-person, three-person, four-person, and five-person mixtures involving kinship.  Further testing needs to be undertaken, but the results also suggest that match statistics can be obtained for mixtures when the contributors are present at different mixture ratios and when there are different relationships between the contributors.  Since match statistics were obtained for all of the tested mixtures, this suggests that an analyst would be able to report a match statistic on a mixture that would otherwise be deemed inconclusive.

## Future Work/Directions

There are numerous avenues that can be pursued with this project.  However, in the interest of time, I will only discuss the future work and directions that pertain to my current data set.  The number of shared alleles has already been investigated in the father

– daughter relationships, but it would be interesting to investigate the number of shared alleles between the father and the mother.  Using the TrueAllele® software, the samples were analyzed using a variety of settings.  It would be helpful if one could determine the optimal settings, to obtain reproducible results, for both the four-person and the five-person kinship mixtures.  In determining the reproducibility of the results, one could concentrate more on the obtained genotype probabilities.  Statistical analysis could also be applied to determine if the results, obtained from different runs, have a difference that is statistically significant.  Additionally, the mixture ratios for the four-person and five-person mixtures were kept constant and the individuals were varied.  It could be interesting to see how the match statistics vary when different mixture ratios are tested.  Finally, for this research, peeling was carried out by mixture weight.  This is not the only way to carry out peeling, so different peeling methods could also be tested.

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