

DNA Mapping the Crime Scene: Do Computers Dream of Electric Peaks?

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Abstract

Items of evidence, from one or more scenes, can fit together to tell a story of crimes and their victims. A hat, a gun, a cell phone or countertop – whatever was touched, handled or worn – can build an invaluable investigative map of criminal activity. In an age of "CSI effects", corroborative DNA may prove vital in persuading a jury.

But this investigative dream has become a crime laboratory's nightmare. Low-level touch DNA mixtures stream in through the door, along with investigator expectations of fast, informative results. People and robots churn through challenging evidence, cranking out complex EPG signals that defy human interpretation. Key DNA is often deemed "inconclusive", and many comparisons must be made to find a probative match.

Enter the computer. In accordance with SWGDAM DNA interpretation guidelines (1, par. 3.2.2), computers can unmix the mixtures, separating out the data into each contributor's (probabilistic) genotype. The stochastic uncertainty of low-level STR peaks can be reliably modeled through statistical computing (2). Probabilistic genotyping can thoroughly examine the data, finding highly informative DNA match statistics.

Comparing many mixture items with many suspects across multiple cases leads to a very large number of potential matches. But perhaps only 10% of these genotype comparisons will actually yield a probative result. A computer can map out crime scenes by comparing evidence genotypes with references to implicate suspects. With computer assistance, a forensic analyst can translate an investigator's dream into evidentiary reality.

In a recent criminal case, a gang was suspected of committing a series of convenience store robberies. As the violence escalated, resolving these crimes became increasingly important. Touch evidence items were collected from five shops, yielding a dozen DNA mixtures. The STR peak heights were low (many under 50 rfu), and the mixture complexity was high (most items having 3 or 4 contributors). Comparisons were needed with ten reference genotypes, half suspect and half victim.

TrueAllele[®] computer interpretation of the touch DNA was able to separate evidence data into probabilistic genotypes. Eight of the twelve mixture items matched a reference, with DNA statistics ranging from thousands to quintillions. Three suspects were identified, including the likely gang leader. Whereas human assessment of the 120 potential DNA matches (12 evidence vs. 10 references) was not feasible, the computer could rapidly pinpoint the 9 probative matches.

Over a hundred criminal cases have been solved in this way, both for prosecution and defense, most involving complex DNA mixtures. Computer separation of mixed data into contributor genotypes preserves the identification information of examined DNA evidence items. Crime labs are now introducing these interpretation systems into their workflow as just one more validated analytical instrument. Their forensic scientists can use computers to convert "inconclusive" findings into solid DNA matches that map out crime scene events.

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Introduction

This paper discusses how computers can map crime scenes through deoxyribonucleic acid (DNA) by dreaming of electric peaks. The running example used here is a gang case I reported on. Some of the details have been changed in order to preserve anonymity.

A crime wave began when a gang was formed and invaded a county. They first raided a food mart, leaving behind a hat and a gun with their DNA (Figure 1, black, west). As the gang committed more crimes and gained experience, they began to physically intimidate witnesses and block their escape.

The gang next robbed a hardware store, with members leaving their DNA on a safe and a phone (Figure 1, purple, southwest). Continuing eastward (Figure 1, blue, central), they struck a jewelry store, depositing their DNA on a counter and on a hat. Their violence escalated as they moved across the county, as seen in a representative video of an armed robbery (3). The gang attacked more stores (Figure 1, green, east), leaving behind keys, hats and clothing as biological evidence items.

The DNA evidence was largely low-level mixtures containing three or four people. We next review DNA mixtures, and how they are solved.

Materials and Methods

Genotypes

DNA evidence comes from genotypes of people (Figure 2). The x-axis shows short tandem repeat (STR) allele size, increasing with allele number (11, 12, 13, 14, ...). The y-axis shows the amount of DNA.

A person's genotype at a locus is an allele pair. In the figure, there is a first genotype 13,14 from the major contributor (blue). A second contributor (green) has a lesser amount of DNA, with an allele pair of 16,18. A third contributor (orange) has even less DNA, and a 17,20 allele pair. These are the genotypes that are actually present as DNA template in a polymerase chain reaction (PCR) tube. Genotypes are deposited in biological evidence, and form the basis of understanding genetic identity.

Data

A crime laboratory processes an STR sample to form an electropherogram (EPG) data signal stored in an electronic computer file (Figure 3). In this D18S51 locus EPG, the x-axis shows the size of the DNA fragments, corresponding to STR allele lengths. The EPG y-axis is measured in relative fluorescence units (RFU). The data RFU peak heights reasonably reflect the DNA quantities left by the three different contributors.

Indicated in blue, green, and orange (circles) are data peaks that arise from adding together the genotype allele amounts, plus some random variation.

In the gang case, the police recovered guns, hats, phones, clothing, and swabs of safes and other surfaces from five different crime scenes (Table 1). The county crime laboratory processed the DNA evidence. They sent the EPG data (as .fsa files) from twelve items to Cybergenetics for TrueAllele® Casework computer interpretation. Cybergenetics also received ten reference items taken from individuals – five victims who were present at these crime scenes, as well as five suspects who were possible gang members.

Consider the DNA interpretation task. There are 12 low-level DNA mixture evidence items, each containing three or four contributors. There are also 10 different references. So 120 (12 times 10) match comparisons must be made. For this series of gang crimes, the lab just sent all their STR data directly to Cybergenetics for computer interpretation.

Human review

Human review of complex data often yields no match results. One reason is the use of thresholds during interpretation (Figure 4). A person draws a threshold (red line). Above this line, data peaks are considered to have equal heights and status. Below threshold, data peaks become invisible to human analysis, treated as if they weren't there at all. As a result, individuals whose genotypes produced evidence peaks under threshold would not be matched. For example, a 16,18 genotype contributor (green arrows) would be incorrectly excluded.

However, computers can dream of electric peaks. They can think about a problem for days, statistically exploring many alternative solutions.

Computer interpretation

DNA interpretation begins with the data. The D18S51 locus EPG shows six allele peaks (Figure 3). The data is a constant in the analysis that does not change (4). A probabilistic genotyping computer does not apply thresholds. Instead, it uses all the quantitative data present in the evidence.

In order to explain the observed data, a statistical computer explores virtually every combination of allele pairs as genotype values for the different contributors. TrueAllele proposes different DNA quantities, mixture weights, PCR stutter, relative amplification, and degraded DNA, while also determining the uncertainty of each variable, including peak height uncertainty (2).

Figure 5 shows a computer-generated peak pattern that hypothesizes a major amount of the first genotype 13,14 (blue), a lesser amount of a second contributor with genotype 16,18 (green), and even less of a third contributor having allele pair 17,20

(orange). Comparing this hypothesized genotype pattern (colored rectangles) with the peak data (gray signal), we (and the computer) see that this genotype hypothesis explains the data very well. Therefore, this pattern explanation and its underlying genotype values have relatively high probability.

The computer also tries out hypotheses where the proposed alleles do not coincide with the data peaks. The computer constantly shuffles around hypothesized allele pairs. For example, it may propose a genotype of 10,11 for the major contributor, which does not cover the allele peaks at 13 and 14. This 10,11 genotype hypothesis does not explain the data well, and therefore has essentially zero probability.

The computer continues its search for a hundred thousand cycles, exploring hundreds of variables within each cycle, and trying out many different peak patterns. The genotype is the one variable we really care about for human identification. Genotypes that better explain the data receive higher probability.

Probabilistic genotypes

Genotypes are computed for each contributor at every locus. For the D18S51 locus, we show a listing of allele pairs for the second contributor (Table 2). TrueAllele can draw pictures of genotype probability distributions, but here we just use a table. Associated with each allele pair is a probability. For example, the probability of a 16,18 for this contributor is 65%.

All DNA mixture methods employ genotype probabilities, even the simple combined probability of inclusion (CPI) (5). However, such allele-based methods spread equal likelihood over many (often infeasible) allele pairs. This artificial probability diffusion reduces DNA match information.

TrueAllele examines STR data very thoroughly, so that statistical sampling can accurately place more probability on the more likely allele pairs. Greater probability is concentrated on those genotypes whose patterns better explain the data (Table 2). Probability describes the extent of our belief in alternative explanations, associating numbers with them that add up to 100% (6).

Match information

The likelihood ratio (LR) is a powerful formulation of the DNA match statistic that works well with genotype probabilities. The LR was used in ABO blood group paternity in the first half of the last century (7). The logarithm of the likelihood ratio (which is the exponent of the statistic, or the number of zeroes after the one) is a standard measure of information in many fields called the "weight of evidence" (8). In forensic science, the LR provides a match statistic that describes DNA identification information (9).

Cybergenetics received the lab's EPG files for the DNA evidence items. We processed these low-level mixtures in a TrueAllele multiprocessor supercomputer to

infer evidence genotypes objectively, without reference to any known genotypes. Afterwards, we received the reference genotypes for comparison. We then compared evidence genotypes with the references in the TrueAllele VUler™ Match environment, and calculated DNA match statistics.

Results

DNA mapping

We show a grid of the LR logarithms (Table 3). TrueAllele made 60 comparisons between 12 evidence items and 5 suspects, identifying 7 matches. In the first row, the food mart handgun matches the fifth suspect with a match score in the tens of thousands. There are 4 zeros in ten thousand, so a number "4" (the logarithm) appears in the top right cell.

For the food mart hat (second row), the computer was able to separate the mixture into two individuals. The match LR to the first suspect is in the thousands, or a 3 (a one followed by three zeros). The match to the fifth suspect is in the tens of thousands, or a 4 (a one followed by four zeros).

We can represent the match table visually as a county map that compares scene evidence to gang suspect (Figure 6). There were five suspects. The gang leader S1 (S1, red) is tied to a hat mixture that was left at a food mart in the west. The second suspect (S2, green) had his DNA found on the counter at a jewelry store. Suspect 5 (S5, purple) was involved in two different crime scenes, both east and west. His role in these crimes was more hands on, as seen from the touch DNA he left on the items at these places. This picture, and superimposed graph, maps out DNA matches at the crime scenes.

Reporting

After interpreting DNA evidence, match results are often presented in court. TrueAllele reporting scientists write a report, bring it to court, and testify about their findings. A mixture interpretation computer can simplify the reporting and presentation of complex DNA evidence (10).

Many forensic practitioners find likelihood ratios difficult to explain (11). However, the LR can be presented in ways that are easily understood (12). A plain language statement is "a match between the evidence and the suspect is a million times more probable than coincidence." All of the words in this LR sentence make sense to nontechnical people, including judges, juries, prosecutors, and defense attorneys. There is no math jargon or conditional probability (transposed or otherwise) to get in the way of clear communication.

Reliability

TrueAllele is a reliable method, based on the many validation studies that have been done. Two peer-reviewed papers have been published (2, 13), and another one is in press (14). Additional studies have been submitted for publication. We summarize results from a study that examined 41 cases containing 81 mixture items, and produced 87 genotype matches.

Sensitivity

We examined the sensitivity of TrueAllele Casework, studying how well the system detects individuals who deposit their DNA at crime scenes. We categorized the 81 DNA items into three groups, based on mixture complexity. The corresponding three histograms show the items arrayed by DNA identification information (Figure 7).

The *simple* mixture items (blue) were primarily two person mixtures, with some having a known reference sample (e.g., victim). The *intermediate* items (green) had two and three person mixtures, with fewer known references. The *complex* items (orange) were more challenging for people to interpret, comprising two and three person mixtures, many with low amounts of DNA.

The histograms describe computer-inferred DNA match information along a log(LR) x-axis. The computer infers informative genotypes (not alleles) to yield informative DNA match statistics. The mean of the simple items (blue) distribution was about 10^{15} , or a quadrillion. The intermediate item (green) histogram shifted to the left, but retained an average match statistic of around 10^{12} , or a trillion. More complex items (orange) shifted their distribution further to the left for an average LR of 10^{10} , or ten billion.

Specificity

We examined the specificity of the TrueAllele Casework system. In addition to identifying the *right* people (sensitivity), we do not want to misidentify the *wrong* people (specificity).

We tested specificity by comparing the inferred genotypes from each case against all the references in the other 40 cases. We found that the expected mismatches accurately produced negative match information. This is seen as histogram bars in the negative region of the log(LR) x-axis (Figure 8). For example, a "-9" means that the LR is one over a billion. That is, a coincidence is far more likely than a specific match.

Likelihood ratios can quantify the extent of *exclusion*, as well as of inclusion. The simple matches (blue) gave LR numbers averaging 10^{-23} . As the mixture items became more complex, their histograms (green, orange) shifted to the right, but TrueAllele still

produced negative log(LR) values. That is, the computer correctly determined when people were not present in the mixture evidence.

Reproducibility

We also established that the TrueAllele method is reproducible. Inferring genotypes from mixture data is a hard statistical problem involving many hundreds of dimensions, so the computer solution is obtained by statistical sampling (15). Reproducibility was assessed as the variation in match information between independent computer solutions on the same mixture data (16).

Ranked by decreasing LR (from left to right) are 87 genotype matches from 81 mixture items (Figure 9). The log(LR) y-axis quantifies the match information. The median LR value across the cases is around 10^{15} . Duplicate computer results (blue, green) are shown for each match. Moving to the extreme right of the graph, match statistics drop below a million and there is more LR variation. Quantifying LR variation over all the replicate computer runs shows a within-group standard deviation of less than one log unit.

Human comparison

The computer's 87 descending match results are again shown, now as a blue background (Figure 10). We compare human review with computer interpretation on the same mixture data (17).

Human review produced four random match probability (RMP) values (gray). RMP focuses on the major contributor to obtain high match scores. There were eight reported combined likelihood ratio (CLR) values (green), where a known reference helped preserve match information. The twelve CPI values (orange) clustered around an average match statistic of a million.

Human review reported on 25 of the 87 matches that the computer found. Hence 70% of the evidence matches were not assigned a match statistic. The bar heights show that when a person did report a CLR or CPI match statistic, their weight of evidence was far less than the computer's LR. These results demonstrate that computers can often preserve more information, make better use of the same data, and produce match statistics when people do not.

Computers in the Courtroom

TrueAllele DNA match reports have been submitted in over a hundred criminal cases. Scientific experts have given TrueAllele testimony in state, federal, military, and foreign courts. The criminal offenses include armed robbery, child abduction or molestation, murder, rape, terrorism, and weapon possession (18).

TrueAllele is often used in cases where there is challenging DNA evidence, and a match statistic is needed, but the lab's report is insufficient (low score or "inconclusive"). The computer can be extremely helpful when a more thorough investigation of the data is needed to infer genotypes and determine a match statistic.

Our work on computerized STR interpretation technology began twenty years ago. My talk at the 2003 Promega ISHI conference in Arizona included a live TrueAllele demonstration on a Macintosh PowerBook laptop computer, solving a DNA mixture problem in under a minute (19). The system has become more powerful over the past decade by expanding the math and testing hundreds of thousands of samples.

The current system is extremely robust, extensively validated, and can account for hundreds of STR variables. A TrueAllele computer now spends much of its time working out the certainty of its results, determining the statistical confidence in its answers. The method accounts for evidence uncertainty by diffusing genotype probability, which appropriately lowers the DNA match statistic.

A TrueAllele precedent was established in *Commonwealth v. Foley*, where sophisticated computing was first used to solve a DNA mixture in a criminal trial. Blairsville dentist Dr. John Yelenic was brutally slashed to death by State Trooper Kevin Foley, the boyfriend of Dr. Yelenic's estranged wife Michelle (20). The key physical evidence was a 7% mixture under the victim's fingernails, from which TrueAllele produced a DNA match statistic of 189 billion to Trooper Foley.

The complete lifecycle of the landmark Foley case has been described, from the crime through the Pennsylvania Superior Court appellate ruling (21). I have given several continuing legal education (CLE) courses on this case. The published Foley precedent has made TrueAllele usage more routine in Pennsylvania.

Investigative Database

Older DNA index systems provided by government are based on allele lists. A crime lab may upload just 10% to 20% of their mixtures (as allele lists) to such index systems. Only the simplest mixtures are uploaded, because of false positive hits that plague the DNA index system association mechanism (22). The majority of informative mixture evidence is never used for DNA investigation.

To make better use of this evidence, Cybergentics provides a TrueAllele genotype database (22). Matching probabilistic genotypes gives essentially complete separation between true and false hits (Figure 11). TrueAllele match *sensitivity* finds the correct criminals on the database. TrueAllele match *specificity* finds the one person (or no one, if they are not there) instead of returning many spurious hits.

Every inferred genotype can be uploaded to the TrueAllele genotype database. The TrueAllele matcher calculates LR match information between all the evidence items

and references. A genotype database hit implies a real DNA match. When no hit is found, there is no statistical support for a match.

Probabilistic genotype databases are far more informative than the older allele list DNA index systems. Their greater accuracy eliminates much of the need for expanding to larger and more expensive STR marker panels. Genotype databases are better suited for identifying criminals, solving cold cases, and exonerating the innocent.

Conclusion

What is the future of DNA evidence?

In 2010, the national Scientific Working Group on DNA Analysis Methods (SWGDM) released guidelines for mixture interpretation that revised much of crime lab DNA practice (1). It was a wake-up call that complex DNA mixtures had to be interpreted in a way that could account for data uncertainty. These new guidelines de-emphasized older, less reliable, human methods of reviewing mixtures, inviting more reliable computer interpretation. Paragraph 3.2.2 acknowledged the role of probabilistic genotyping methods, and the role of computers in solving mixture problems.

The international forensic DNA community concurs with this assessment (23). The December 2012 *Forensic Science International: Genetics* journal is a focus issue on interpreting complex and low template DNA samples. The editors write "The statistical evaluation of the weight of the evidence is probably the most important single advance in the history of forensic science, and undoubtedly the most significant contribution of forensic genetics to forensic science as a whole" (24). The issue includes articles and recommendations about probabilistic genotyping (25).

We are now in the DNA computer age. Cybergenetics TrueAllele provides forensic science with an objective, reliable, truth-seeking tool that solves the DNA mixture problem. The system resolves five and six contributor mixtures (and, of course, simpler ones having fewer contributors). The computer handles low copy and degraded DNA. Its DNA match statistics are accurate and reproducible, and computer processing helps automate the data review process.

Some crime labs have installed their own in-house TrueAllele Casework system. Others (e.g., police, prosecutors, laboratories and defense attorneys) send their STR data to Cybergenetics for out-sourced TrueAllele analysis. TrueAllele can help eliminate backlogs, reduce forensic costs, solve crimes, find criminals, convict them, free the innocent, and create a safer society. A TrueAllele parallel processing supercomputer works 24/7, mapping DNA crime scenes as it dreams of electric peaks.

Resources

More information about probabilistic genotyping is at Cybergenetics website under "www.cybgen.com/information". The site provides courses, newsletters, and a newsroom that describes recent cases. There are downloadable conference presentations and publications, including book chapters, magazine articles, validation studies and other materials.

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Tables

Table 1. *Laboratory processing.* There were 12 evidence items at 5 crime scenes. Of the 10 reference items, 5 were victims and 5 were suspects.

12 evidence items		10 reference items	
Scene 1	<ul style="list-style-type: none">• gun• hat	5 victims	
Scene 2	<ul style="list-style-type: none">• safe• phone	<ul style="list-style-type: none">• V1• V2• V3	
Scene 3	<ul style="list-style-type: none">• counter• safe	<ul style="list-style-type: none">• V4• V5	
Scene 4	<ul style="list-style-type: none">• keys• tape	5 suspects	
Scene 5	<ul style="list-style-type: none">• hat 1• hat 2• overalls• shirt	<ul style="list-style-type: none">• S1• S2• S3• S4• S5	

Table 2. Probabilistic genotype. A probability distribution over allele pairs is shown for the inferred genotype of the second (minor) contributor at the D18S51 locus. The probabilities are listed in descending order, given at a 99% level.

Allele pair	Probability
16, 18	65%
14, 18	12%
13, 18	10%
18, 20	8%
17, 18	4%

Table 3. TrueAllele match statistics. DNA match statistics are shown as log(LR) values in a grid comparing 12 evidence items (rows) and 5 suspect references (columns). (The 5 victim references are not shown.) Seven matches were reported. There was no statistical support for a match in the other 53 comparisons (blank entries).

log(LR)	Suspect 1	Suspect 2	Suspect 3	Suspect 4	Suspect 5
1. Gun					4
1. Hat	3				4
2. Safe					
2. Phone					
3. Counter		6			
3. Safe					
4. Keys					
4. Tape					
5. Hat 1					6
5. Hat 2					
5. Overalls					11
5. Shirt					3

Figures

Figure 1. *Evidence from multiple scenes.* Five crime scenes are overlain on a county map. The gang's attacks proceeded from west to east, chronologically shown by color (black, purple, blue, and green). The items of evidence found at each scene are listed.

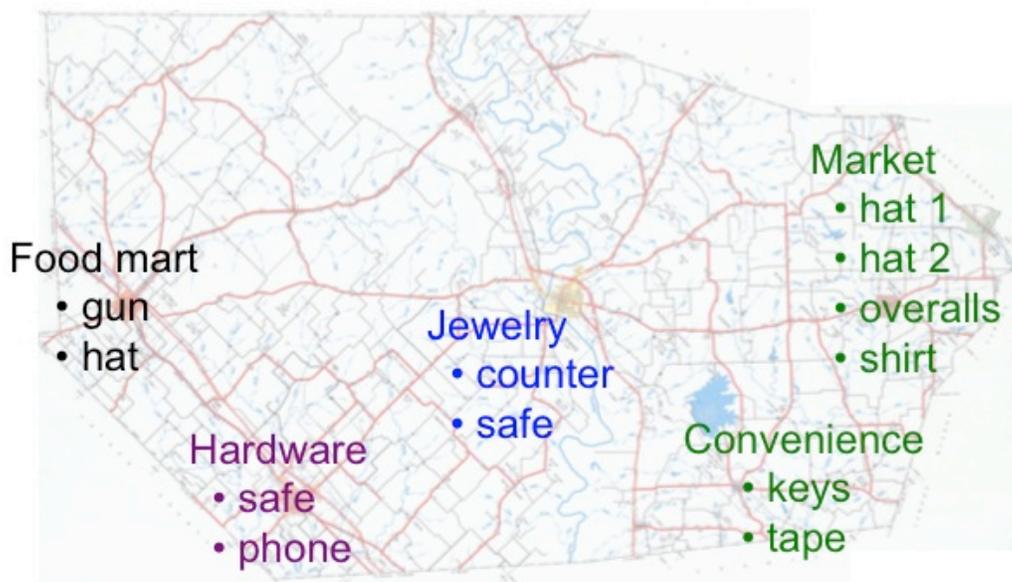


Figure 2. DNA evidence genotypes. The data schematic shows DNA amount (y-axis) versus allele size (x-axis) at an STR locus. Each contributor is shown in a different color (blue, green, orange). The allele pair of each contributor genotype is indicated by size location (x-axis), while the relative genotype amount is indicated by bar height (y-axis).

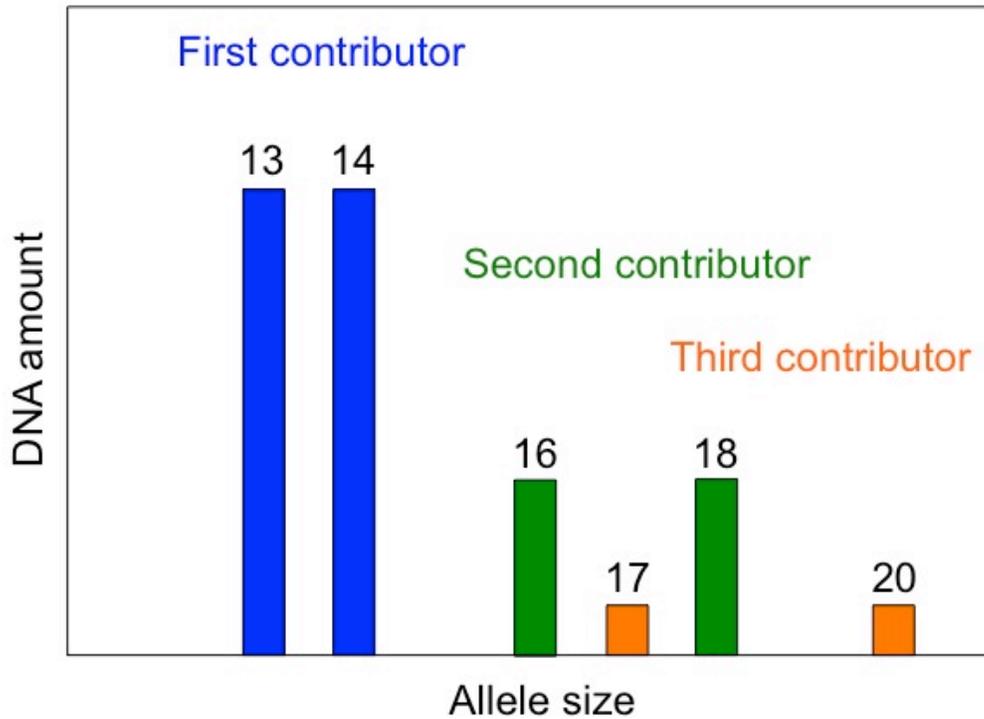


Figure 3. Develop STR data. The electrophoretogram (EPG) data trace for this three contributor mixture shows one data peak for each allele. The x-axis gives allele size (in base pairs), while the y-axis measures peak heights on a relative fluorescence unit (RFU) scale. The allele pair for each genotype (blue, green, orange) has peaks with comparable heights. Each contributor corresponds to a genotype shown in Figure 2.

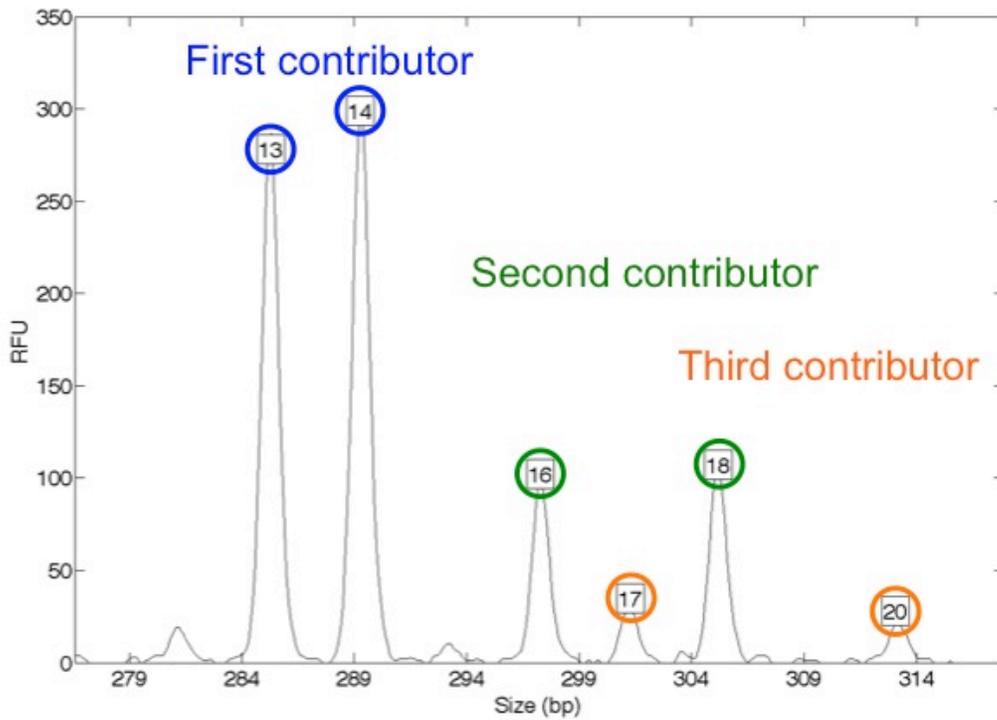


Figure 4. Human review gives no results. A threshold (red line) of height 150 RFU is applied to the quantitative STR locus data of Figure 3. This threshold reduces the 13,14 genotype peak heights to equal all-or-none events (blue bars). The threshold also eliminates from DNA data the two minor contributors having genotypes 16,18 (green arrows) and 17,20.

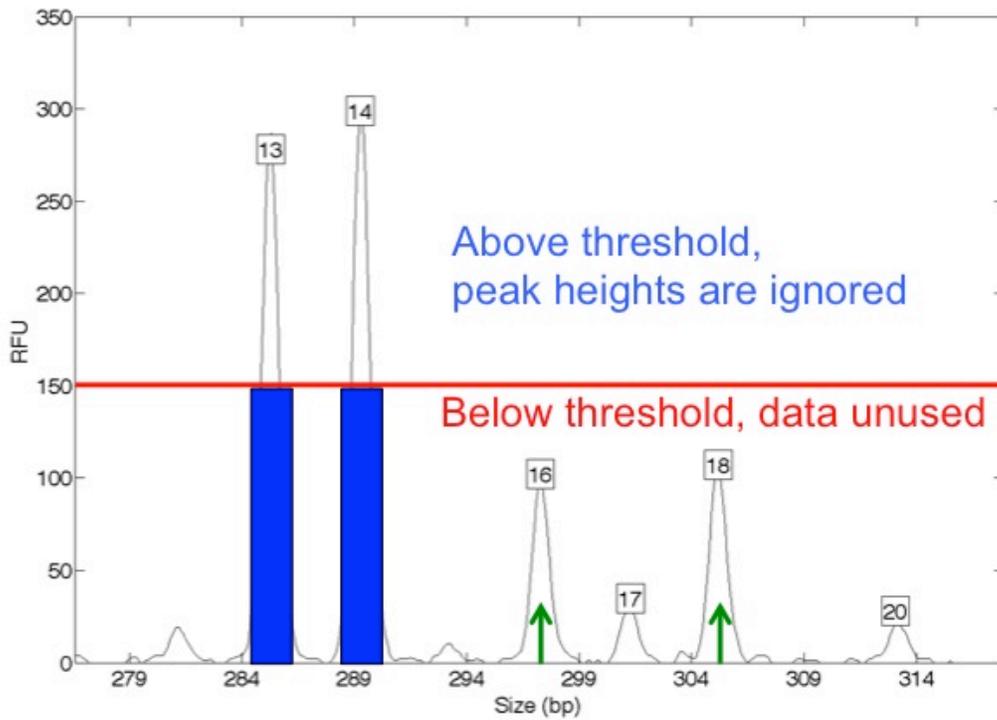


Figure 5. *Computers dream of electric peaks.* Quantitative computer modeling can explain the observed data. Better explanations of the data confer higher probabilities to their underlying genotypes. In the explanation shown, an allele pair is represented by two colored rectangles (blue, green, orange). The placement and heights of the allele pairs explain the observed data peaks well, and so these genotypes have a high probability.

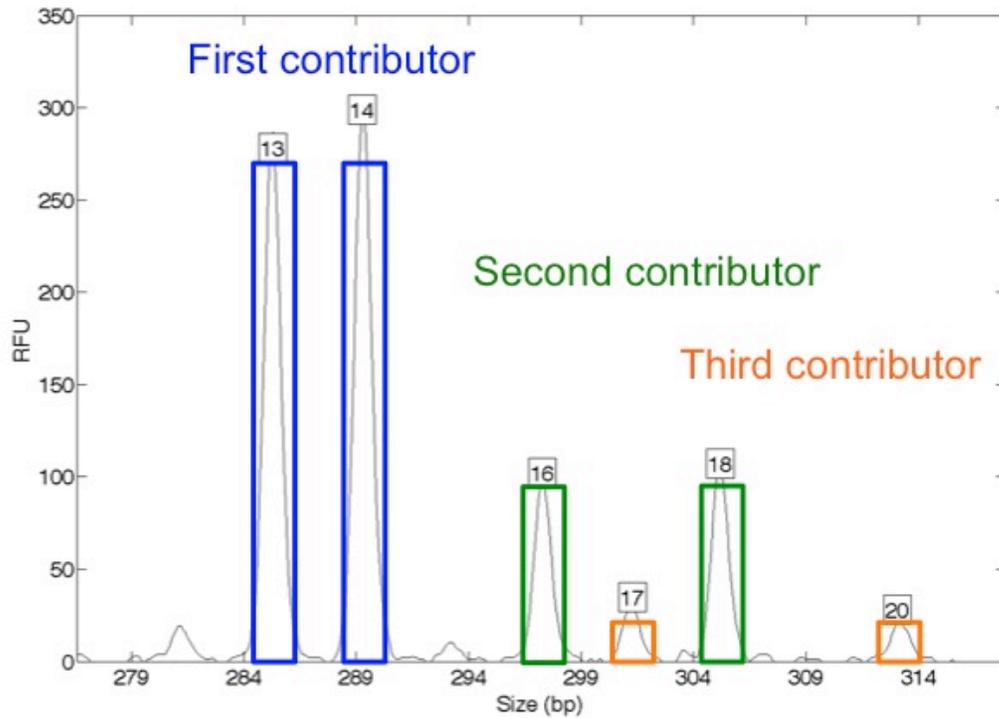


Figure 6. DNA mapping the crime scene. TrueAllele DNA matching associates suspects with evidence items found at crime scenes. The first suspect (S1, red) matches a DNA mixture from a hat found at the food mart. The fifth suspect (S5, purple) also matches the hat mixture, which places both suspects at this scene at the same time. He also matches the food mart gun, and three clothing items left at the market. The second suspect (S2, green) matches the jewelry store counter.

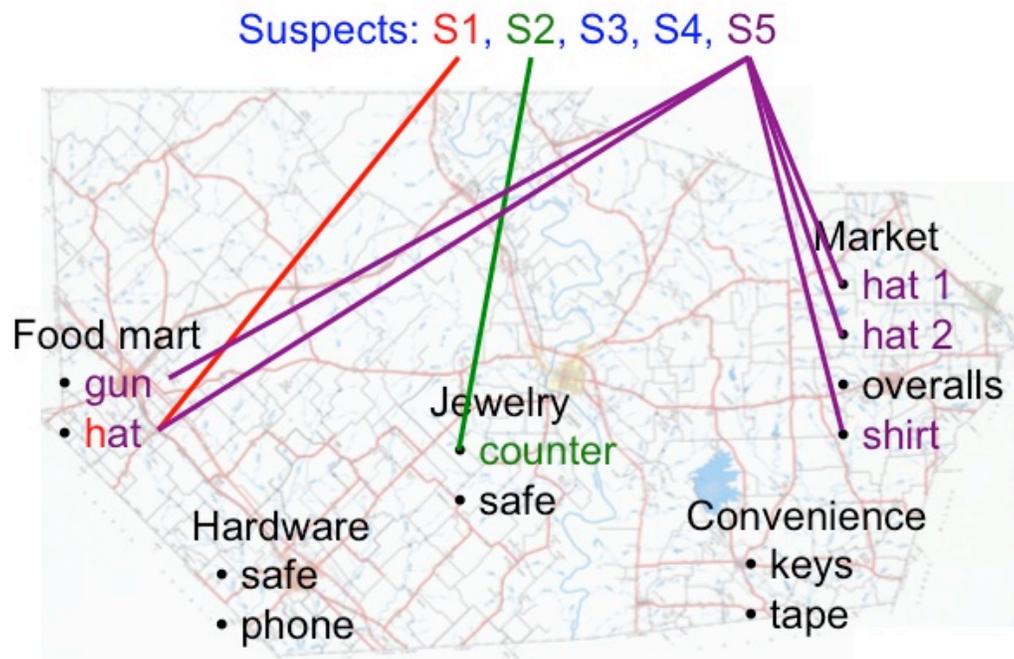


Figure 7. *TrueAllele sensitivity.* The distribution of genotype match information (as inferred by TrueAllele) is shown as a log(LR) histogram of counts for each complexity category, with theta at 1%. The simple items (blue) are distributed more to the right than the intermediate items (green). The leftmost distribution is for the complex items (orange), which tend to be less informative.

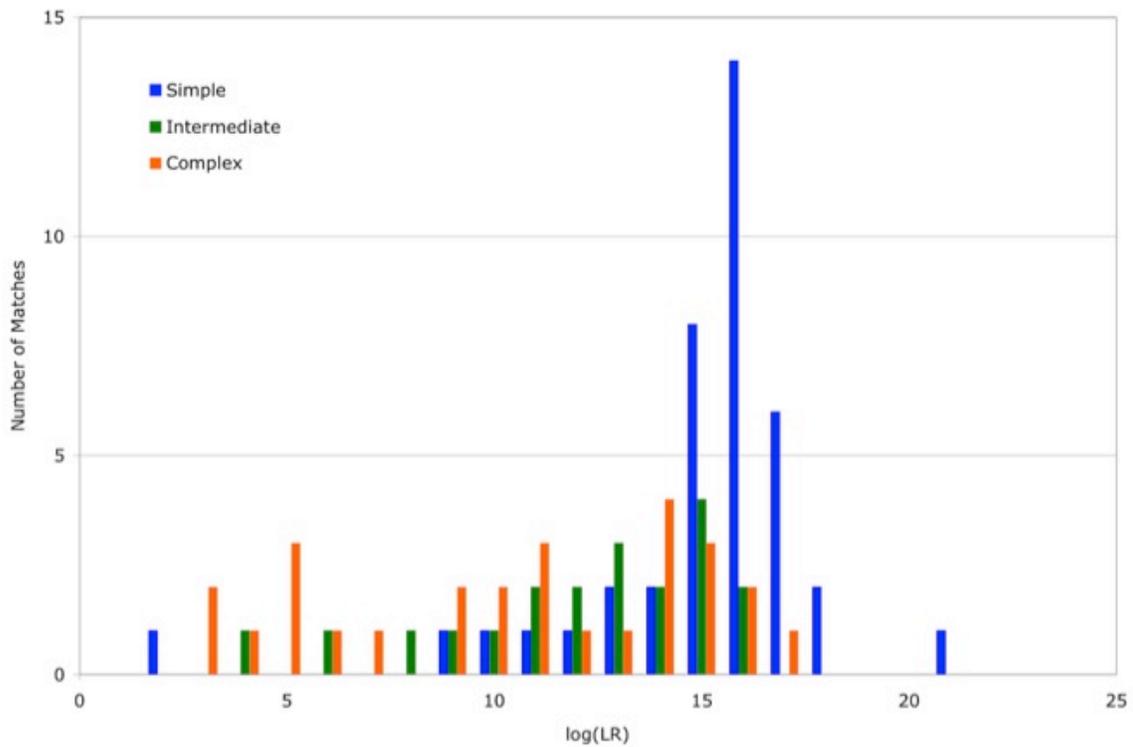


Figure 8. *TrueAllele specificity.* The distribution of genotype mismatch comparison information (as inferred by TrueAllele) is shown as a log(LR) histogram of counts for each complexity category, with theta at 1%. The simple items are most specific (blue), distributed more to the left than the intermediate items (green). The rightmost distribution is for the complex items (orange), which are the least specific.

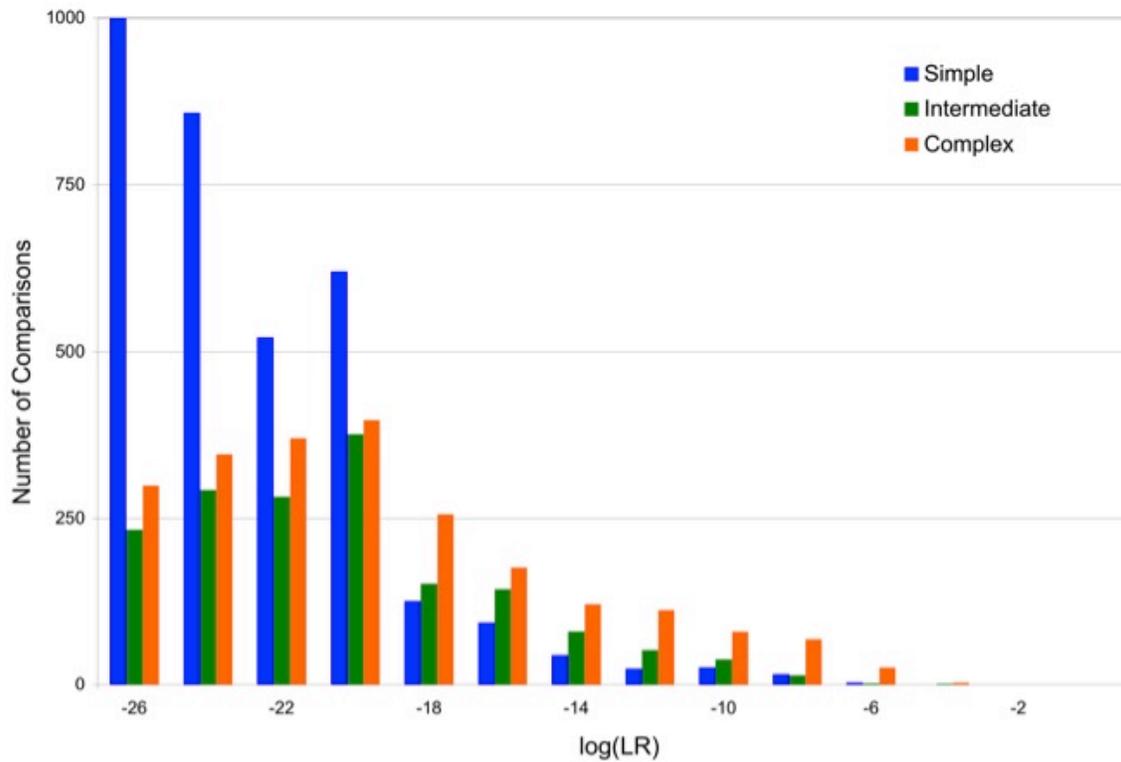


Figure 9. *TrueAllele reproducibility.* The inferred genotypes are sorted by descending match information, with theta at 1%. For each matched genotype, the first (blue bar) and second (green bar) independent TrueAllele computer runs' match statistics are shown on a log(LR) scale. The information difference between the two genotype replicates is shown (red line).

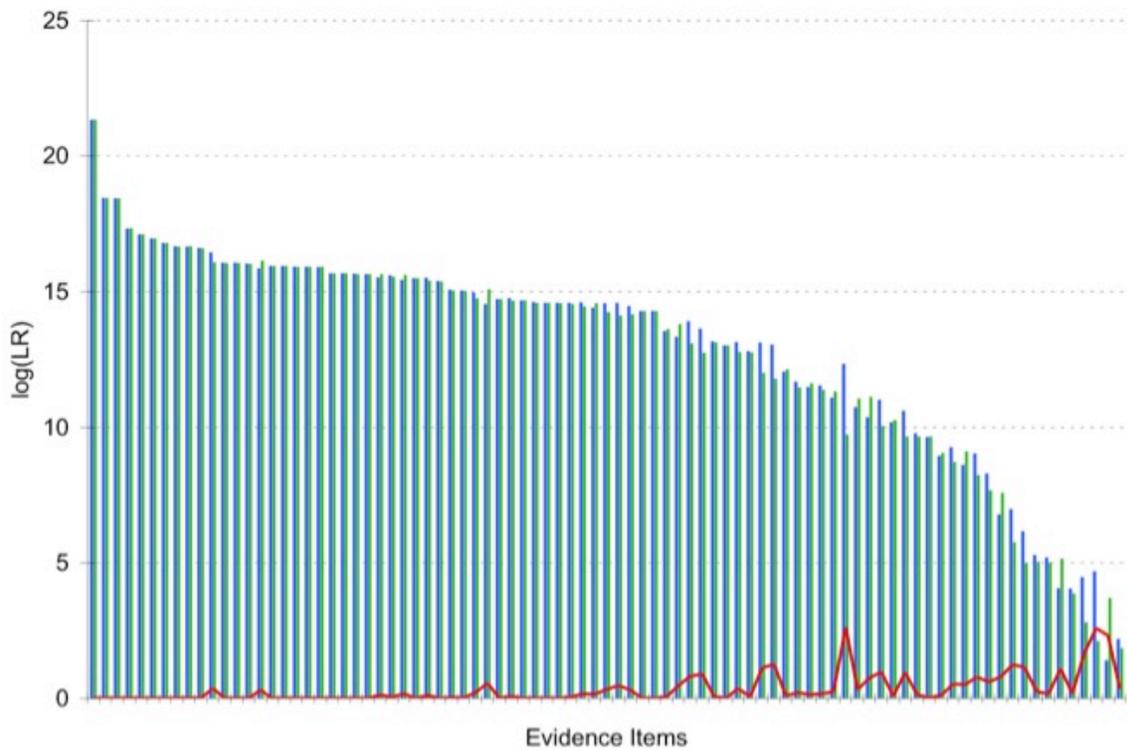


Figure 10. Human comparison. The blue background shows the computer-inferred match information for each genotype, as in Figure 9. The foreground bars show logarithms of human match statistics obtained on the same DNA mixture genotypes. The human review methods used were CMP (gray), CLR (green) and CPI (orange). The TrueAllele LR_s were calculated with theta at 0% for CLR and CPI, and at theta = 3% for CMP, to correspond to the reported laboratory results.

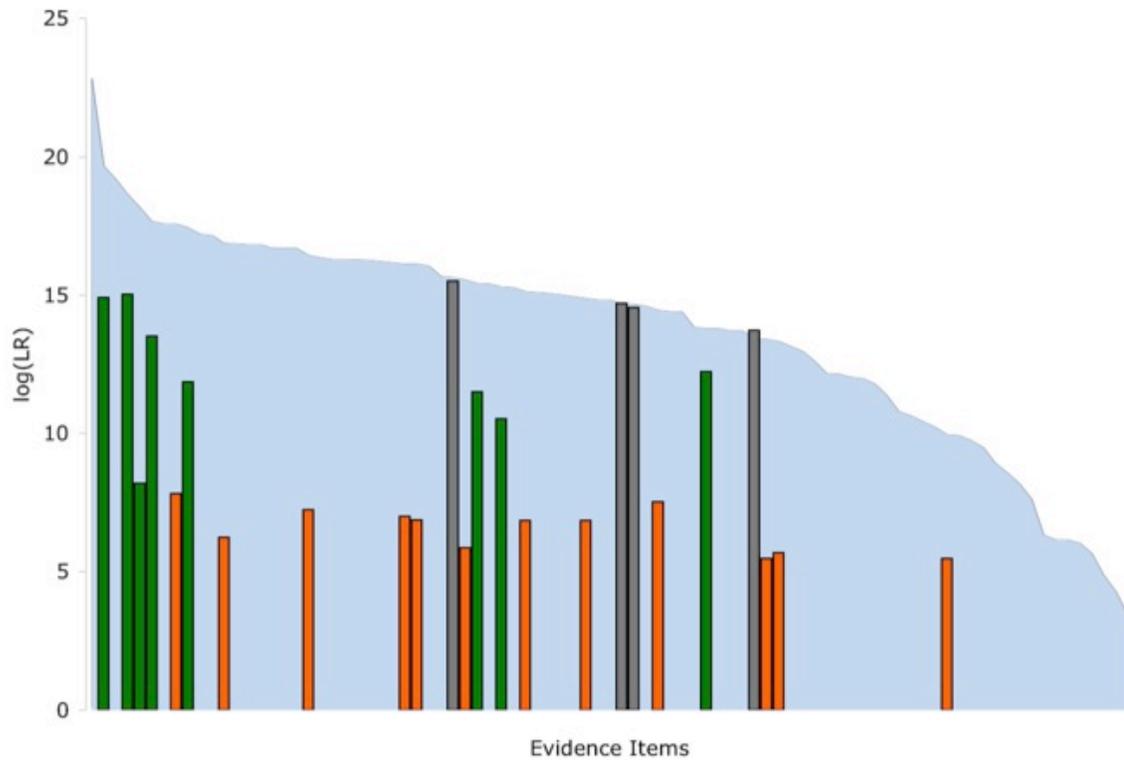


Figure 11. Genotype information database. Histograms show the relative frequency (y-axis) of match information for 40 two person mixtures on a log(LR) scale (x-axis). TrueAllele inferred the genotypes, and matched them using its genotype database. (a) *Sensitivity* (blue, right). The computer-inferred genotypes were compared with known reference genotypes. All 40 genotype matches had $LR > 1$, with average information (inclusion) around 18, or quintillion. (b) *Specificity* (red, left). The computer-inferred genotypes were compared with 1,000 randomly generated genotypes. The average negative information (exclusion) was around -24, or a septillionth. The false positive rate was under 0.01%. The TrueAllele genotype database clearly separates real DNA matches (blue) from spurious ones (red).

