Getting more from less: low-level DNA mixtures on cartridges

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Abstract

Cartridge casings are the empty shells left behind after a gun was fired and they're typically made of different metals and have different calibers. Nearly 200,000 cartridge cases are recovered annually at United States crime scenes.[1] The crime scenes where cartridges can be collected can range from homicides, aggravated assaults, robberies, and gang-related crimes. It has been shown that cartridges that were fired degrade any DNA that was left and have significantly less DNA[2]. Items with less DNA make it harder for crime labs to interpret the data and for crime scene investigators to collect DNA.

The cartridge study was designed to determine how much single source identification information can be recovered from firearm cartridges, as well as the best collection method and most informative cartridge type. Our study examined DNA data from seven different casing materials for a total of 910 cartridge samples. The cartridge samples were then collected using five different DNA collection methods. Once the DNA was collected, both manual interpretation and a probabilistic genotyping software, TrueAllele® Casework, were used to analyze the cartridge data.

Once the single source cartridge samples were created, George Washington University lab manually interpreted the DNA data. The lab used a threshold and an allele counting method. The laboratory counted how many alleles matched the reference that was expected to be present in the sample. The laboratory found the reference sample was present in 205 of the cartridge samples.

A TrueAllele trained analyst set up single source requests for the program to solve. Upon review of the data, it was determined that the cartridge data was low level mixtures. There was a total of 202 samples that were low level. There was a total of 431 mixtures present, which made up 47% of the total samples.

The presence of additional contributors was an unexpected result, so a TrueAllele analyst did further processing considering multiple contributors, with the number of contributors that was observed in the data. The total number of contributors for these mixtures ranged from 2-5.

TrueAllele measured more information by looking at the lower-level data and mixtures and found a previously unidentified contributor. The unknown profile was informative with an expected genotype statistic of 30.36 ban. The unknown contributor was also found in 138 of the samples, ranging across the seven different cartridge types. Manual interpretation did not consider this unknown person as the method focused on the reference's allele pair only, limiting their interpretation of the data.

Based on the results of the study, the known reference was found in 351 cartridge samples using TrueAllele, compared to 251 samples using manual interpretation. The unknown person was found in 138 samples using TrueAllele where manual interpretation ignored the unknown person.

The most informative collection methods based on high genotype statistics were wet:wet or wet:dry. The least informative collection methods were scraping and soak and sonicate. The most informative cartridge types based on high genotype and inclusionary statistics were aluminum and steel. The least informative cartridge type was 45 fired.

Overall, TrueAllele was able to use more DNA information than manual interpretation from cartridge casings, especially low-level and mixture data.

Methods & Materials

An individual touched various cartridge types to create single source evidence samples. The study included 910 total cartridge samples across 7 different cartridge types.

Table 1 below shows the cartridge type and number of each cartridge case.

Material	Total
45 Fired	90
45 Unfired	90
Aluminum Unfired	150
Brass Fired	130
Brass Unfired	150
Nickel Unfired	150
Steel Unfired	150

Collection Method

Five different collection methods were utilized to collect. The five methods with a description of each method can be seen below in Table 2.

Collection	Description
Wet:Wet	Wet cotton swab followed by another wet cotton swab
Wet:Dry	Wet cotton swab followed by a dry cotton swab
Soak and Sonicate	Cartridge is placed into a solution and then placed in a sonicator
Tape Lift	Tape dot stuck to microscope slide and cartridge is rotated over the tape dot
Scraping	Scrape material off of the cartridge with a sterile razor bale

Each collection method was used on each of the cartridge types, with the exception of 45 Fired and Unfired. The 45 caliber cartridges did not undergo tape lift or scraping collection methods. Table 3 below shows the number of each cartridge sample and the collection method.

	Collection				
Material	Wet:Wet	Wet:Dry	Soak and Sonicate	Tape Lift	Scraping
45 Fired	30	30	30	N/A	N/A
45 Unfired	30	30	30	N/A	N/A
Aluminum Unfired	30	30	30	30	30
Brass Fired	30	30	10	30	30
Brass Unfired	30	30	30	30	30
Nickel Unfired	30	30	30	30	30
Steel Unfired	30	30	30	30	30

Organic Extraction

The organic extraction method was used to remove the DNA from the cartridge casings. This method uses organic solvents to denature proteins. The denatured proteins are removed by a series of wash steps, and DNA remains.

The extracted DNA was amplified using the Applied Biosystems GlobalFiler™ PCR Amplification kit. The amplicons were separated using an Applied Biosystems® 3500 Genetic Analyzer.

Data Interpretation

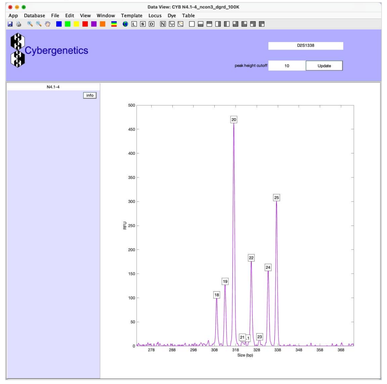
The George Washington University Laboratory developed the cartridge DNA data. The laboratory also manually interpreted the data.

After first applying a data threshold, the laboratory viewed the electropherograms (EPGs) to determine if the reference's DNA profile was present in the cartridge samples. Alleles that matched the reference's genotype were counted.

Manual interpretation found the expected reference in 205 cartridge samples.

Electropherogram (EPG) Data

Figure 1 below shows the EPG data for locus D2S1338 from nickel cartridge N4.1-4. The presence of additional peaks indicate that more than one person's DNA is present on the cartridge sample.



TrueAllele Casework

Cybergenetics' (Pittsburgh, PA) TrueAllele Casework (TA) probabilistic genotyping system uses Bayesian probability modeling and Markov chain Monte Carlo (MCMC) statistical sampling to interpret DNA data. The objective TrueAllele process uses all of the DNA data to separate out genetic types for each contributor in a DNA sample. The software learns from evidence data and does not need to be calibrated on previously developed laboratory data.

The TA technology calculates the Kullback-Leibler (KL) statistic and likelihood ratio (LR) match statistic. KL quantifies DNA data information as the expected log(LR) for the true (but unknown) contributor. log(LR) evaluates match information at a known reference. These values are expressed in logarithmic units (the number of zeros after the 1). For example, 6 log units represent a million.

Table 4 below shows the KL statistics for the single source processing of the cartridge data. The relatively high KL values indicate that the DNA data are informative.

	Collection				
Cartridge Type	Wet:Wet	Wet:Dry	Soak:Sonicate	Tape Lift	Scraping
45 Fired	16.60	13.89	14.34	N/A	N/A
45 Unfired	15.45	17.88	11.37	N/A	N/A
Aluminum Unfired	24.58	25.27	16.95	27.14	18.41
Brass Fired	24.33	20.43	10.29	18.96	13.79
Brass Unfired	19.10	18.74	4.88	25.54	16.17
Nickel Unfired	21.70	23.10	8.21	23.30	9.96
Steel Unfired	25.01	24.03	21.28	22.89	22.85

TrueAllele Casework

The EPGs showed that some samples appeared to be low-level mixtures. Manual interpretation found these low-level mixtures to be uninformative. Table 5 shows the percentage of low-level cartridge samples for wet:wet and wet:dry collection methods.

Percentage of Low-Level Samples		
	Collection	
Material	Wet:Wet	Wet:Dry
45 Fired	40.00	73.33
45 Unfired	40.00	36.67
Aluminum Unfired	0.00	16.67
Brass Fired	3.33	16.67
Brass Unfired	6.67	30.00
Nickel Unfired	6.67	13.33
Steel Unfired	30.00	10.00

Manual interpretation failed to consider low-level data, additional contributors, and peaks below the threshold. The method only focused on the reference's allele pairs.

In total, there were 431 cartridge samples, or 47% of the samples, that were mixtures. Allele counting would not have considered the other contributors in the mixtures. Table 6 below shows the total number of mixtures for each cartridge type.

	Collection				
Material	Wet:Wet	Wet:Dry	Soak and Sonicate	Tape Lift	Scraping
45 Fired	10	7	8	N/A	N/A
45 Unfired	13	15	9	N/A	N/A
Aluminum Unfired	24	17	9	27	11
Brass Fired	16	12	1	16	10
Brass Unfired	14	15	0	29	15
Nickel Unfired	16	20	1	26	6
Steel Unfired	19	21	9	22	13

TrueAllele processed these mixtures assuming 2 to 5 contributors (as indicated by the peak data). log(LR) match statistics were calculated for the known reference. TrueAllele statistically included the reference in 351 samples. Table 7 below shows the total inclusions for each method and cartridge type for the reference.

	Collection				
Material	Wet:Wet	Wet:Dry	Soak and Sonicate	Tape Lift	Scraping
45 Fired	1	0	9	N/A	N/A
45 Unfired	12	19	6	N/A	N/A
Aluminum Unfired	26	18	9	29	11
Brass Fired	5	13	1	3	3
Brass Unfired	8	7	1	22	0
Nickel Unfired	15	24	0	18	3
Steel Unfired	18	22	17	17	14

Additionally, TrueAllele found an unknown person. The 30 ban KL value indicates that the profile is informative. We used TrueAllele to compare this unknown profile with all the cartridge samples. Table 8 below shows the total number of inclusions of the unknown person for each cartridge type.

	Collection				
Material	Wet:Wet	Wet:Dry	Soak and Sonicate	Tape Lift	Scraping
45 Fired	1	2	2	N/A	N/A
45 Unfired	10	2	8	N/A	N/A
Aluminum Unfired	4	4	0	9	0
Brass Fired	14	3	1	5	3
Brass Unfired	9	1	0	10	1
Nickel Unfired	9	3	1	10	0
Steel Unfired	6	1	3	13	3

A Nickel Example

There are a total of 31 combinations of collection method and material types. The inclusionary TrueAllele LR values range from 10's of billions to 10's of quadrillions. Table 9 shows the statistics for Nickel Unfired Wet:Wet. The average KL and log(LR) for the reference and unknown person are shown. A blank entry indicates that there wasn't any data for that contributor assumption.

	ref inclusion		unknown inclusion	
# of contrib	KL	Log(LR)	KL	Log(LR)
1 contrib	26.86	15.08		
2 contrib	15.53	10.27	22.71	16.14
3 contrib	13.94	10.06	14.64	11.38
4 contrib				
5 contrib				

The log(LR) value of 10.06 ban from the table above equals an LR of 11.5 billion, a large inclusionary match statistic to the reference. 10.06 ban is the average log(LR) value across the 3-person mixture set.

Conclusions

The cartridge study was designed to examine single source samples. However, low-level mixture samples were later discovered. Therefore, the study was extended to examine these low-level mixtures, and to compare their genotypes with the reference and unexpected unknown profile. The unknown profile was informative for comparisons across all cartridge samples. The identity of the unknown person remains unknown.

Manual interpretation was limited to counting alleles, find the reference in 205 samples. On the same data, TrueAllele made more identifications, and could further consider other contributors to the mixtures. TrueAllele calculated LR match statistics for the reference and unknown contributor. Sample-to-sample comparisons developed the unknown profile from the cartridge casings. TrueAllele found the expected reference in 351 cartridge samples, more often than using manual review. The computer found the unknown person in 138 samples.

The most informative DNA collection methods for cartridges with organic extraction were wet:wet and wet:dry. The least informative method was scraping & soak and sonicate. The most informative cartridge type was aluminum or steel; the least informative was 45 Fired.

In summary, TrueAllele can develop DNA information from cartridge casings that older manual methods cannot. Moreover, TrueAllele can examine low-level data and identify minor contributors. This more powerful DNA interpretation capability is important, since cartridges are common evidence items at crime scenes.

References

[1] "Shelling out Evidence: NIST Ballistic Standard Helps Tie Guns to Criminals." *NIST*, 23 Jan. 2023, www.nist.gov/news-events/news/2012/08/shelling-out-evidence-nist-ballistic-standard-helps-tie-guns-criminals.

[2] Prasad, Elisha, et al. "Touch DNA recovery from unfired and fired cartridges: Comparison of swabbing, tape lifting and soaking." *Forensic Science International*, vol. 330, Jan. 2022, p. 111101, https://doi.org/10.1016/j.forsciint.2021.111101.

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