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

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Cybergenetics

Background

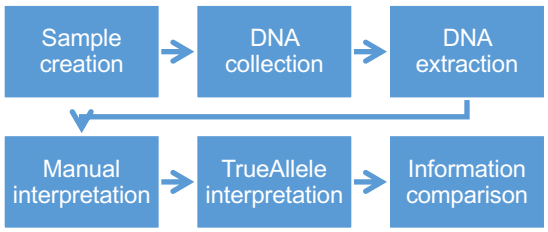
- Cartridge casings are the empty shells left behind after a gun was fired[1]
- Nearly 200,000 cartridge cases are recovered annually at U.S. crime scenes[1]
- Cartridges that were fired degrade any DNA that was left and have significantly less DNA[2]
- Caliber of the firearm did not have any impact on the amount of DNA recovered[2]

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.

Cartridge Study Main Questions

- Can manual interpretation obtain DNA information from cartridge data?
- Can TrueAllele® Casework interpretation obtain DNA information from cartridge data?
- Which collection method is the most informative for cartridge data?
- Which cartridge type produces the most DNA information?

Study Design



Sample Creation

- Single source data
- The reference individual touched various cartridge types
  - 910 total cartridge casing samples
  - Across 7 different cartridge types

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

DNA Collection

- DNA was collected using five collection types
  - Wet:wet
  - Wet:dry
  - Soak and sonicate
  - Tape lift
  - Scraping

	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

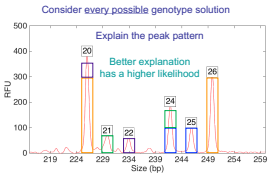
DNA Extraction

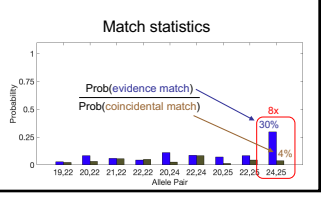
- Organic extraction
  - Organic solvents are used for denaturation
  - Denatured proteins are removed then washed
- DNA sequencer
  - Applied Biosystems® 3500 Genetic Analyzer
- STR kit
  - Applied Biosystems GlobalFiler™

Manual Interpretation

- George Washington University Laboratory manually interpreted the data
- A peak height threshold was applied to EPG data to form allele events
- *Allele counts*: how many EPG allele events match a reference

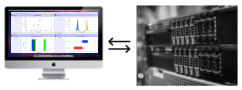
TrueAllele Casework Interpretation





TrueAllele Casework Interpretation – Part 1

- Cybergenetics generated TrueAllele requests assuming the samples were single source
- Completely objective and unbiased
  - TrueAllele processes DNA data without knowing a reference
- *Kullback-Leibler* (KL) genotype statistic
  - Quantifies the identification information in a genotype
  - The expected log(LR) to the true contributor
- *Likelihood Ratio* (LR) match statistic
  - Compares genotype to known reference



Information Comparison – Part 1

- Reviewed KL information from single source runs
- Most cartridges had a high KL: the DNA was 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

But …

Problem?

Mixtures and Low-Level Data – Oh no!

- Much of the data were mixtures
  - Locus EPGs with 3 or more peaks
- Low-level data, little DNA: uninformative manual interpretation
  - Percentage of low-level samples for each cartridge type (Table)
- Manual review couldn't handle more contributors and sub-threshold peaks

Percentage of Low-Level Samples		
	Collection	
Material	Wet:Wet	Wet:Dry
45 Fired	40	73
45 Unfired	40	36
Aluminum Unfired	0	16
Brass Fired	3	16
Brass Unfired	6	30
Nickel Unfired	6	13
Steel Unfired	30	10

Mixtures – TrueAllele to the rescue!

- 431 (of 910) samples were found to be mixtures
  - 47% of the samples were mixtures
  - Allele counting couldn't handle more than one contributor

	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 Interpretation – Round 2

- Cybergenetics created requests for the mixture data
- TrueAllele Casework processed the requests
  - Some items had multiple contributor assumptions
  - Samples contained 2 to 5 contributors
- TrueAllele found an unknown person in many of the cartridges
  - We compared the cartridge samples with the unknown profile

Information Comparison – Round 2

- All cartridge samples compared to reference
- LR match statistic calculated for each comparison

Known reference inclusionary counts

	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

Information Comparison – Round 2

- All cartridge samples were compared to the unknown profile

Unknown profile inclusionary counts

	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

Information Comparison – Round 2

- The unknown profile was in many samples
  - Found in **138 of the 910** cartridge samples
- The unknown profile was informative
  - Its KL was **30.36** ban

Example - Nickel Unfired Wet:Wet Collection

- 31 combinations of the collection and material type
  - Table: statistics for one combination (Unfired Nickel + Wet:Wet)
  - KL and log(LR) inclusionary averages for the reference and unknown person
  - Blank entry: no data available
- KL and log(LR)
  - The number of zeros after the 1 in the match statistic (ban)
  - The inclusionary LR values ranged from 10's of billions to 10's of quadrillions (really, really informative)

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

LR Example (Reference Inclusion – 3 Contributor)

- A log(LR) of 10.0 ban is **10,000,000,000**
  - Large inclusionary DNA match statistic
  - TrueAllele average from 3-person mixtures

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

But human review got no information at all!

Who is the unknown?

- Who's DNA is in the unknown profile?
  - We don't know
- Maybe it's someone who had handled the gun
  - Same person across multiple cartridge types
  - Not restricted to a specific cartridge type

Study Conclusions

- Manual interpretation used allele counting and thresholds
  - Could only find the known reference
- TrueAllele considered additional mixture contributors
  - The computer calculated match statistics for both the reference and unknown profile
  - The computer's developed unknown enabled comparison between the different cartridge mixtures
  - The known reference was found in 351 samples (205 manually)
  - The unknown person was found in 138 samples (0 manually)

Study Conclusions

- More informative **collection method**
  - Wet:Wet or Wet:Dry
  - Tape lift was close
- Least informative was Scraping / Soak and Sonicate

- Most informative **cartridge type** was Aluminum / Steel
- The least informative was 45 Fired

Conclusion

- TrueAllele can develop informative data from cartridges
  - All DNA data is used, none discarded
  - Handles low-level data and minor contributors
  - Cartridges are common crime scene evidence
  - TrueAllele motivates gathering cartridge evidence
- Methods that use less data are less informative
- TrueAllele has done almost a hundred cartridge cases, getting more DNA information from crime lab data

Future Directions

- Analyze **more cartridge data**
- Study **other extraction methods** (PrepFiler™ and QIAamp) on these cartridge types and collection methods
- Determine the **best collection method** for each extraction
- **Compare TrueAllele** Casework with other interpretation methods using KL and log(LR) information

Questions?

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Cybergenetics

www.cybgen.com