

The Science and Law of Automated DNA Evidence Interpretation

National Forensic Science University
Gandhinagar, Gujarat, India
February, 2023

Mark W Perlin, PhD, MD, PhD
Cybergenetics



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What is Truth?

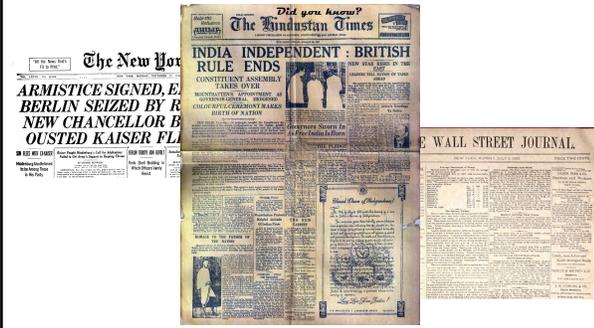
"Beauty is truth, truth beauty,—that is all
Ye know on earth, and all ye need to know." - Keats



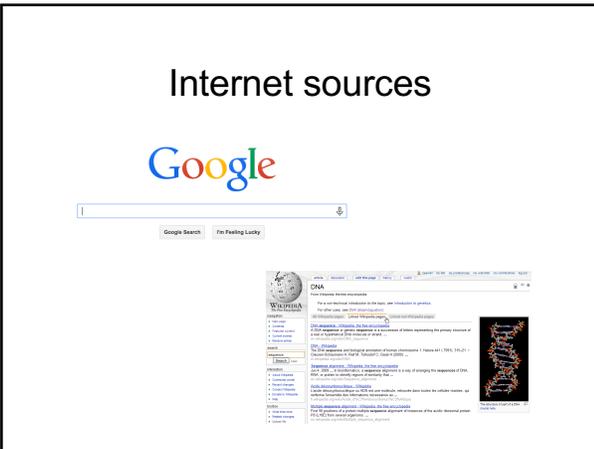
Religious texts



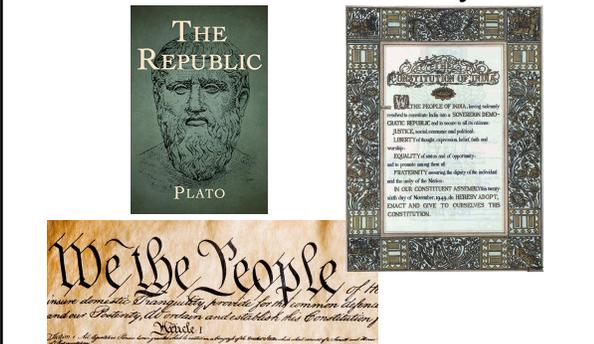
News reporting



Internet sources



Government authority



Judicial decisions



Philosophical reasoning

Truth is based on what you:

Think – Rene Descartes

See – David Hume

Know – Immanuel Kant

Do – Ludwig Wittgenstein

“To be is to do” – Socrates

“To do is to be” – Sartre

“Do Be Do Be Do” – Sinatra

What is Science?

The search for truth, based on empirical testing.

Scientific method

Propose explanatory hypothesis

Collect experimental data

Test hypothesis on the data

Natural selection

Virus mutates

Vaccines adapt

Immunity learns

How to represent Uncertainty?

Probability
 $\Pr\{\text{hypothesis}\}$

Conditional probability
 $\Pr\{\text{hypothesis} \mid \text{data}\}$

Likelihood – explain the data
 $\Pr\{\text{data} \mid \text{hypothesis}\}$

How to quantify Information?

Karl Popper – falsify theory
 $\Pr\{\text{data} \mid \text{hypothesis}\} = 0$

Thomas Kuhn – confirm paradigm
 $\Pr\{\text{data} \mid \text{hypothesis}\} = 1$

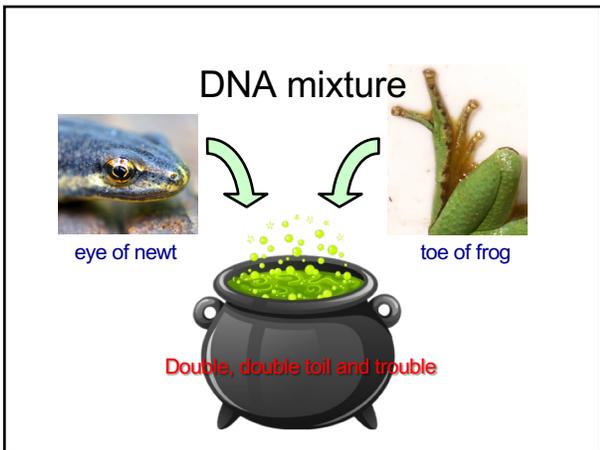
Thomas Bayes – posterior probability
 $\Pr\{\text{hypothesis} \mid \text{data}\}$

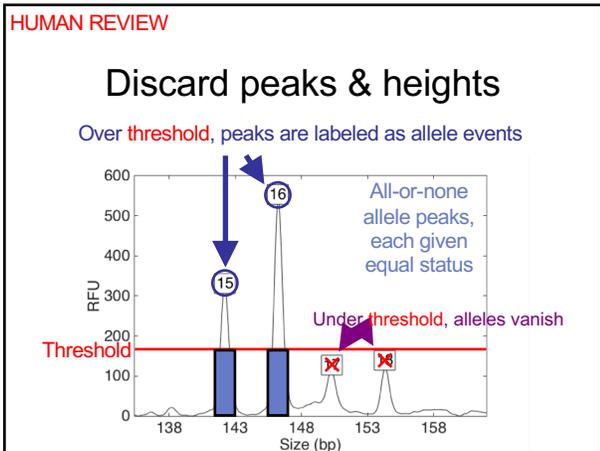
Alan Turing – likelihood ratio (LR)
 $\Pr\{\text{data} \mid \text{hypothesis}\} / \Pr\{\text{data} \mid \text{alternative}\}$

California v. Lopez

Man accused of rape and murder of girlfriend's toddler son

- Facing the death penalty, or life in prison.
- The child was 2 years and 10 months old.
- There were bruises to his face, genitals, and rectum.
- An autopsy showed brain swelling, skull fracture, cheek bruises, and asphyxia.
- A rectal swab from the boy showed semen.
- The swab matched the defendant's DNA.





HUMAN REVIEW

Simplify data to interpret mixture

1. Apply threshold to find "alleles"
2. Add allele frequencies ($f_1 + f_2 + \dots$)
3. Square sum, take reciprocal
4. Locus "probability of inclusion" (PI)
5. Multiply locus PI values
6. **Combined (CPI, RMNE) match statistic**

HUMAN REVIEW

Unreliable DNA mixture statistics

NIST (US Commerce Department) study in 2005
Two contributor mixture data, known victim

Some Differences in Reporting Statistics

LabID	Kits Used	Caucasians	African-Americans	Hispanics
80	ProPlus/Cofiler	1.19E+15	2.13E+14	3.09E+15
34	ProPlus/Cofiler	2.40E+11	2.00E+10	9.80E+10
33	ProPlus/Cofiler	2.94E+08	1.12E+08	1.74E+09
6	ProPlus/Cofiler	40,000,000	3,600,000	280,000,000
9	ProPlus/Cofiler	1.14E+07	1.97E+07	1.54E+08
79	ProPlus/Cofiler	930,000	47,886	1,360,000
16	ProPlus/Cofiler	438,600	51,710	999,100

Remember that these labs are interpreting the same MIX05 electropherograms

When not "inconclusive":
213 trillion (14)
31 thousand (4)

Forensic science put on notice 15 years ago

HUMAN REVIEW

Biased DNA workflow

Choose data Person decides Calculate statistic



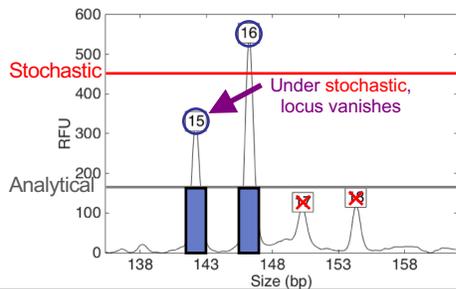
- Put people in the process
- To overcome software limits
- And introduce human bias



HUMAN REVIEW

NIST: Stochastic threshold

Under threshold, discard the locus DNA test entirely



HUMAN REVIEW

NIST: Thresholds misidentify

MIX13 Case 5 Outcomes with Suspect C
(whose genotypes were not present in the mixture)

# Labs	Report Conclusions	Reasons given
6	Exclude Suspect C	detailed genotype checks (ID+); TrueAllele negative LR (ID+); assumed major/minor and suspects did not fit (ID+); 3 labs noted Penta E missing allele 15 (PP16HS)
3	Inconclusive with C only (A & B included)	All these labs used PP16HS
21	Inconclusive for A, B, and C	
70	Include & provide CPI statistics	All over the road...

Range of CPI stats for Caucasian population:
FBI allele frequencies: **1 in 9** to **1 in 344,000**

HUMAN REVIEW

Statistics lack scientific basis

CPI/RMNE just counts the number of reported loci

J Pathol Inform

Research Article
Inclusion probability for DNA mixtures is a subjective one-sided match statistic unrelated to identification information

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HUMAN REVIEW

Mixture statistics shut down labs

“National accreditation board suspends all
DNA testing at D.C. crime lab”
The Washington Post April 27, 2015
Did not comply with FBI standards

“New protocol leads to reviews of
'mixed DNA' evidence”
The Texas Tribune September 12, 2015
24,468 lab tests affected

HUMAN REVIEW

Manual mixture interpretation

- Incomplete. Discard data, apply thresholds
- Inaccurate. Disagrees with true information
- Subjective. Workflow introduces human bias
- Inoperative. Hundreds of thousands of cases
- Opaque. Choices use only some of the data
- Biased. Can only include – or give no answer

Inconclusive

COMPUTER REVIEW

TrueAllele® computer technology

- Complete. Use all data, no thresholds
- Accurate. 42 validation studies, 8 published
- Objective. Workflow removes human bias
- Accepted. Reported in 46 states, WTC, labs
- Transparent. Give math, software (4GB DVD)
- Neutral. Can statistically include or exclude

Informative

PCR variation measures identity



Mark W. Perlin,¹ Ph.D., M.D., Ph.D. and Beata Szabady,¹ Ph.D.

Linear Mixture Analysis: A Mathematical Approach to Resolving Mixed DNA Samples

(12) **United States Patent** (10) **Patent No.:** **US 8,898,021 B2**
 Perlin (45) **Date of Patent:** **Nov. 25, 2014**

(54) **METHOD AND SYSTEM FOR DNA MIXTURE ANALYSIS** 6,274,317 B1 * 8/2001 Hiller et al. 435/6
 6,750,011 B1 * 6/2004 Perlm 435/6.11
 6,807,490 B1 * 10/2004 Perlm 702/20

(76) Inventor: **Mark W. Perlin**, Pittsburgh, PA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

FOREIGN PATENT DOCUMENTS

EP 0812621 A1 * 12/1997

OTHER PUBLICATIONS

(21) Appl. No.: **09/776,096**

(22) Filed: **Feb. 2, 2001**

Gill et al. (Forensic Science International (1998) 91: 41-53)*
 Clayton et al. (Forensic Science International (1998) 91: 55-70)*

Peer-reviewed validation studies

Perlin MW, Sinelnikov A. An information gap in DNA evidence interpretation. *PLoS ONE*. 2009;4(12):e8327.

Ballantyne J, Hanson EK, Perlin MW. DNA mixture genotyping by probabilistic computer interpretation of binomially-sampled laser captured cell populations: Combining quantitative data for greater identification information. *Science & Justice*. 2013;53(2):103-114.

Perlin MW, Hornyak J, Sugimoto G, Miller K. TrueAllele® genotype identification on DNA mixtures containing up to five unknown contributors. *Journal of Forensic Sciences*. 2015;60(4):857-868.

Greenspoon SA, Schiermeier-Wood L, Jenkins BC. Establishing the limits of TrueAllele® Casework: a validation study. *Journal of Forensic Sciences*. 2015;60(5):1263-1276.

Bauer DW, Butt N, Hornyak JM, Perlin MW. Validating TrueAllele® interpretation of DNA mixtures containing up to ten unknown contributors. *Journal of Forensic Sciences*. 2020; 65(2):380-398.

Perlin MW, Legler MM, Spencer CE, Smith JL, Allan WP, Belrose JL, Duceman BW. Validating TrueAllele® DNA mixture interpretation. *Journal of Forensic Sciences*. 2011;56(6):1430-1447.

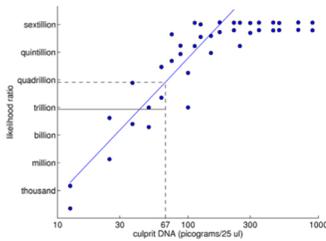
Perlin MW, Belrose JL, Duceman BW. New York State TrueAllele® Casework validation study. *Journal of Forensic Sciences*. 2013;58(6):1458-1466.

Perlin MW, Dormer K, Hornyak J, Schiermeier-Wood L, Greenspoon S. TrueAllele® Casework on Virginia DNA mixture evidence: computer and manual interpretation in 72 reported criminal cases. *PLoS ONE*. 2014;9(3):e92837.

TrueAllele predictability

An Information Gap in DNA Evidence Interpretation

Mark W. Perlin^{1*}, Alexander Sinelnikov²  2009



JOURNAL OF FORENSIC SCIENCES 

PAPER *J Forensic Sci*, November 2011, Vol. 56, No. 6
doi: 10.1111/j.1556-4029.2011.01859.x
Available online at: onlinelibrary.wiley.com

CRIMINALISTICS

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Validating TrueAllele® DNA Mixture Interpretation^{*†}

Heliyon

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Revised: 18 September 2018
Accepted: 24 September 2018

Efficient construction of match strength distributions for uncertain multi-locus genotypes

Mark W. Perlin, PhD, and
Construction of match strength distributions for uncertain multi-locus genotypes

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California v. Lopez

Man accused of rape and murder of girlfriend's toddler son

- Facing the death penalty, or life in prison.
- The child was 2 years and 10 months old.
- There were bruises to his face, genitals, and rectum.
- An autopsy showed brain swelling, skull fracture, cheek bruises, and asphyxia.
- A rectal swab from the boy showed semen.
- The swab matched the defendant's DNA.

Computer Interpretation of Quantitative DNA Evidence

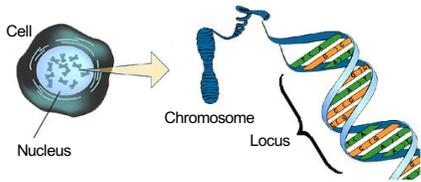
People of California v. Manuel Lopez
March, 2020
San Jose, CA

Jennifer M. Bracamontes, MS
Mark W. Perlin, PhD, MD, PhD
Cybergenetics, Pittsburgh, PA

Cybergenetics

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DNA biology



Short tandem repeat



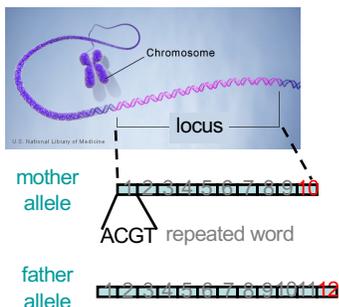
23 volumes in
cell's
DNA encyclopedia

DNA locus paragraph

Take me out to the ball game
take me out with the crowd
buy me some peanuts and Cracker Jack
I don't care if I never get back
let me
root root root root root root root root root
for the home team,
if they don't win, it's a shame for it's one, two,
three strikes, you're out
at the old ball game

"root" repeated 10 times, so
allele length is 10 repeats

DNA genotype



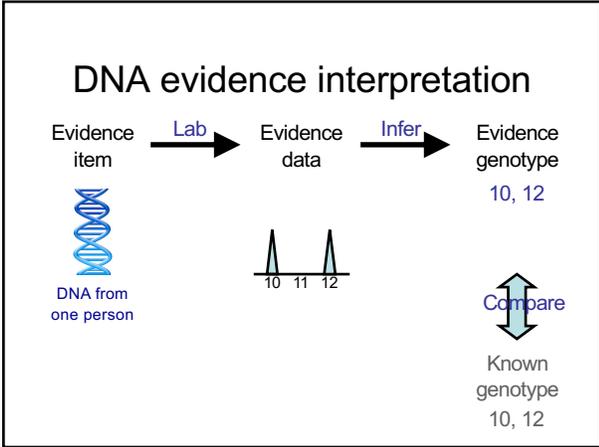
A genetic locus has
two DNA sentences,
one from each parent.

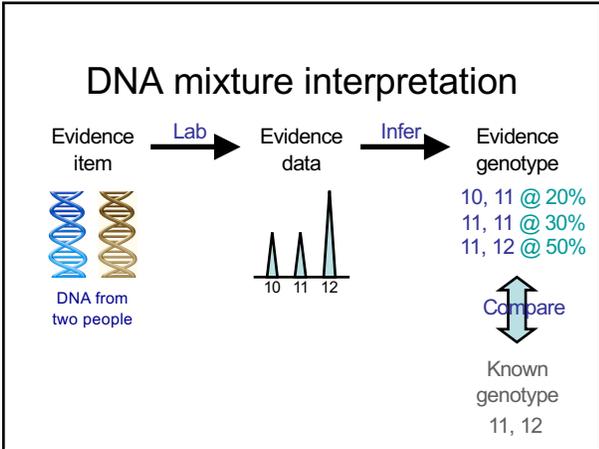
An **allele** is the number of
repeated words.

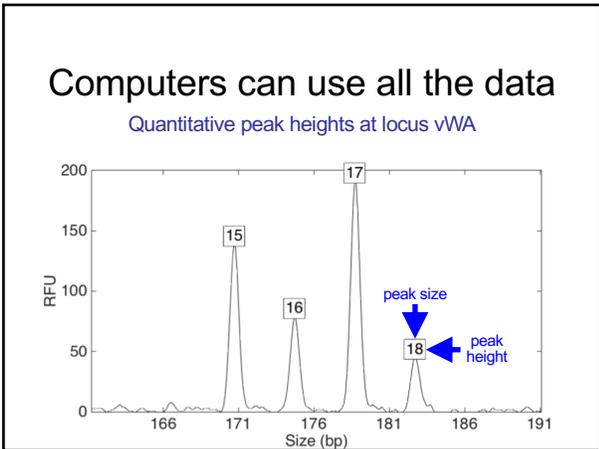
A **genotype** at a locus
is a pair of alleles.

10, 12

Many alleles allow for
many many allele pairs.
A person's genotype
is relatively unique.



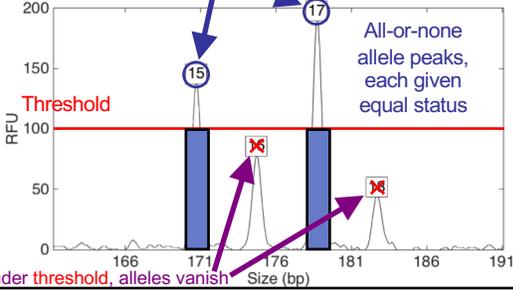




HUMAN REVIEW

People may use less of the data

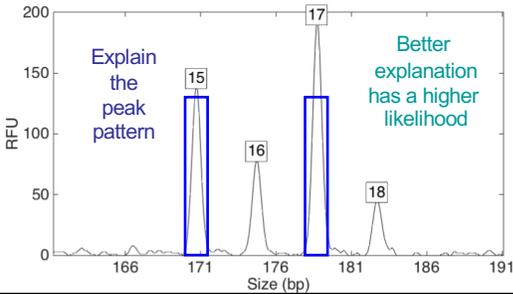
Over **threshold**, peaks are labeled as allele events



COMPUTER REVIEW

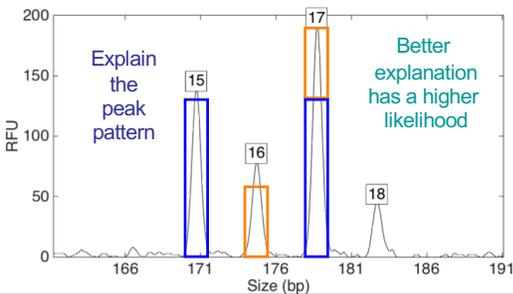
How the computer thinks

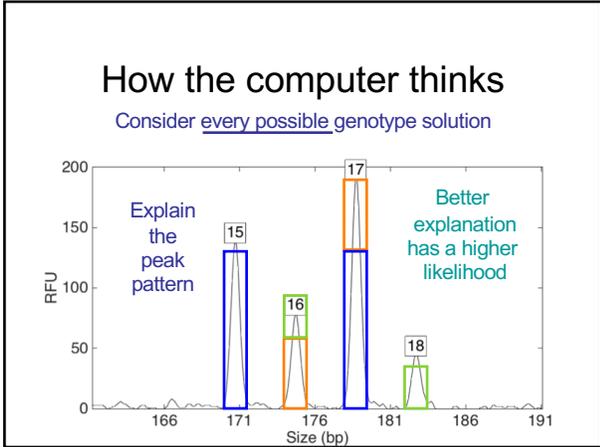
Consider every possible genotype solution

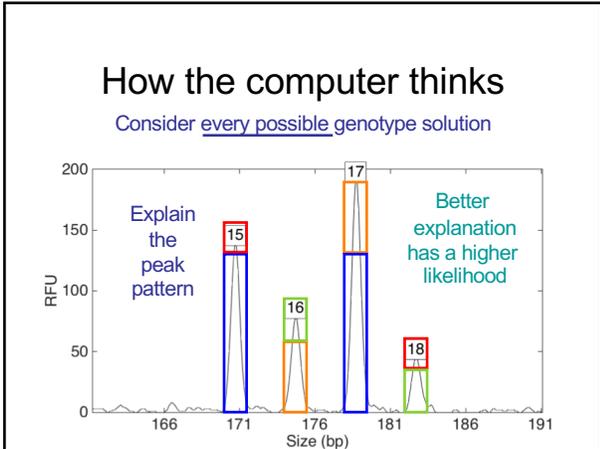


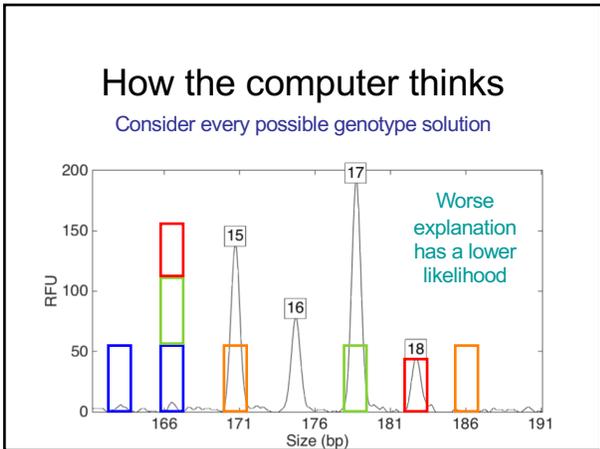
How the computer thinks

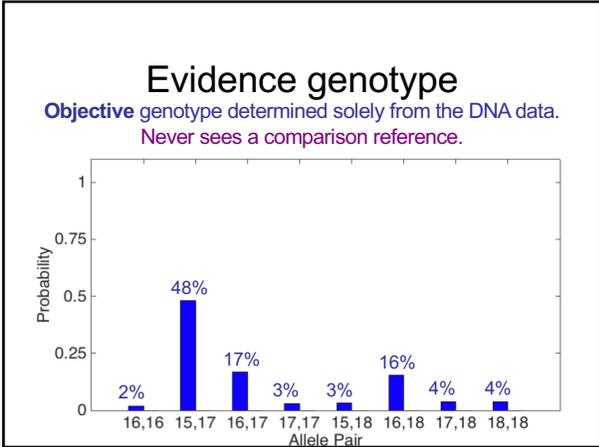
Consider every possible genotype solution

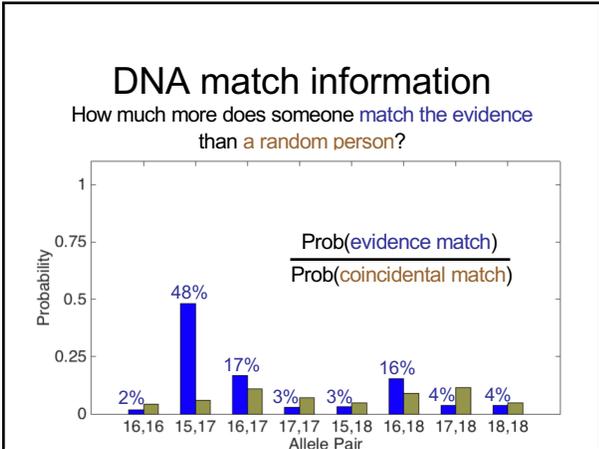


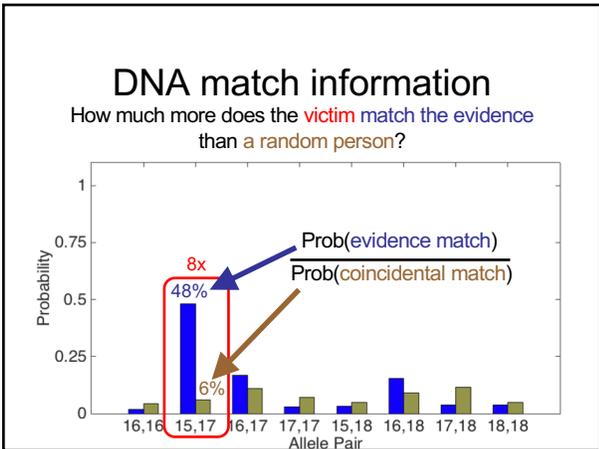


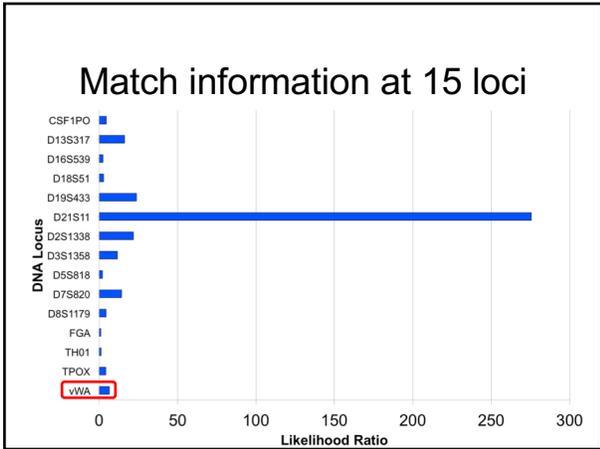












Is the victim in the evidence?

A match between the folded areas adjacent to bag knot and Apollo Torres is:

- 213 trillion times more probable than a coincidental match to an unrelated African-American person
- 3.09 trillion times more probable than a coincidental match to an unrelated Caucasian person
- 5.08 trillion times more probable than a coincidental match to an unrelated Southeast Hispanic person
- 5.15 trillion times more probable than a coincidental match to an unrelated Southwest Hispanic person

Is the reference in the evidence?

A match between the folded areas adjacent to bag knot and Arnulfo Torres Jr. is:

- 910 thousand times more probable than a coincidental match to an unrelated African-American person
- 71.9 thousand times more probable than a coincidental match to an unrelated Caucasian person
- 94.6 thousand times more probable than a coincidental match to an unrelated Southeast Hispanic person
- 14.8 thousand times more probable than a coincidental match to an unrelated Southwest Hispanic person

COMPUTER REVIEW

Match statistics



7-S2

folded areas adjacent to bag knot

			Person in	
2 REF	Iris Torres	X	21A EC on 17 items	2.62
3 REF	Odin Torres	X	21D SP on 7 items	X
23	Samantha Torres	X	7B-4 SP on 8 items	X
24	Manuel Lopez	X	8C SP on 8 items	3.42
25	Arnulfo Torres Jr.	4.17		
35	Apollo Torres	12.76		

COMPUTER VS. HUMAN

Match statistics



7-S2

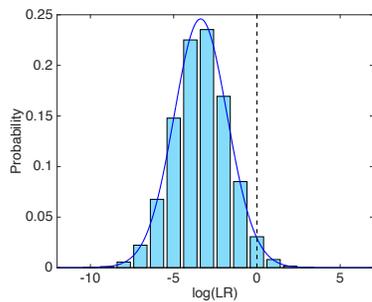
folded areas adjacent to bag knot

		CYB	KRCL	SCCCL
2 REF	Iris Torres	X	X	
3 REF	Odin Torres	X		
23	Samantha Torres	X		
24	Manuel Lopez	X	X	
25	Arnulfo Torres Jr.	4.17		
35	Apollo Torres	12.76	11.53	

COMPUTER REVIEW

Noncontributor Analysis

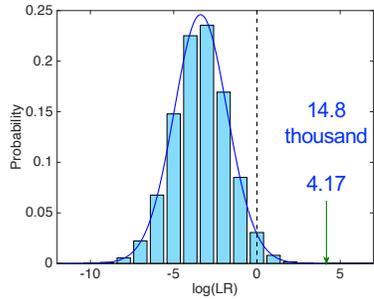
Perlin, M.W. "Efficient construction of match strength distributions for uncertain multi-locus genotypes." *Heligen*, 4(10):e00824, 2018.



COMPUTER REVIEW

Error Rate

Only 1 in 191 thousand people would match as strongly



Two different views

Prosecution. The defendant raped and killed a two-year old boy who lived in his house.

Defense. An abused toddler died. The defendant had nothing to do with his death.

Defendant's hair on clothes bag?



COMPUTER REVIEW

Hair match statistics (DNA,PG)



H1

hair root found on the bag

		Person in	
2 REF Sister	X	21A EC on 17 items	X
3 REF Brother	X	21D SP on 7 items	X
23 Mother	X	7B-4 SP on 8 items	X
24 Defendant	X	8C SP on 8 items	X
25 Brother	X		
35 Victim	X		

Def's semen in victim's rectum?

Weakly positive?



First in hospital

Entirely negative



Later at autopsy

COMPUTER REVIEW

Rectal swabs at hospital (DNA)

16A/B SP

first set of rectal swabs sperm fraction

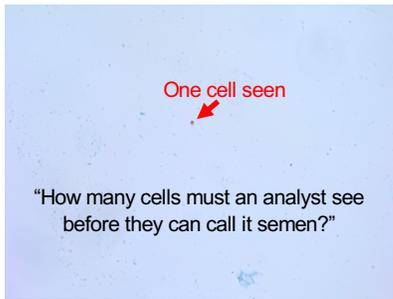
		Person in	
2 REF Sister	X	21A EC on 17 items	X
3 REF Brother	X	21D SP on 7 items	X
23 Mother	X	7B-4 SP on 8 items	X
24 Defendant	3.81	8C SP on 8 items	X
25 Brother	X		
35 Victim	X		

COMPUTER REVIEW

Rectal swabs at autopsy (DNA)

39A/B SP second set of rectal swabs sperm fraction	2 REF Sister	X	21A EC on 17 items	X
	3 REF Brother	X	21D SP on 7 items	X
	23 Mother	X	7B-4 SP on 8 items	X
	24 Defendant	X	8C SP on 8 items	X
	25 Brother	X		
	35 Victim	2.79		

Def's sperm on victim's penis?



COMPUTER REVIEW

Penile swabs (STR)

38A/B SP second set of penile swabs sperm fraction	2 REF Sister	X	21A EC on 17 items	X
	3 REF Brother	X	21D SP on 7 items	X
	23 Mother	X	7B-4 SP on 8 items	X
	24 Defendant	X	8C SP on 8 items	X
	25 Brother	X		
	35 Victim	7.57	Y-STR positive	

Forensic DNA evidence

Crime Laboratory

STR analyzed 97 evidence items
Reported 43 matches
Discovered 1 unknown person

Cybergenetics

Processed 77 items using TrueAllele®
Reported 138 matches
Discovered 5 unknown people

Targeted DNA – manual review

CPI/RMNE just counts how many loci an analyst reported

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Research Article

Inclusion probability for DNA mixtures is a subjective one-sided match statistic unrelated to identification information

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PAPER

CRIMINALISTICS

Untargeted DNA computer review

Mark W. Perlin,¹ Ph.D., M.D.; Jennifer M. Hornyak,¹ M.S.; Garrett Sugimoto,² M.S.; and Kevin W.P. Miller,² Ph.D.

TrueAllele® Genotype Identification on DNA Mixtures Containing up to Five Unknown Contributors*

PAPER

CRIMINALISTICS

David W. Bauer,¹ Ph.D.; Nasir Butt,² Ph.D.; Jennifer M. Hornyak,¹ M.S.; and Mark W. Perlin,¹ Ph.D., M.D., Ph.D.

Validating TrueAllele® Interpretation of DNA Mixtures Containing up to Ten Unknown Contributors*

J Forensic Sci. July 2015, Vol. 60, No. 4
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Available online at: onlinelibrary.wiley.com

J Forensic Sci. 2019
doi: 10.1111/1556-4029.14204
Available online at: onlinelibrary.wiley.com

Crime lab vs. TrueAllele information comparison

First 3 charts
County Crime Lab
Manual review

Second 3 charts
Cybergenetics
TrueAllele PG

Log(LR)	
25 to 30	
19 to 24	
13 to 18	
7 to 12	
1 to 6	
Inclusion	

County Crime Lab (1 of 3)

Item	Sister	Brother	Mother	Defendant	Brother	Victim	Person in	Person in	Female in	Person in	Person in	Male in
	2 REF	3 REF	23	24	25	35	21A-EC	21D-SP	45D-EC	7B-4-SP	8C-SP	7-S1
5A												
7-S1												
7-S2												
7A-2-EC												
7B-1-SP												
7B-2-SP												
7B-3-EC												
7B-4-EC												
7B-4-SP												
7B-5-EC												
8A												
8B-EC												
8B-SP												
8C-EC												
8C-SP												
8D-EC												
8D-SP												
8E-EC												
8E-SP												
8F-EC												
8G-EC												
8G-SP												
8H-EC												
8H-SP												
8I-EC												

County Crime Lab (2 of 3)

Item	Sister	Brother	Mother	Defendant	Brother	Victim	Person in	Person in	Female in	Person in	Person in	Male in
	2 REF	3 REF	23	24	25	35	21A-EC	21D-SP	45D-EC	7B-4-SP	8C-SP	7-S1
15A/B-EC												
15A/B-SP												
16A/B-EC												
16A/B-SP												
17A/B												
18A												
18B												
21A-EC												
21A-SP												
21B-EC												
21B-SP												
21C-EC												
21C-SP												
21D-EC												
21D-SP												
21E-EC												
21E-SP												
34A-EC												
34A-SP												
34B-EC												
34B-SP												
34C-EC												
34D-EC												
37-EC												
37-SP												

Cybergenetics TrueAllele (3 of 3)



Two strange puzzles

- **Where's Mom's DNA?**
Lots of different people left lots of DNA, but the primary caretaker left none.
- **Rectal DNA conflict**
Why was the defendant's DNA found in the **initial** hospital rectal swabs (Item 16), but **not later** at autopsy (Item 39)?

Two different views

Same DNA evidence, different conclusions

Prosecution. The forensic evidence shows that the defendant raped and killed the two-year old boy who lived in his house.

Defense. An abused toddler died. The forensic evidence shows that the defendant had nothing to do with his death.

Confirmation bias
Assume H, conclude H.
Assume ~H, conclude ~H.



Mother's DNA masked

Her hands were covered in defendant's semen

The child was in toilet training.
The child's primary caregiver had the defendant's semen on her hands. Swamping her own DNA as she attended to her child. Which is why we couldn't detect her DNA.
The mother was spreading the defendant's semen: from her hands, to baby wipes, to garbage bags, and whatever she touched – like to her child.
There was no probative value in this expected DNA.



Rectal DNA conflict

Q And in this particular investigation at the hospital, did you collect DNA swabbing from the victim's anus?

Pathologist Yes, sir.

Q And how did you do that?

A The same way we did for his genitals. Swabs were broken from a sterile package, sterile water was applied. I would place the swabs around the skin of anus in a circular manner, insert an inch to two inches, pull them out, and then they would have been handed off to law enforcement.

Q Okay. So you never stuck the swab into the rectum or into the anus more than two inches?

A Right. The beginning part of the anus and rectum is sufficient.



Rectal/anal cleaning swab

At the hospital. The mother had transferred the defendant's semen from her hands to the toddler's bottom. The first pathologist swabbed his anus, cleaning the external (anal) semen onto the swab. Before the swab was rectally inserted, the so-called "rectal swab" already contained external (anal) semen.

At the autopsy. The toddler's bottom had been cleaned by the first hospital swabbing. No more external (anal) semen; and there never was any internal (rectal) semen. So the second "rectal swab" was devoid of DNA. No external semen to collect.

Misleading terminology: "anal/rectal" swab, not "rectal" swab.

Final verdict

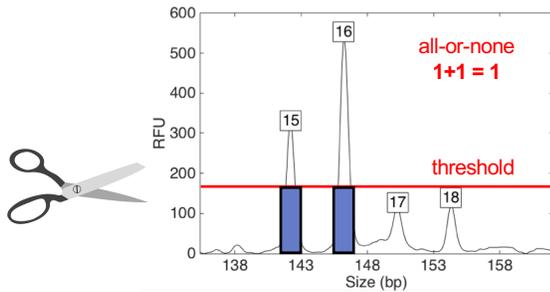
The prosecution was target-driven.
The defense was nontarget-driven.
Forensic experts educated the jury.

The nontargeted scenario better explained the evidence.
The jury acquitted the defendant of all charges.

The county no longer seeks the death penalty.

HUMAN REVIEW

Crime labs don't use all DNA data



Wolfe sisters homicide

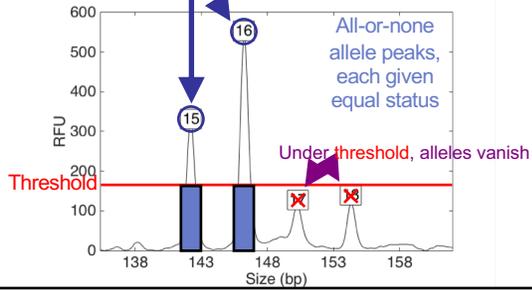


On February 6, 2014, Susan Wolfe (44)
and her younger sister Sarah (38, left)
were killed in their East Liberty home in Pittsburgh.

HUMAN REVIEW

Crime lab didn't use all DNA data

Over threshold, peaks are labeled as allele events



Pennsylvania v. Allen Wade

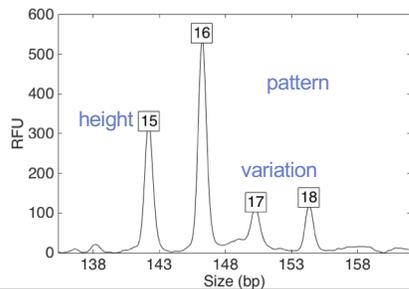
Thresholds failed to interpret most DNA mixtures

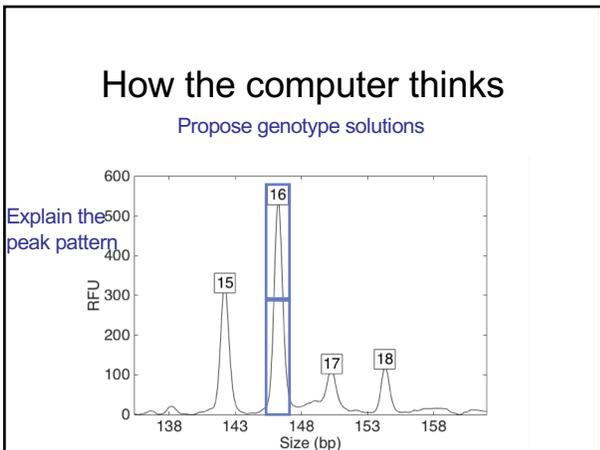
- | | |
|-------------|----------------------------------|
| Hat | No conclusions |
| Cup | Insufficient data |
| Fingernails | Contamination, insufficient data |
| Gear shift | Insufficient data |
| Seat lever | Cannot be excluded |
| Knit hat | Insufficient data |
| Sock | Too complex, no conclusions |

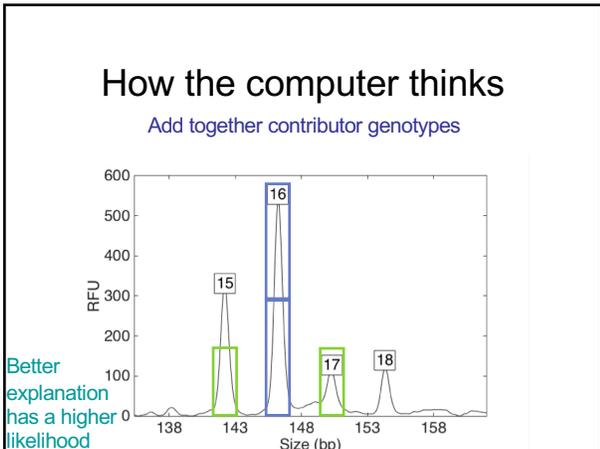
COMPUTER REVIEW

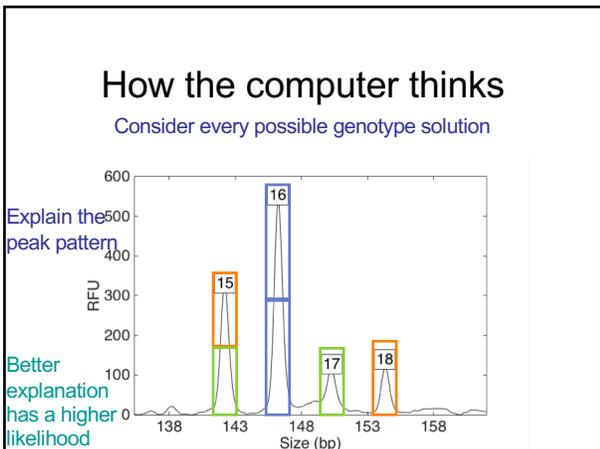
Computers can use all the data

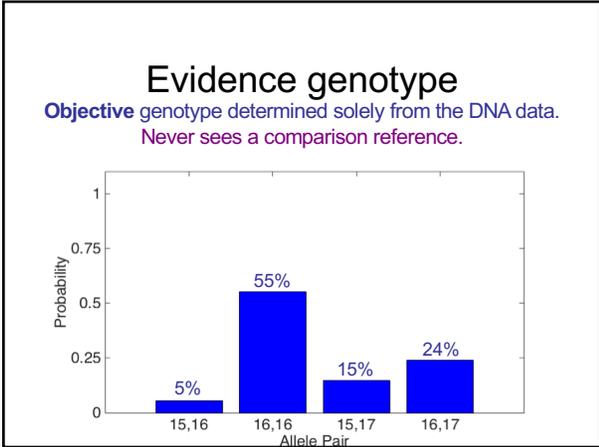
Quantitative peaks at locus vWA

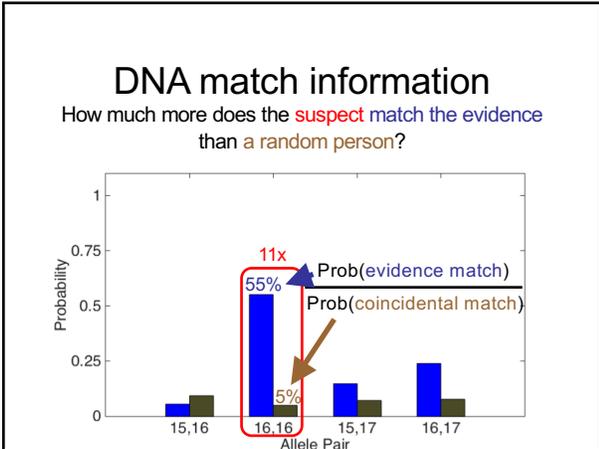


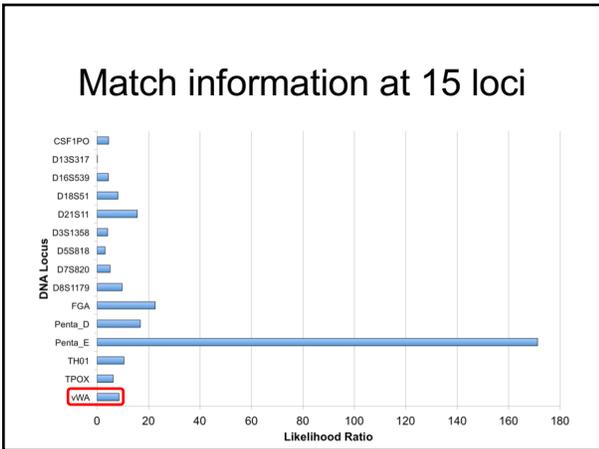












Is the suspect in the evidence?

A match between the right fingernails and Allen Wade is:

6.06 trillion times more probable than a coincidental match to an unrelated Black person

32.5 trillion times more probable than a coincidental match to an unrelated Caucasian person

8 trillion times more probable than a coincidental match to an unrelated Hispanic person

Pennsylvania v. Allen Wade

The crime lab reported 5 DNA mixture matches
TrueAllele found 17 matches on the same data

Hat	65.3 thousand	Allen Wade
Cup	20.5 thousand	Susan Wolfe
Fingernails	6.06 trillion	Allen Wade
Gear shift	9.37 million	Sarah Wolfe
Seat lever	385 billion	Sarah Wolfe
Knit hat	25.7 thousand	Allen Wade
Sock	300	Sarah Wolfe

Allen Wade Found Guilty On All Counts In East Liberty Sisters' Slaying

CBS News, May 23, 2016

PITTSBURGH (KDKA/AP)

- A man accused of killing two sisters who lived next door to him in East Liberty has been found guilty on all counts.
- Allen Wade was accused of shooting Sarah and Susan Wolfe after they returned from work on Feb. 6, 2014, apparently to steal a bank card.
- On Monday morning, a jury found Wade guilty of first-degree murder, robbery, burglary and theft by unlawful taking.

HUMAN REVIEW

Pennsylvania v. Allen Wade

Thresholds failed to interpret DNA mixture
TrueAllele succeeded on the same data

A hat left from a burglary of the Wolfe sister's home
six weeks before the murder matched
Allen Wade with a 65.3 thousand statistic



Preventable Crime

HUMAN REVIEW

No information from mixture

Crime laboratory DNA report
Crime lab user fee: \$5,000

Conclusions:

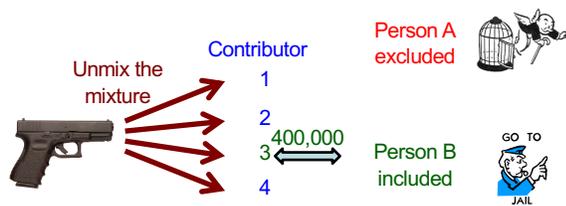
Item 1 – Swab of textured areas from a handgun

The data indicates that DNA from four (4) or more contributors was obtained from the swab of the handgun.
Due to the complexity of the data, no conclusions can be made regarding persons A and B as possible contributors to this mixture.

COMPUTER REVIEW

Computer reanalysis

Cybergenetics TrueAllele report
Match statistics provide information



TrueAllele today

Invented math & algorithms	25 years
Developed computer systems	20 years
Support users and workflow	10 laboratories
Routinely used in casework	400 agencies
Validate system reliability	42 studies
Educate the community	100 talks
Train or certify analysts	800 students
Admissibility challenges	37 rulings, 15 states and federal
Testify about LR results	110 trials
Educate lawyers and public	1,000 people
Make the ideas understandable	1,100 cases, 46 states

More information

<http://www.cybgen.com/information>



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