Forensics @ NIST
December 7, 2010 – Gaithersburg, MD

Low-Level DNA & Kinship

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Outline of Topics to Discuss

- Introduction and historical perspective of Low Template (LT) DNA testing
- Challenges and limitations with LT-DNA testing
  - Approaches to genotyping low template DNA
  - NIST LT-DNA studies and Sensitivity Comparisons
  - LT-DNA mixture data
- Conclusions and future NIST studies

Some Definitions of Low Template (LT) DNA

- Working with **<100-200 pg genomic DNA**
- Considered to be data below stochastic threshold level where PCR amplification is not as reliable (determined by each laboratory; typically 150-250 RFUs)
- Enhancing the sensitivity of detection (increasing PCR cycles, PCR product clean-up, increasing CE injection/voltage)
- Having too few copies of DNA template to ensure reliable PCR amplification (allelic or full locus drop-out)
- Can often be the minor component of mixture samples consisting of low level DNA template amounts

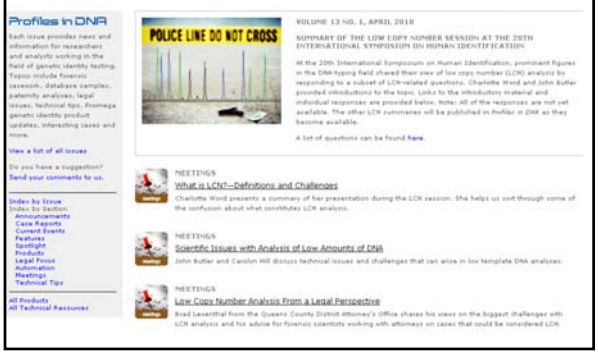
Recent LT-DNA Court Rulings

- "…a challenge to the validity of the method of analysing Low Template DNA by the LCN process should no longer be permitted at trials where the quantity of DNA analysed is above the stochastic threshold of 100-200 picograms…"
  - United Kingdom: Crown vs. Reed & Reed, Dec. 21, 2009
- LT-DNA testing is "…generally accepted as reliable in the forensic scientific community under the standard enunciated in Frye…"
  - NYC OCME: People vs. Megnath, Feb. 8, 2010
- "LCN DNA evidence is not inherently unreliable."
  - New Zealand: Crown vs. Wallace, Mar. 3, 2010

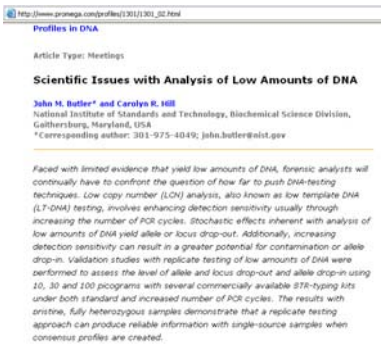
The judge in the Wallace case quotes from John Butler's *Fundamentals of Forensic DNA Typing* in drawing the court's conclusion

Profiles in DNA (April 2010)

http://www.promega.com/profiles/



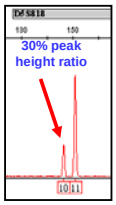
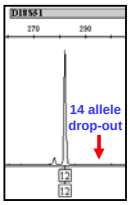
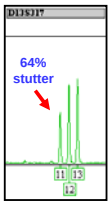
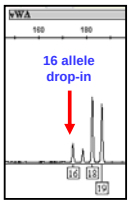
Profiles in DNA Article on Low Level DNA



Stochastic (Random) Effects with LT-DNA

When Combined with Higher Sensitivity Techniques

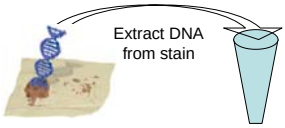
Loss of True Signal (False Negative) | *Gain of False Signal (False Positive)*

Severe **Peak Imbalance**	Allelic **Drop-out**	**High Stutter**	Allelic **Drop-in**
			
Identifiler, 30 pg DNA, 31 cycles	Identifiler, 30 pg DNA, 31 cycles	Identifiler, 10 pg DNA, 31 cycles	Identifiler, 10 pg DNA, 31 cycles
Correct genotype: **10,11**	**12,14**	**12,13**	**18,19**

Suggestions for Optimal Results with LT-DNA

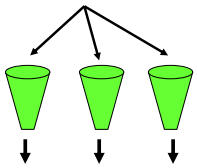
- Typically at least 2 – 3 PCR amplifications from the same DNA extract are performed to obtain consensus profiles
- An allele cannot be scored (considered real) unless it is present at least twice in replicate samples
- Extremely sterile environment is required for PCR setup to avoid contamination from laboratory personnel or other sources

Typical LT-DNA Analysis Procedure



Extract DNA from stain

Quantify Amount of DNA Present



Perform 3 Separate PCR Amplifications

Interpret Alleles Present

Develop a Consensus Profile

(based on replicate consistent results)

Comparison of Approaches

What "LCN Labs" Are Doing

Replicate Amplification with Consensus Profile

Low amount of DNA examined

Stochastic effects

Amplification #1
Amplification #2
Amplification #3

Consensus Profile Developed
(from repeated alleles observed)

Interpretation Rules Applied
(based on validation experience)
e.g., specific loci may dropout more

Result can be and usually is Reliable & Reproducible

Single Amplification

Low amount of DNA examined

Stochastic effects

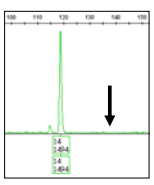
Amplification #1 (only a single test)

Result can be Unreliable

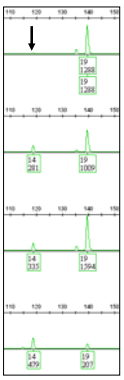
Individual results may vary but a consensus profile is reproducible (based on our experience with sensitivity studies and replicate amplifications)

D3S1358 replicates with 3 extra cycles

False Homozygote with 1494 RFUs, well above any stochastic thresholds



Allele "19" drop-out



*Any combination of 3/5 replicates gives the correct genotype (14,19)

Identifiler Plus, 32 cycles, 10 pg DNA

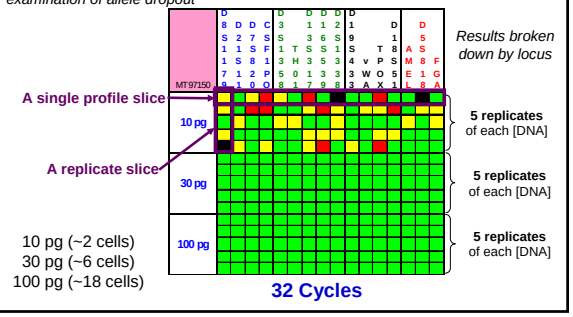
Experimental Design to Study LT-DNA Issues

- Pristine DNA Samples
  - 2 single-source samples
  - **heterozygous for all loci tested** (permits peak height ratio studies)
- **Low DNA Template Amounts**
  - Dilutions made after DNA quantitation against NIST SRM 2372
  - **100 pg, 30 pg, and 10 pg** (1 ng tested for comparison purposes)
- Replicates
  - **5 separate PCR reactions** for each sample
- STR Multiplex Kits
  - **Identifiler Plus and PowerPlex 16 HS** (half-reactions)
- **Increased Cycle Number**
  - Identifiler Plus (**29 cycles and 32 cycles**; 28 for 1 ng)
  - PowerPlex 16 HS (**31 cycles and 34 cycles**; 30 for 1 ng)

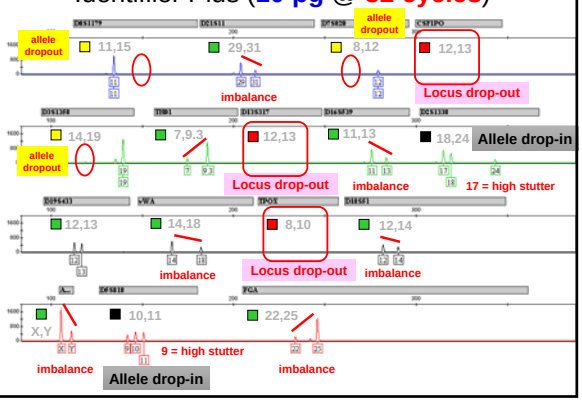
Sensitivity Comparison

Green = full (correct) type
Yellow = allele dropout
Red = locus dropout
Black = drop-in

Tested sample is heterozygous
(possesses 2 alleles) at every locus, which permits an examination of allele dropout



Identifiler Plus (10 pg @ 32 cycles)

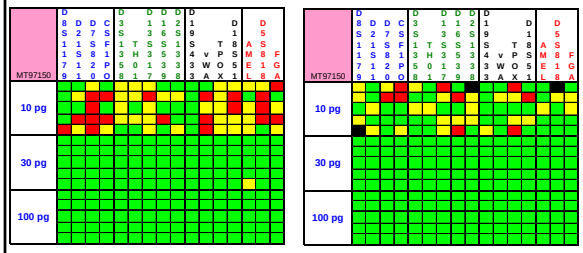


Impact of Three More PCR Cycles

Identifiler Plus

Green = full (correct) type
Yellow = allele dropout
Red = locus dropout
Black = drop-in

*Full type = both alleles above 50 RFU (does not account for peak imbalance)



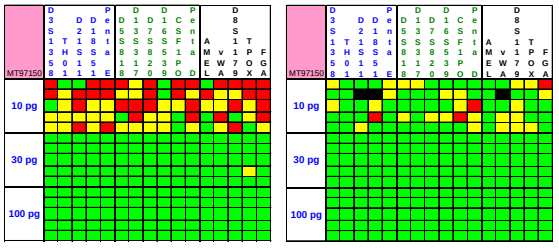
33% vs. 53% full profiles

38% improvement with 3 extra cycles

Sensitivity & Performance

PowerPlex 16 HS

Green = full (correct) type
Yellow = allele dropout
Red = locus dropout
Black = drop-in



16% vs. 60% full profiles

73% improvement with 3 extra cycles

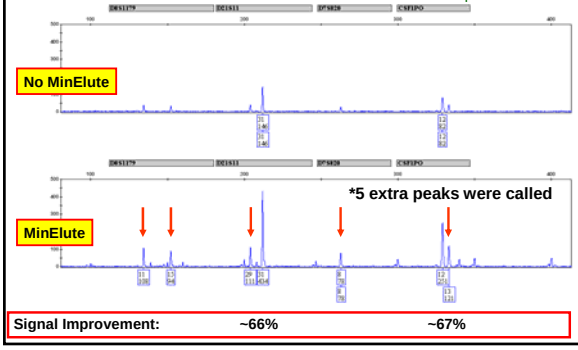
Additional Methods of LT-DNA Testing and Future Studies at NIST

- Signal enhancing techniques
  - MinElute PCR purification kit (Qiagen) for salt removal in final product – example shown
  - Increasing CE injection voltage and time
  - Reduced volume PCR (concentrates amplicon)
- Degraded DNA studies
- LT-DNA mixture studies (preliminary results shown)

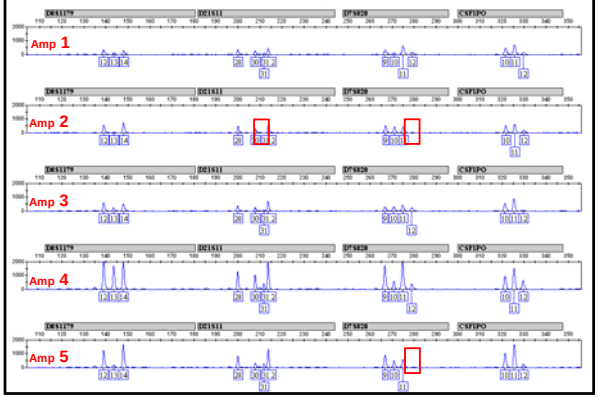
MinElute PCR Purification Kit

*96 well plates with vacuum protocol used

Identifiler Plus, 29 cycles, 10 pg



GT:OT, 1:3, 50 pg @ 31 cyc



Summary of LT-DNA Testing

- More and more labs are “pushing the envelope” and attempting LT-DNA testing.
- LT-DNA testing has been “generally accepted as reliable” in many recent court cases.
- Our results demonstrate that replicate testing can produce reliable information with single source samples at low levels of DNA when consensus profiles are created.

The NIST Human Identity Project Team

(Forensic DNA & DNA Biometrics)

Funding from the **National Institute of Justice (NIJ)** through the NIST Office of Law Enforcement Standards and the **FBI S&T Branch** through the NIST Information Access Division

…Bringing traceability and technology to the scales of justice…

John Butler — *Project Leader, Forensic DNA*
Erica Butts
Mike Coble
Dave Duewer
Becky Hill
Margaret Kline
Kristen Lewis O’Connor
Jan Redman
Pete Vallone — *Project Leader, DNA Biometrics*

Workshops & Textbooks | Mixtures, mtDNA & Y | Concordance & LT-DNA | Kinship Analysis | Rapid PCR & Biometrics

Direct PCR & DNA Extraction | Software Tools & Data Analysis | Variant alleles & Cell Line ID | STRBase Support

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
becky.hill@nist.gov
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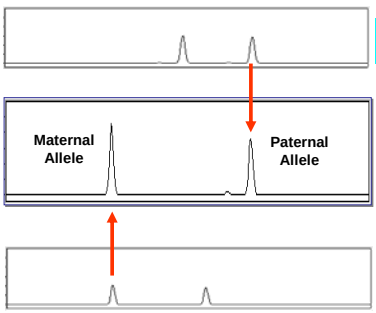
Questions to Be Addressed

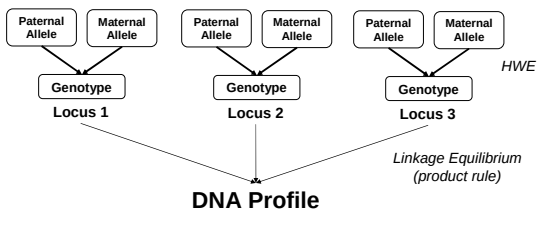
- How does kinship analysis relate to forensic DNA typing?
- What are the challenges of kinship analysis?
- What has NIST accomplished with kinship analysis?
- Where can one learn more about these topics?

Inheritance Pattern of DNA Profiles



Result from a Single Locus (specific region of DNA)

Composition of a DNA Profile



John M. Butler (2009) Fundamentals of Forensic DNA Typing. Figure 11.3

Elements Going into the Calculation of a Rarity Estimate for a DNA Sample

2 Population allele frequencies

1 DNA Profile (with specific alleles)

→ Rarity estimate of the specific DNA profile

Appropriate genetic formulas

3

There are different ways to express the profile rarity

NIST Sample Set

- NIST U.S. population samples
  - 254 African American, 261 Caucasian, 139 Hispanic
- U.S. father/son samples
  - 178 African American, 198 Caucasian, 190 Hispanic, 198 Asian
- Extended family samples
  - 6 sets of 3-4 generations
  - 165 total samples

www.cstl.nist.gov/strbase/

Autosomal STR Markers

46 unique STR loci have been characterized at NIST

	U.S.	Europe	NIST 26plex Assay (non-commercial)
13 CODIS loci	TPOX		D1GATA113
	CSF1PO		D1S1627
	D5S818		D1S1677
	D7S820		D2S1776
	D13S317	*European Standard Set = ESS*	D3S3053
	FGA	FGA	D3S4529
	vWA	vWA	D4S2364
	D3S1358	D3S1358	D4S2408
	D8S1179	D8S1179	D5S2500
	D18S51	D18S51	D6S474
	D21S11	D21S11	D6S1017
	TH01	TH01	D8S1115
	D16S539	D16S539	D9S1122
	D2S1338	D2S1338	D9S2157
	D19S433	D19S433	D10S1435
	Penta D		D11S4463
	Penta E		D12ATA63
			D14S1434
			D17S974
			D17S1301
			D2S441
			D10S1248
			D22S1045
			D18S853
			D20S482
			D20S1082

7 ESS loci

5 loci adopted to expand to 12 ESS loci

D12S391, D1S1656, D2S441, D10S1248, D22S1045, SE33

Elements Going into the Calculation of a Rarity Estimate for a DNA Sample

2 Population allele frequencies

1 DNA Profile (with specific alleles)

→ Rarity estimate of the specific DNA profile

Appropriate genetic formulas

3

There are different ways to express the profile rarity

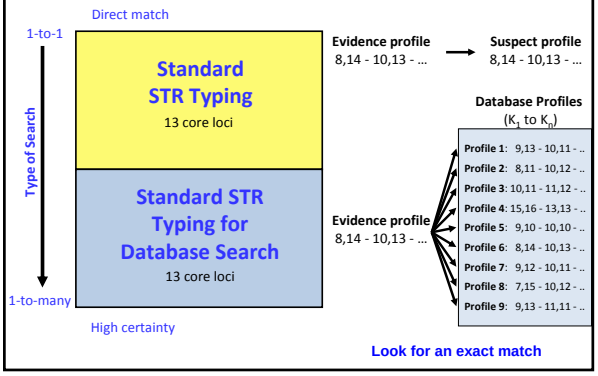
Table 11.3 Random match probability for a 13-locus STR profile using the U.S. Caucasian allele frequencies found in Table 11.1.

	Allele 1	Allele 2	Allele 1 Frequency (p)	Allele 2 Frequency (q)	Formula	Expected Genotype Frequency
D13S317	11	14	0.33940	0.04801	2pq	0.0326
TH01	6	6	0.23179		p^2	0.0537
D18S51	14	16	0.13742	0.13907	2pq	0.0382
D21S11	28	30	0.15894	0.27815	2pq	0.0884
DGS1358	16	17	0.25331	0.21523	2pq	0.1090
D5S818	12	13	0.38411	0.14073	2pq	0.1081
D7S820	9	9	0.17715		p^2	0.0314
D8S1179	12	14	0.18543	0.16556	2pq	0.0614
CSF1PO	10	10	0.21689		p^2	0.0470
FGA	21	22	0.18543	0.21854	2pq	0.0810
D16S539	9	11	0.11258	0.32119	2pq	0.0723
TPOX	8	8	0.53477		p^2	0.2860
VWA	17	18	0.28146	0.20033	2pq	0.1128
AMEL	X	Y				
Product rule						1.20×10^{-15}
Combined frequency						1 in 8.37×10^{14}

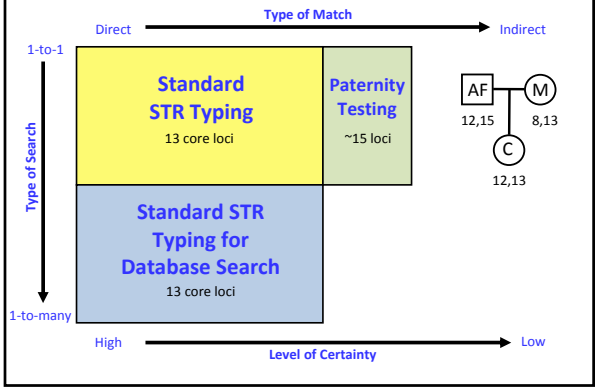
1 in 837 trillion

John M. Butler (2009) Fundamentals of Forensic DNA Typing, Table 11.3

Forensic Core Competency

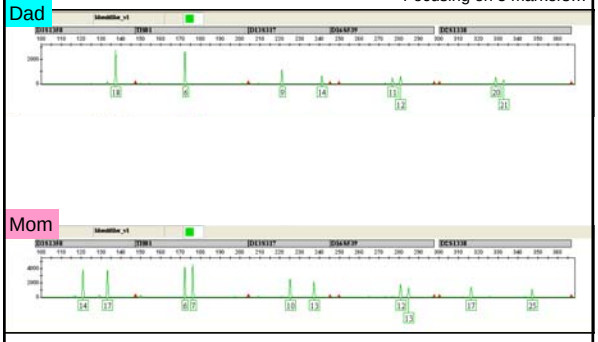


Expanding the Forensic Core Competency



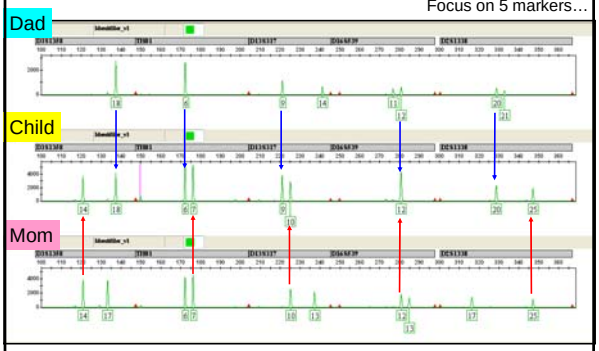
Paternity Testing

Focusing on 5 markers…



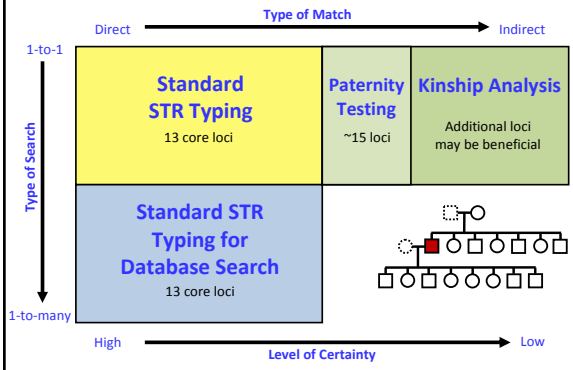
Fundamentals of Paternity Testing

Focus on 5 markers…



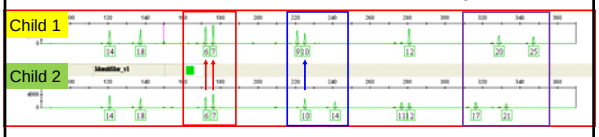
Parent-offspring will share **one** allele at every locus

Expanding the Forensic Core Competency



Kinship Analysis: Full Siblings

Focusing on 5 markers…



Full siblings may share **two**, **one**, or **zero** alleles at a locus

For more distant familial relationships, allele sharing decreases

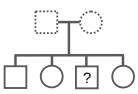
Uncertainty Increases

What is kinship analysis?

Evaluation of relatedness between individuals

Applications

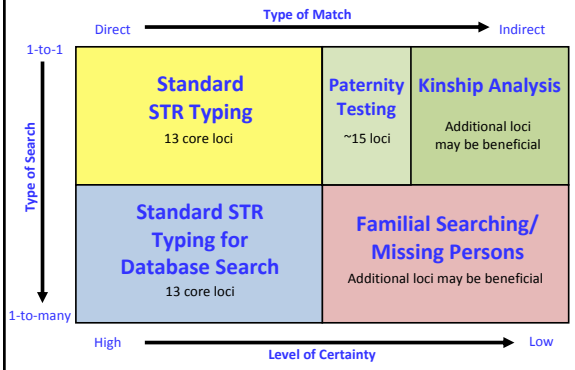
Parentage testing (civil or criminal)
Missing persons identification
Familial searching
Disaster victim identification
Immigration



Forensic Applications of Kinship Analysis

Missing Persons Identification
Familial Searching

Expanding the Forensic Core Competency

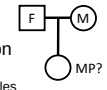


Missing Persons Identification

Missing Persons Identification

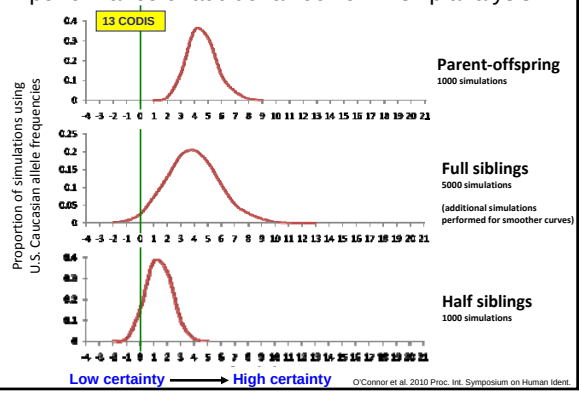
- National Missing Persons DNA Database
  - DNA from unidentified human remains
  - DNA from family members of a missing person
  - At least 8 forensic markers are required
  - Family reference samples are necessary

- False positives and negatives occur with one-to-many searches



- Additional markers can improve kinship identification
  - Y-chromosome loci
    - Identify all relationships between males who are linked by males
  - Mitochondrial DNA
    - Identify all relationships between any persons, male or female, who are linked by females
- Need research to evaluate the power of different types of loci

Simulation experiments to assess expected performance of additional loci for kinship analysis



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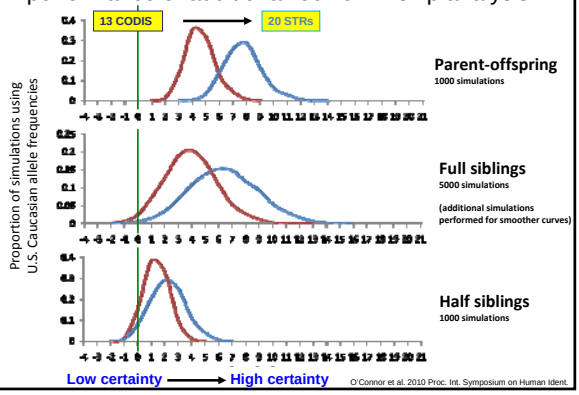

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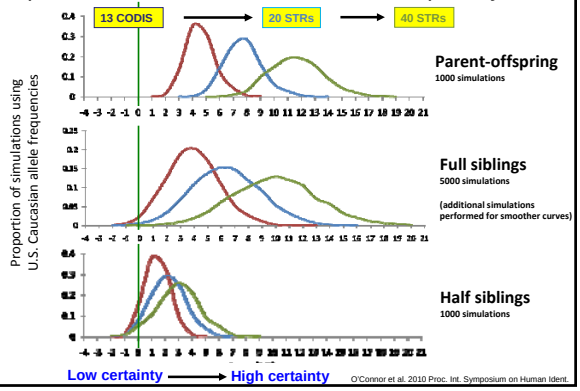


O'Connor et al. 2010 Proc. Int. Symposium on Human Ident.

How can uncertainty in kinship determination be reduced?

Improve the measurement technique

- Add more family references
- Add more loci
  - Autosomal STRs* improve identification of **parent-offspring and full siblings**
    *** More chances for mutation**
  - Lineage markers or SNP arrays may improve identification of more distant relatives

Nothnagel M, Schmidtke J, Krawczak M. (2010) Potentials and limits of pairwise kinship analysis using autosomal short tandem repeat loci, *Int. J. Legal Med.* 124(3):205-15.

Familial Searching

Familial Searching

- Search unknown evidence profile against offender database to identify a close relative
- No suspect cases, cold cases, violent crimes
- Success in the United Kingdom
  - 2004-2010: 176 submitted, 131 searches, 35 successes
- Recent familial searching programs in the U.S.
  - Colorado (all forensic unknowns, 19 leads, 1 conviction)
  - California (13 searches, 1 arrest)

Victims of the Grim Sleeper

http://www.laweekly.com/2008-08-28/news/eleven-lives-stolen-and-one-lucky-survivor/

The Grim Sleeper's Victims

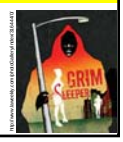
1)Debra Jackson (age 29) – August 10, 1985
2)Henrietta Wright (age 35) – August 12, 1986
3)Thomas Steele (age 36) – August 14, 1986
4)Barbara Ware (age 23) – January 10, 1987
5)Bernita Sparks (age 25) – April 15, 1987
6)Mary Lowe (age 26) – October 31, 1987
7)Lachrica Jefferson (age 22) - January 30, 1988
8)Monique Alexander (age 18) – September 11, 1988
9)Enietra Washington (raped but survived) – November 1988

10) Princess Berthomieux (age 14) – March 19, 2002
11) Valerie McCorvey (age 35) – July 11, 2003
12) Janecia Peters (age 25) – January 1, 2007

Ballistics on bullets recovered from the victim's bodies matched

DNA evidence recovered

Over a 13 year gap in detected crimes, hence the "Sleeper" nickname



http://blogs.laweekly.com/informer/crime/grim-sleeper-son-dna-trail-led/

Building Tools to Identify the Grim Sleeper

- DNA evidence from the perpetrator was searched against the national offender database
  - No direct match found
  - Dead end?

- California Dept. of Justice initiated a research program to evaluate the use of familial searching
  - Developed and validated the program using NIST Population data of forensic and Y-STR markers
  - Data are freely available to the forensic community on the STRBase website
    www.cstl.nist.gov/strbase/
- October 2008: First familial search of the California database yielded no strong possibilities in the Grim Sleeper case

Putative Relative Is Found

- June 30, 2010: Second familial search of the California database yielded one likely relative
- Database profile belonged to Christopher Franklin (31 years old)
  - Profile added to the database in 2009 after a felony weapons possession charge
- Grim Sleeper profile matched C. Franklin's profile with one allele at all 15 loci
- Both individuals shared the same Y-STR profile, indicating a possible paternal relationship

Identifying the Grim Sleeper

- Given that the murders spanned at least 25 years, the paternal relationship was likely father-son
- Undercover police shadowed C. Franklin's father, Lonnie David Franklin, Jr., who lived in the vicinity of the murders
- Police collected a DNA sample from Lonnie Franklin
  - **Direct match between L. Franklin and the Grim Sleeper**

CRIME & COURTS July 7, 2010

Arrest Made in L.A. 'Grim Sleeper' Killings

Published July 07, 2010 | Associated Press

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LOS ANGELES -- A one-time police mechanic was arrested and charged Wednesday in the serial killing of 10 people over 25 years after a DNA sample from his son was found to bear a close resemblance to DNA found on the victims.

Lonnie Franklin Jr., 57, was charged with 10 counts of murder, one count of attempted murder and special circumstance allegations of multiple murders that could make him eligible for the death penalty if convicted, District Attorney Steve Cooley said.

He is charged with 10 counts of murder and one count of attempted murder for a series of killings that date back to 1985.

Lonnie David Franklin Jr.

Familial Searching in the U.S.

High-profile success in the Grim Sleeper case has led other states to consider familial searching

Experts say Texas might solve Twilight Serial Rapist cases with family DNA

http://www.examiner.com/law-enforcement-in-wichita-falls/experts-say-texas-might-solve-twilight-serial-rapist-cases-with-family-dna

DNA DATABASE

Milwaukee police on hunt for serial killer linked to 7 deaths

May 19, 2009 http://articles.cnn.com/2009-05-19/justice/wisconsin.serial.killer_1_dna-technology-dna-database-prostitutes?_s=PM:CRIME

Familial DNA hunt sought in East Coast rape case

http://www2.insidenova.com/news/2010/aug/04/familial_dna_hunt_sought_in_east_coast_rape_case-ar-428231/

Wednesday, December 1, 2010

Virginia could become 3rd state to use familial DNA searches

Some concerned practice could stigmatize those related to criminals

http://www.fairfaxtimes.com/cms/story.php?id=2600

Research Underpins Familial Searching

Forensic Sci Int Genet. 2010 Nov 3. [Epub ahead of print]

Searching for first-degree familial relationships in California's offender DNA database: Validation of a likelihood ratio-based approach.

Myers SP, Timken MD, Piucci ML, Sims GA, Greenwald MA, Weigand JJ, Konzak KC, Buonaccorsi MR.

California Department of Justice Jan Bashinski DNA Laboratory, 1001 West Cutting Blvd., Suite 110, Richmond, CA 94804, United States.

Abstract

A validation study was performed to measure the effectiveness of using a likelihood ratio-based approach to search for possible first-degree familial relationships (full-sibling and parent-child) by comparing an evidence autosomal short tandem repeat (STR) profile to California's ~1,000,000-profile State DNA Index System (SDIS) database. Test searches used autosomal STR and Y-STR profiles generated for 100 artificial test families. When the test sample and the first-degree relative in the database were characterized at the 15 Identifiler(®) (Applied Biosystems(®), Foster City, CA) STR loci, the search procedure included 96% of the fathers and 72% of the full-siblings. When the relative profile was limited to the 13 Combined DNA Index System (CODIS) core loci, the search procedure included 93% of the fathers and 61% of the full-siblings. These results, combined with those of functional tests using three real families, support the effectiveness of this tool. Based upon these results, the validated approach was implemented as a key, pragmatic and demonstrably practical component of the California Department of Justice's Familial Search Program. An investigative lead created through this process recently led to an arrest in the Los Angeles Grim Sleeper serial murders.

Research is necessary to evaluate the performance of searching algorithms in different state databases

- Size of database
- Number of loci typed
- Types of relationships
- Autosomal vs. lineage markers
- False positives vs. false negatives

What is NIST doing to improve kinship analysis?

Evaluation of new loci

Allele frequencies for U.S. population samples

Concordance testing of new multiplexes

Developed a new website to support kinship analysis

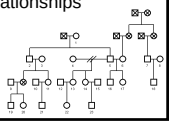
Kinship Resource Page on STRBase

www.cstl.nist.gov/strbase/kinship.htm

NIST Standard Reference Family Data

Aid **validation** of algorithms, software, and loci selection for **kinship analysis**

- Use genotypes with known inheritance
- Compare LRs from algebraic and software calculations
- Test algorithms for mutation, rare alleles, null alleles, incest
- Evaluate use of additional loci to detect relationships



The NIST Human Identity Project Team

(Forensic DNA & DNA Biometrics)

Funding from the **National Institute of Justice (NIJ)** through the NIST Office of Law Enforcement Standards and the **FBI S&T Branch** through the NIST Information Access Division

...Bringing traceability and technology to the scales of justice...

John Butler (*Project Leader, Forensic DNA*) | Erica Butts | Mike Coble | Dave Duewer | Becky Hill | Margaret Kline | Kristen Lewis O'Connor | Jan Redman | Pete Vallone (*Project Leader, DNA Biometrics*)

Workshops & Textbooks | Mixtures, mtDNA & Y | Concordance & LT-DNA | Kinship Analysis | Rapid PCR & Biometrics

Direct PCR & DNA Extraction | Software Tools & Data Analysis | Variant alleles & Cell Line ID | STRBase Support

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

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