Research Practices Applied to and Born from the World Trade Center Tragedy

<table>
<thead>
<tr>
<th>Presenter</th>
<th>Institution</th>
<th>Position</th>
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<tbody>
<tr>
<td>John Butler</td>
<td>NIST</td>
<td>Applied Genetics Group Leader</td>
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<tr>
<td>Barbara Butcher</td>
<td>NYC OCME</td>
<td>Chief of Staff</td>
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<td>Bradley Adams</td>
<td>NYC OCME</td>
<td>Director of Forensic Anthropology</td>
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<tr>
<td>Elias Kontanis</td>
<td>NTSB</td>
<td>Coordinator for Medicolegal Operations</td>
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The NIJ Conference
June 21, 2011 – Crystal City, VA

WTC Kinship and Data Analysis Panel (KADAP) & DNA Technology Innovations

John M. Butler
National Institute of Standards and Technology
Dedication of this Presentation in Memory of George Carmody

- Member of WTC KADAP
- Member of Hurricane Katrina Expert Group
- Member of SWGDAM Mixture Committee
- Colleague, fellow teacher, and friend…

March 29, 1938 – June 13, 2011
We shared co-authorship on an important article that brings us together today…

Policy Forum
Epidemiology

DNA Identifications After the 9/11 World Trade Center Attack


Presentation Outline

• WTC Kinship and Data Analysis Panel (KADAP)

• DNA Innovations **Applied to** WTC Effort
  – **Assays:** miniSTRs, SNPs, HT-mtDNA sequencing
  – **Software:** M-FISys, DNA View, MDKAP
  – **Information:** family DNA brochure, sample collection form

• DNA Innovations **Born from** WTC Effort
  – New assays: miniSTRs (MiniFiler) & new loci
  – **Software:** OSIRIS
  – Lessons Learned document (NIJ Sept 2006 publication)
    • Family DNA brochure has been used by many states for missing persons programs
Acknowledgments & Disclaimers

Funding: Interagency Agreement 2008-IJ-R-029 between the National Institute of Justice and NIST Office of Law Enforcement Standards

Points of view are mine and do not necessarily represent the official position or policies of the US Department of Justice or the National Institute of Standards and Technology.

Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology or the National Institute of Justice nor does it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose for which they were used.
Special Circumstances of WTC Samples

• Destructive Energy of 9/11/01 Attack
  – Kinetic energy and fuel load of airplanes
  – Kinetic energy of collapse
• Two Boeing 767 airplanes (fueled with 10,000 gallons each) traveling at 429 to 586 mph
• Two towers 110 floors each, 1362 ft high
• Towers reduced to 70 ft hill, 16 acres, 1.7 million tons debris
• Subterranean fires until December 2001

See also http://wtc.nist.gov

The challenge of an “open” system vs. a “closed” system like an airplane crash

Source: Mecki Prinz (NYC OCME) ISFG presentation, Sept 11, 2003
World Trade Center Victim Identification Efforts

Without DNA
736 Victims Identified
Finished May 2002

Source: Mecki Prinz (NYC OCME) ISFG presentation, Sept 11, 2003
DNA Analysis Requires Comparisons (Unknowns are compared to references samples)

- DNA results from human remains (unknown sample) are compared against DNA results from samples of known origin (reference samples).
- If a match occurs, statistical analysis is performed and a report is issued to the appropriate individual.

Slide courtesy of Dr. Amanda Sozer, SNA International
Number of Remains Identified as of 2004

Statistics (July 26, 2004)

Total Reported Missing: 2,749
Number of Remains: 19,915
Number Identified: 1,560 (5 pending)
Whole Bodies Recovered: 239

WTC MFISys Statistics (4.30.2004)

52,528 STR profiles (including miniSTR data)
31,155 mtDNA sequences
10,799 SNP profiles

Free Press (2005)
Kinship and Data Analysis Panel (KADAP)

- **Developed & funded by the National Institute of Justice**
- Group of ~25 subject matter experts gathered to advise NYC OCME on WTC DNA identification matters
- Met almost every other month for the two years following Sept 11, 2001 usually in DC or NY to review data, make recommendations, and discuss methodologies and innovations
- Prepared a *Lessons Learned document* to aid with future mass disaster DNA identification efforts and published a Nov 2005 article in *Science* on the WTC DNA Identifications

KADAP Leaders

Lisa Forman  
*National Institute of Justice*

Amanda Sozer  
*NIJ Contractor*

Steve Niezgoda  
*NIJ Contractor*
NIJ WTC KADAP
(Kinship and Data Analysis Panel)

- Robert Shaler, Ph.D., Sc.D. **NYC OCME**
- Howard Baum, Ph.D. **NYC OCME**
- Fred Bieber, M.D, Ph.D. **Harvard Med**
- Bruce Budowle, Ph.D. **FBI**
- George Carmody, Ph.D. **Carleton U.**
- Ken Kidd, Ph.D. **Yale**
- Mike Conneally, Ph.D. **Indiana U.**
- Art Eisenberg, Ph.D. **U. North Texas**
- Mark Dale **NY State Police**
- Barry Duceman, Ph.D. **NY State Police**
- Dennis Gaige **NY State Police**
- Steve Swinton **NY State Police**
- Anne Walsh, Ph.D. **NY State Dept Public Health**
- Jack Ballantyne, Ph.D. **U. Central Florida**
- Joan Bailey-Wilson, Ph.D. **NIH**
- Leslie Biesecker, Ph.D. **NIH**
- Lisa Forman, Ph.D. **NIJ**
- Benoit Leclair, Ph.D. **Myriad Genetics**
- Steve Niezgoda, MBA **NIJ Contractor**
- Tom Parsons, Ph.D. **AFDIL**
- Elizabeth Pugh, Ph.D. **NIH/CIDR**
- Steve Sherry, Ph.D. **NIH/NCBI**
- Mandy Sozer, Ph.D. **NIJ Contractor**
- Lois Tully, Ph.D. **NIJ**
- Charles Brenner, Ph.D. **DNA View**
- Mike Hennessy **GeneCode Forensics**
- Judy Nolan, Ph.D. **GeneCode Forensics**
- **John Butler, Ph.D. NIST**

Met in NYC, Albany, DC, Baltimore

A “Lessons Learned” document was published by NIJ in September 2006…
Efforts for WTC Victim Identification Using DNA Testing

Government/Corporate/University Participation

- OCME Staff
- NYSP
- NYPD
- NIJ
- FBI
- NCBI
- NIH
- NIST
- NYSDOH
- AFDIL
- Myriad Genetics
- Bode Technology Group
- Gene Codes Forensics
- Celera Genomics
- Orchid Biosciences
- Johns Hopkins University
- SAIC
- Harvard University
- NYU Med. School
- Columbia Med. School
- Porter-Lee
WTC Lessons Learned

- Available at http://massfatality.dna.gov
- 142 pages
- 14 chapters
- 9 appendices
Because DNA technology is of such interest to the public, there are likely to be many DNA-related questions from the media. To minimize the potential for misunderstandings, there should be a single point of contact between the laboratory and the press, and laboratory staff should be instructed on how to respond if contacted directly by the media. Through press briefings, the laboratory director can help educate the public and manage expectations by providing a realistic picture of what DNA analysis can—and cannot—do.
APPENDIX F

Issues to Consider When Outsourcing Reference Samples

There are many issues a laboratory director must consider when making the decision to send mass fatality samples to an outside vendor for short tandem repeat (STR) analysis testing. This list of issues is not meant to be inclusive; rather, it is offered as a starting point to aid in considering the use of a vendor laboratory to test personal items, reference samples, or remains samples.

- Requirements that changes in the vendor’s key personnel (specific personnel) be approved.
- Protocols and procedures for making analysis of the samples, quality control documents, and validation documentation available for review, inspection, and monitoring, including onsite reviews of the vendor’s facility and records.

WTC KADAP Lessons Learned: http://massfatality.dna.gov (p. 101)
Material Flow Between Laboratories Involved in Processing World Trade Center DNA Samples

Reference Samples (Direct or Relatives)

Victims Remains (Bones & Tissue)

Extract
Swab
Personal Effect
Bone
Tissue
DNA Innovations Used in WTC

- Improved assays to handle degraded DNA
  - miniSTRs (NIST/OhioU → Bode)
  - SNPs (Orchid Cellmark) – used on an experimental basis
  - High-throughput mtDNA CR sequencing (Celera)

- Improved DNA extraction from bone
  - Bode Technology Group (refined AFDIL methods?)

- New or modified software for data analysis
  - M-FISys (Gene Codes Forensics) – created from scratch for WTC
  - MDKAP (Benoit Leclair – Myriad Genetics)
  - DNA-View new module (Charles Brenner – consultant)
Comparison of Full vs. Partial DNA Profiles

DNA size (bp) relative to an internal size standard (not shown)

(a) Full Profile (Reference sample)

(b) Partial Profile (Casework sample)

Loss of signal from larger size PCR products

Bode Technology Group – WTC Phase I summary

- 12,392 Bone samples processed
- 3,405 Full profiles (13 STR loci)
- 2,143 High partial profiles (>7 STR loci)
- 2,670 Low partial profiles (<7 STR loci)
- 4,174 No loci

Reduced-sized PCR products (aka miniSTRs) provide an opportunity to recover this information

>50% of samples tested
**Advantages of Approach:**
Size reduction enhances success rate with degraded DNA
Retains same marker information (database compatibility)
Uses highly polymorphic STR loci (high discriminatory power)

**Conventional STR test** (COfiler kit)

**MiniSTR assay** (using Butler et al. 2003 primers)

This miniSTR is 150 bp smaller
Most of the miniSTR Primers Came from Previous NIJ-Funded Work with Mass Spectrometry

NIJ Funded Project Began June 1997

United States Patent

<table>
<thead>
<tr>
<th>Patent Number:</th>
<th>6,090,558</th>
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<tr>
<td>Date of Patent:</td>
<td>Jul. 18, 2000</td>
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Butler et al.

[54] DNA TYPING BY MASS SPECTROMETRY WITH POLYMORPHIC DNA REPEAT MARKERS

[75] Inventors: John M. Butler, Menlo Park; Jia Li, Union City; Joseph A. Monforte, Berkeley; Christopher H. Becker, Palo Alto, all of Calif.


[21] Appl. No.: 09/157,177

[22]Filed: Sep. 18, 1998


Most of the new miniplex primer sequences had already been described in the NIJ report and US Patent 6,090,558 (originally designed for use with STR typing by mass spectrometry)

http://www.ojp.usdoj.gov/nij/pubs-sum/188292.htm
Brief Timeline on Development of miniSTR Assays

- Project begun in November 2001 at the request of Bob Shaler to aid WTC DNA identifications
- Primers were designed to come as close as possible to the repeat region to generate the smallest possible PCR products for optimal recovery of information from degraded DNA
- Collaboration with Bruce McCord (then at Ohio University)
- Focus was on testing to demonstrate that equivalent genotypes could be produced compared with commercial STR kits
- Information supplied to Bode Technology Group in April 2002 and developed into two “BodePlexes” during summer 2002
- “BodePlexes” were used to increase success rates with bone samples during remaining WTC testing
NIST/OhioU miniplex Primer Sets

Dye combinations were chosen because matrix is commercially available and works well on ABI 310/3100

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<th>6FAM</th>
<th>VIC</th>
<th>NED</th>
<th>PET</th>
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<tr>
<td><strong>Miniplex 1</strong></td>
<td>TH01</td>
<td>CSF1PO</td>
<td>TPOX</td>
<td>D3S1358</td>
</tr>
<tr>
<td><strong>Miniplex 2</strong></td>
<td>D5S818</td>
<td>D8S1179</td>
<td>D16S539</td>
<td>Penta D</td>
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<tr>
<td><strong>Miniplex 3</strong></td>
<td>FGA</td>
<td>D21S11</td>
<td>D7S820</td>
<td>Penta E</td>
</tr>
<tr>
<td><strong>Miniplex 4</strong></td>
<td>VWA</td>
<td>D18S51</td>
<td>D13S317</td>
<td>D2S1338</td>
</tr>
<tr>
<td><strong>Miniplex 5</strong></td>
<td>Penta D</td>
<td>Penta E</td>
<td>D2S1338</td>
<td></td>
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"Big Mini" | TH01, FGA | CSF, D21 | TPOX, D7 |

Testing can be performed in 4-dye or 5-dye combinations using either ROX or LIZ labeled internal size standards

Only Big Mini supplied to OCME per recommendation of KADAP
Comparison: Profiler Plus/COfiler vs. BodePlexes

BodePlex 1

BodePlex 2

D13  D21  TPOX

D7  FGA

D16  CSF  D7  D18

The Bode Technology Group, Inc.
Improved DNA Extraction from Bone Samples

Probably had the biggest impact in improving success rates of DNA information recovery in the end but took a while to develop…
Information Technology Needed

Exhibit 14: Information Technology in a DNA Laboratory

- Sample Accessioning
- Sample Analysis
- Data Interpretation
- Quality Assurance
- Matching and Statistics
- Laboratory Information Management System (LIMS); either commercial or built in-house
- Commercial software created by DNA instrument vendors
- Software to call alleles
- Usually custom software
- CODIS; custom software

WTC KADAP Lessons Learned: http://massfatality.dna.gov (p. 54)
M-FI Sys Software
Mass Fatality Identification System

M-FI Sys worked through a direct match algorithm and helped in collapsing and sorting data sets to obtain identifications.

Howard Cash and Gene Codes Forensics
MDKAP
Mass Disaster Kinship Analysis Program

Software had been developed initially for Swiss Air Flight 111 (RCMP; 1998)

MDKAP performed **kinship analyses** through **pairwise comparisons**

*Benoit LeClair*
*Myriad Genetics*

Now available as **Bloodhound** from Ananomouse


Benoit Leclaire, Ph.D.; Robert Shaler, Ph.D.; George R. Carmody, Ph.D.; Kristilyn Eliason, B.Sc.; Brant C. Hendrickson, M.Sc.; Thad Judkins, B.Sc.; Michael J. Norton, B.Sc.; Christopher Sears, Ph.D.; and Tom Scholl, Ph.D.

Bioinformatics and Human Identification in Mass Fatality Incidents: The World Trade Center Disaster*

DNA-View
(new module for disaster matching)

http://dna-view.com/

DNA View deduced *kinship by pedigree analyses*; re-written to handle large WTC data sets

Charles Brenner
Consultant

Available online at www.sciencedirect.com


Issues and strategies in the DNA identification of World Trade Center victims

C.H. Brenner\textsuperscript{a,}\textsuperscript{*} and B.S. Weir\textsuperscript{b}

\textsuperscript{a}6508 Sobrante Road, Oakland, CA 94611-1123, USA

\textsuperscript{b}Program in Statistical Genetics, Department of Statistics, North Carolina State University, Raleigh, NC 27695-7566, USA

Received 11 November 2002
Benefits of DNA Innovations **Born from WTC**

- **Improved software for missing persons**
  - **OSIRIS** (NIH-developed open-source software) for improved genealogical information to aid in rapid acceptance of data. Achieving quality metrics for example vendor data for reference samples to be accepted or reanalyzed prior to trying to make an identification from an RM sample.


- **Improved DNA assays and extraction**
  - Commercially available miniSTR kits (MiniFiler)
  - New miniSTR loci have led to new European and U.S. core loci (D10S1248 and D2S441)

- **Improved disaster victim identification (DVI) recommendations and preparations**
A number of miniSTR articles have been published based on initial WTC efforts…

John M. Butler, Ph.D.; Yin Shen, Ph.D.; and Bruce R. McCord, Ph.D.

The Development of Reduced Size STR Amplicons as Tools for Analysis of Degraded DNA*

Denise T. Chung, B.S.; Jiří Drábek, Ph.D.; Kerry L. Opel, M.A.; John M. Butler, Ph.D.; and Bruce R. McCord, Ph.D.

A Study on the Effects of Degradation and Template Concentration on the Amplification Efficiency of the STR Miniplex Primer Sets*

Kerry L. Opel, M.A.; Denise T. Chung, Ph.D.; Jiří Drábek, Ph.D.; John M. Butler, Ph.D.; and Bruce R. McCord, Ph.D.

Developmental Validation of Reduced-Size STR Miniplex Primer Sets*

Bruce McCord
NIJ Grant 2002-IJ-CX-K007
MiniFiler™ Kit

Put a face to your cold case
Now Available: Proven miniSTR technology in an easy-to-use kit.

TECHNICAL NOTE

Carolyne R. Hill,¹ M.S.; Margaret C. Kline,¹ M.S.; Julio J. Mulero,² Ph.D.; Robert E. Lagace,² B.A.; Chien-Wei Chang,² Ph.D.; Lori K. Hennessy,² Ph.D.; and John M. Butler,¹ Ph.D.

Concordance Study Between the AmpFISTR® MiniFiler™ PCR Amplification Kit and Conventional STR Typing Kits*

J Forensic Sci. July 2007, Vol. 52, No. 4
Available online at: www.blackwell-synergy.com
At NIST, we expanded to non-CODIS (NC) loci...

**Characterization of New MiniSTR Loci to Aid Analysis of Degraded DNA**

*Michael D. Coble, Ph.D. and John M. Butler, Ph.D.*

**Characterization and performance of new MiniSTR loci for typing degraded samples**

_M.D. Coble, C.R. Hill, P.M. Vallone, J.M. Butler_

_National Institute of Standards and Technology, Biochemical Sciences Division, 100 Bureau Drive, Mail Stop 8311, Gaithersburg, MD, 20899-8311, USA_

**Characterization of 26 MiniSTR Loci for Improved Analysis of Degraded DNA Samples**

_Carolyn R. Hill, M.S.; Margaret C. Kline, M.S.; Michael D. Coble, Ph.D.; and John M. Butler, Ph.D._

**A 26plex Autosomal STR Assay to Aid Human Identity Testing**

_Carolyn R. Hill, M.S.; John M. Butler, Ph.D.; and Peter M. Vallone, Ph.D._
European Use and Promotion of miniSTRs

At concurrent meetings held on 4–5 April, 2005, in Glasgow, UK by the EDNAP and ENFSI groups, as a result of collaborative exercises and a review of the literature, the following recommendations were made:

1. Mini-STRs to be adopted as the way forward to increase both the robustness and sensitivity of analysis.
The April 2011 announcement of new core U.S. loci includes several miniSTR systems:

- **D2S441**
- **D10S1248**
We are better prepared for the future...

DNA Commission of the International Society for Forensic Genetics (ISFG): Recommendations regarding the role of forensic genetics for disaster victim identification (DVI)

M. Prinz\textsuperscript{a,*}, A. Carracedo\textsuperscript{b}, W.R. Mayr\textsuperscript{c}, N. Morling\textsuperscript{d}, T.J. Parsons\textsuperscript{e}, A. Sajantila\textsuperscript{f}, R. Scheithauer\textsuperscript{g}, H. Schmitter\textsuperscript{h}, P.M. Schneider\textsuperscript{i}
Acknowledgments

• Funding from the National Institute of Justice over the years with our many DNA-related projects

• **Bruce McCord & Yin Shen** (then at Ohio University) – initial miniSTR collaborators

• Pete Vallone & Gordon Spangler (NIST) – Orchid SNP validation work

• Mike Coble & Becky Hill (NIST) – 26 new NC miniSTR loci

• Bob Shaler & Lisa Forman – for getting me involved in WTC efforts

• Lisa Forman Neall, Amanda Sozer, & Mecki Prinz – input on slides

• Fellow WTC KADAP members
Thank you for your attention!

Picture taken at Ground Zero on September 10, 2002