Potential Application of Forensic DNA Testing Methods to Cancer Diagnostics

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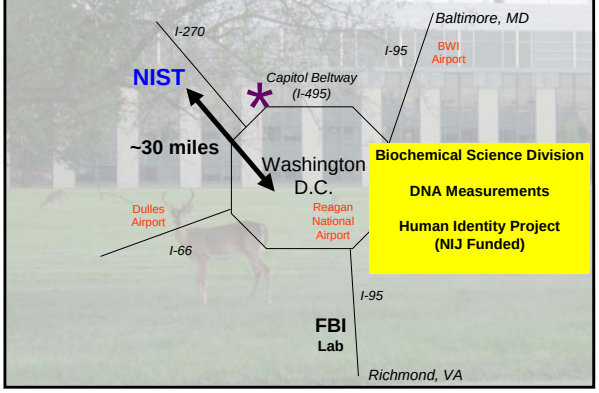
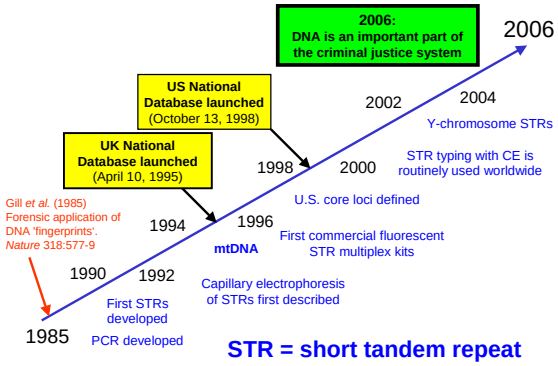
Outline

- STR loci and kits
- Real Time PCR for DNA quantitation
- SNP markers
- Case example
- Interlaboratory Studies
- NIST Standard Reference Materials (SRMs)
- miniSTRs
- Sample Quality Control

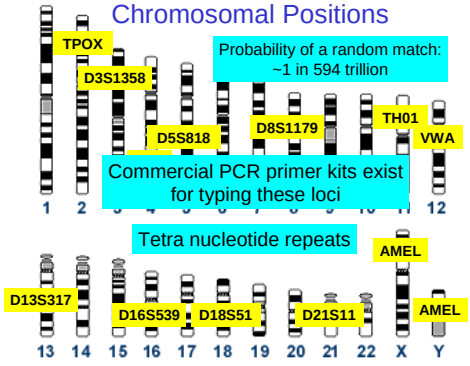
Location of NIST (Gaithersburg, MD)



A Brief History of Forensic DNA Typing



13 CODIS Core STR Loci with Chromosomal Positions



Forensic DNA Testing

- Probe subsets of genetic variation in order to differentiate between individuals
- DNA typing must be done efficiently and reproducibly (information must hold up in court)
- Typically, we are not looking at genes – little/no information about race, predisposal to disease, or phenotypical information (eye color, height, hair color) is obtained

Applications for Human Identity Testing

- Forensic cases - **matching suspect with evidence**
- Paternity testing - **identifying father**
- Historical investigations
- Missing persons investigations
- Mass disasters - **putting pieces back together**
- Military DNA "dog tag"
- Convicted felon DNA databases
- Genealogy (Y-STRs, Y-SNPs, mtDNA)

Common Challenges

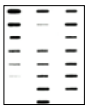
- Sample extraction
- DNA Quantitation (0.5 - 2ng)
- PCR inhibition (humic acid, Ca^{++})
- Degraded Samples (fragmented)
- Low copy number (stochastic effects)
- Contamination
- Mixtures
- Other

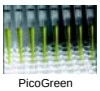
Steps in DNA Analysis

Collection
Extraction
Quantitation
Genotyping
Interpretation of Results
Database
Storage & Searching


Collection & Storage (Blood Stain)

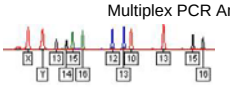

DNA Extraction


Slot Blot


PicoGreen

DNA Quantitation


Multiplex PCR Amplification

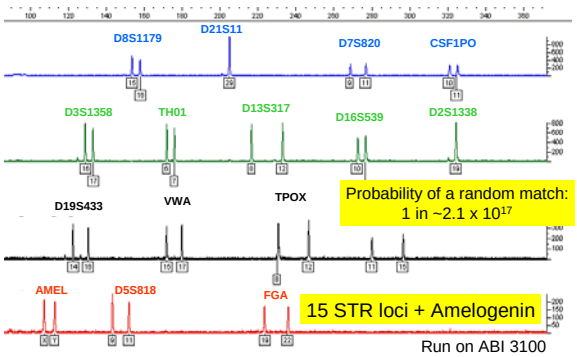

STR Typing

Male: 13,14-15,16-12,13-10,13-15,16
Interpretation of Results


DNA Database

Identifiler STR Kit

Information is tied together with multiplex PCR and data analysis



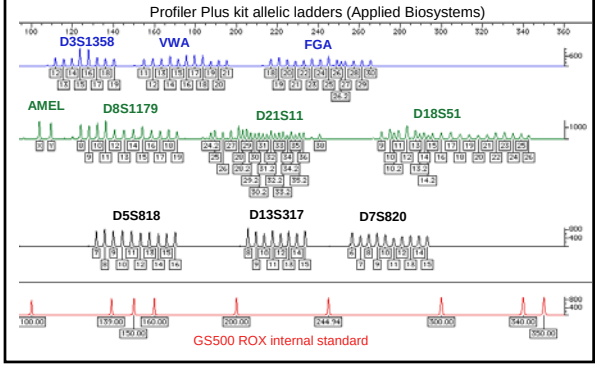
Probability of a random match: 1 in ~2.1 x 10^{17}

15 STR loci + Amelogenin

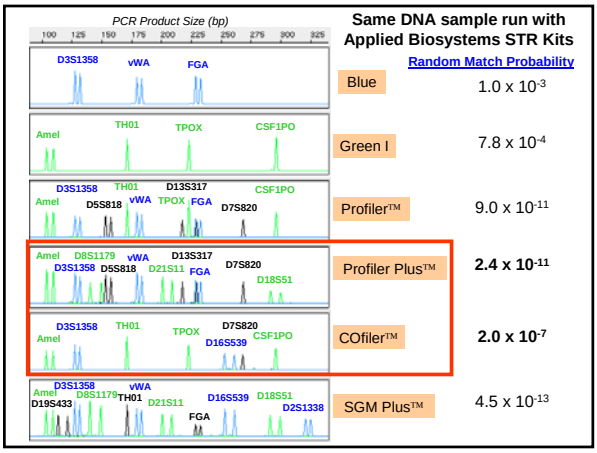
Run on ABI 3100

Companies Supply **Allelic Ladders** in STR Kits to **Aid Interlaboratory Consistency**

Profiler Plus kit allelic ladders (Applied Biosystems)



GS500 ROX internal standard



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Steps in DNA Analysis

Steps in DNA Analysis

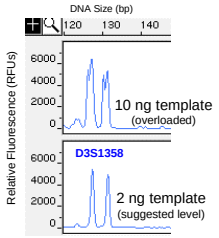
- Collection
- Extraction
- Quantitation
- Genotyping
- Interpretation of Results
- Database
  Storage & Searching

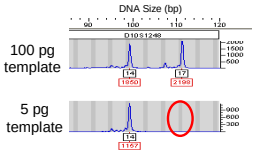
- It is important that the optimal amount of DNA is added to the PCR
- Typically 0.5 ng to 2 ng works well
- Too much or too little DNA will result in artifacts obscuring data interpretation
- DNA can be quantitated using UV spectroscopy, fluorescence (after the addition of an intercalation dye), hybridization with a probe, and qPCR

Impact of DNA Amount into PCR

Reason that DNA Quantitation is Important Prior to Multiplex Amplification

- Too much DNA
  - Off-scale peaks
  - Split peaks (+/-A)
  - Locus-to-locus imbalance
- Too little DNA
  - Heterozygote peak imbalance
  - Allele drop-out
  - Locus-to-locus imbalance





Stochastic effect when amplifying low levels of DNA produces allele dropout

Real Time qPCR

- Various methods/kits exist for qPCR
  - Currently evaluating
- SYBR green and TaqMan
- Single and multi copy loci
- All results rely on a Calibrant of a "known" concentration
- Duplex/Triplex for nuclear, mito, and Y
- We are in the process of developing a DNA quantitation reference material

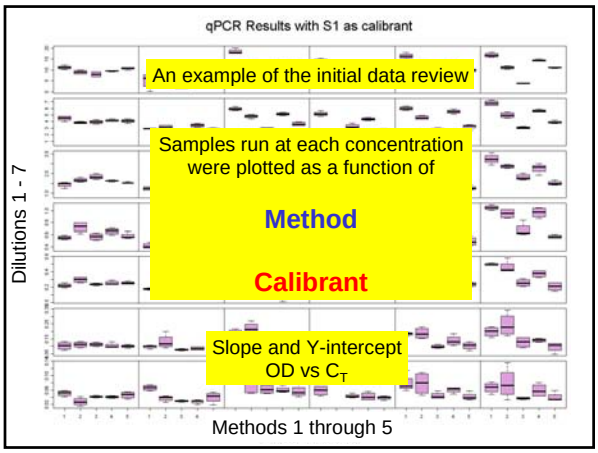
qPCR Methods Evaluated at NIST

- Quantifiler Human (TaqMan MGB)
- Quantifiler Y Male (TaqMan MGB)
- Alu (SYBR Green)
- CA DOJ nDNA (TaqMan BHQ)
- CFS HumTH01 (TaqMan MGB)

1. Quantifiler™ Human DNA Quantification Kit PN4343895
2. Quantifiler™ Y Human Male Quantification Kit PN4343906
3. Nicklas J, Buel E. J Forensic Sci 2003; 48:936-944.
4. Timken M, Swango K, Orrego C, Buoncristiani M. J Forensic Sci, (Sept 2005)
5. Richard ML, Frappier RH, Newman JC. J Forensic Sci 2003;48:1041-1046.

qPCR Methods

Method	Amplicon (bp)	Target
Quantifiler Human	62	Human telomerase reverse transcriptase gene (hTERT), 5p15.33
Quantifiler Y Male	64	Sex determining region Y gene (SRY)
Alu	124	Alu , Ya5 Subfamily (**multi copy**)
CA DOJ	170-190	TH01, 11p15.5
CFS HumTH01	62	Flanking region of TH01, 11p15.5



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SNP Typing Instrumentation

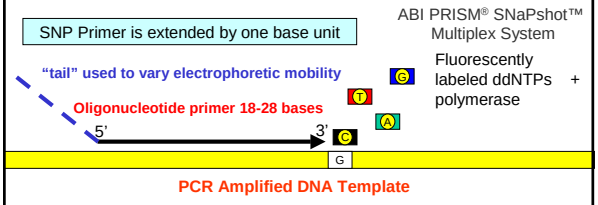
Multi-Color Capillary Electrophoresis (ABI 310 or 3100)

Luminex 100 Flow Cytometer

Time-of-Flight Mass Spectrometer

ABI 7000/7500 SDS

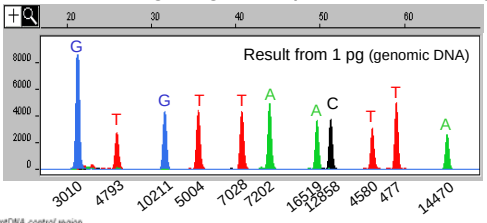
Allele-Specific Primer Extension

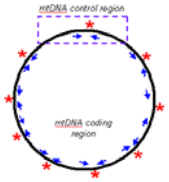


Multiplex PCR is used to amplify 1-2 ng of gDNA

Loci are typed with multiplex primer extension reactions

mtDNA Coding Region 11plex ASPE Assay





11plex PCR and 11plex SNP detection

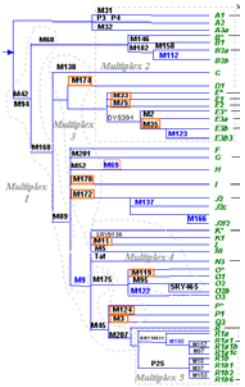
Sites are polymorphic in Caucasians (H1) and useful in resolving most common HV1/HV2 types

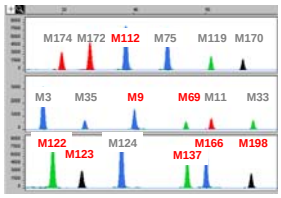
Multiplex PCR used to co-amplify all regions of interest at once

PCR product sizes kept under 200 bp to enable success with degraded DNA samples

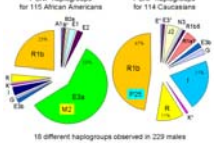
Vallone, P.M., Just, R.S., Coble, M.D., Butler, J.M., Parsons, T.J. (2004) *Int. J. Legal Med.*, 118: 147-157

Development of Y-SNP Assays





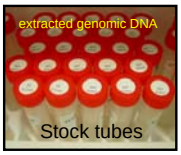
Multiplexing done at both PCR and SNP levels



U.S. Population Dataset

260 Caucasians, 260 African Americans, 140 Hispanics, 3 Asians = **663 males**

DNA extracted from whole blood (anonymous; self-identified ethnicities) received from Interstate Blood Bank (Memphis, TN) and Millennium Biotech Inc. (Ft. Lauderdale, FL)



To date: (>100,000 allele calls)

Identifiler (15 autosomal markers + Amelogenin) (10,608)
Roche Linear Arrays (HV1/HV2 10 regions) (6,630)
Y STRs 22 loci—27 amplicons (17,388)
Y STRs 27 new loci (14,535)
Yfiler kit 17 loci (11,237)
Y SNPs 50 markers on sub-set of samples (11,498)
Orchid 70 autosomal SNPs on sub-set (13,230)
miniSTR testing-new loci and CODIS concordance (9,228)
New miniSTR loci – for 11 loci, 7,293 genotypes
mtDNA full control region sequences by AFDIL

Genotypes with various human identity testing markers

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Case Example: Typing SNPs for a Collaborator

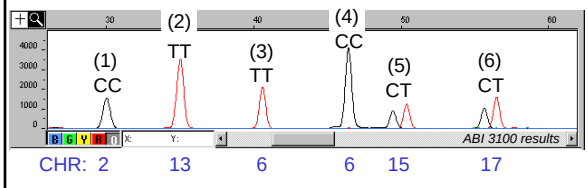
Typing Autosomal SNPs to determine F_{ST} values
- 12-plex assay
- 12 multiplex panels (70 SNPs)

The samples were from different world population groups
- Biaka Pygmy
- Cambodian
- Chinese_Taiwan
- Hausa
- Ibo
- Maya
- Euro_American

Summary of Samples Sent and NIST Sample Handling

- Four 96-well sample plates containing 375 samples
- The samples were rehydrated in 100 μL of nanopure water, centrifuged and allowed to equilibrate overnight
- Samples were handled with care to avoid contamination
- Based on information provided from collaborator, the nominal DNA template concentrations should be 1-2 ng/μL

6-plex SNP Assays



CHR: 2 13 6 6 15 17

Extension primers for 6-plex

(1) TTTTTAGCTCCTAATTTCTTGATGGG
(2) TTTTTTTTTTCATCTGATGCCATGAGAAAGC
(3) TTTTTTTTTTTTTTTGTTCTGCTTTAATACAAAACCAG
(4) TTTTTTTTTTTTTTTTTTTTTTTATAAAGGGCAGAATGAGGATTA
(5) TTTTTTTTTTTTTTTTTTTTTTTTTAGAAAGTATCTTGCAAAAGGTCCA
(6) TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCATAATCACAGCTTTTTTCTCCCAA

Vallone, P.M., Decker, A.E., Butler, J.M. (2005) Allele frequencies for 70 autosomal SNP loci with U.S. Caucasian, African American, and Hispanic samples. *Forensic Sci. Int.* 149: 279-286.

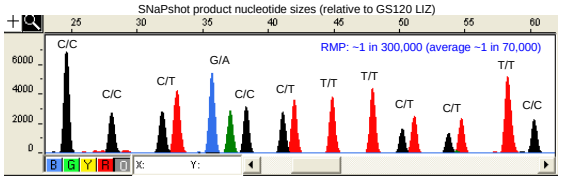
Vallone, *et al.* (2005) *Progress in Forensic Genetics 11, in press*

Autosomal 12plex SNP Assay

12plex PCR and 12plex ASPE from 0.5 ng DNA template

all PCR products <90 bp

SNaPshot product nucleotide sizes (relative to GS120 LIZ)



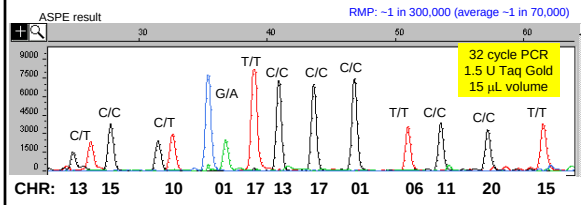
locus:	1	2	3	4	5	6	7	8	9	10	11	12
chr:	5	15	10	1	17	13	17	1	6	11	20	11

"Best" 12 SNPs selected from 70 originally tested;
>0.45 heterozygosity in U.S. Caucasians, Hispanics, and African Americans

Vallone, P.M., Decker, A.E., Butler, J.M. (2005) Allele frequencies for 70 autosomal SNP loci with U.S. Caucasian, African American, and Hispanic Samples. *Forensic Sci. Int.* 149:279-286.

Population data has been collected on >1,000 samples (662 U.S. and 375 world samples) **from 10 different population groups**

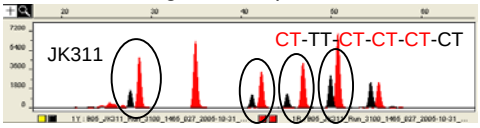
Autosomal 12-plex SNP Assay



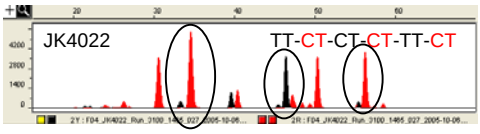
12-plex PCR followed by 12-plex ASPE
Fragments separated on a ABI 3100 in 35 minutes
The 12-plex assay has been run on over 1000 samples
Works well on 0.5 to 1 ng of template

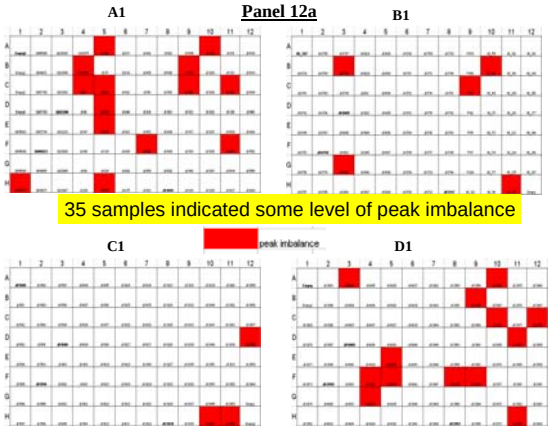
Poor heterozygote balance makes conclusive typing difficult in some of the 6-plex assays

Ambiguous call panel 12a



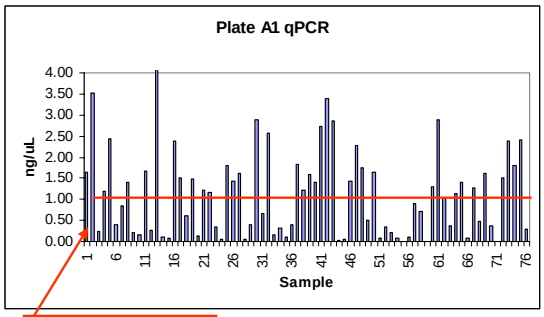
Ambiguous call panel 14a





Overview of Sample Quality and Quantity Issues

- Autosomal STR typing experiments exhibited mixtures that may indicate well to well contamination. Some of the STR profiles indicated a 1:1 mixture ratio.
- In addition, qPCR human DNA quantitation experiments conducted at NIST indicate that the actual concentrations varied from 100 pg to almost 10 ng.
- The low concentration samples are a concern because of the possibility of stochastic imbalance during PCR (allele drop-in or drop-out) and thus potential unreliable SNP results.



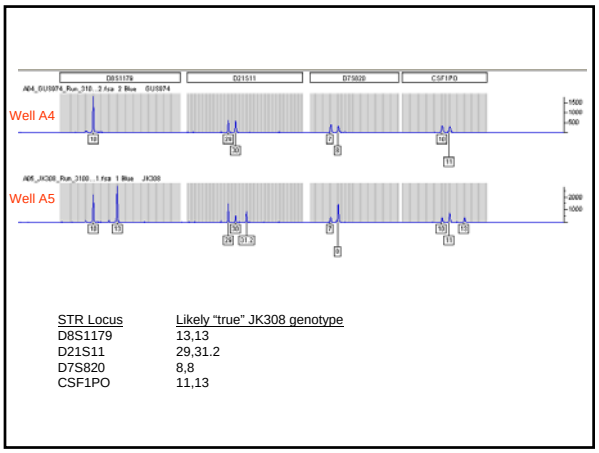
Of the 80 samples examined in Plate A1 with the Alu qPCR human DNA quantitation assay,13 samples were observed with <100 pg/uL DNA rather than the expected 1-2 ng/uL based on the sample information provided.

Autosomal STR Typing Data Confirm the Presence of Mixtures

- Obvious mixtures were identified
- Partial profiles indicate low template concentration
- Low mixtures and low signal/mixtures were also identified

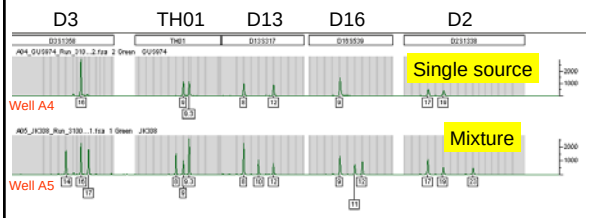
Mixtures (major component) were observed

8 A1 (18 low, 5 possible mixture, 4 low/mixture)
1 B1 (6 low, 0 possible mixture, 8 low/mixture)
2 C1 (2 low, 0 possible mixture, 8 low/mixture)
1 D1 (14 low, 3 possible mixture, 3 low/mixture)

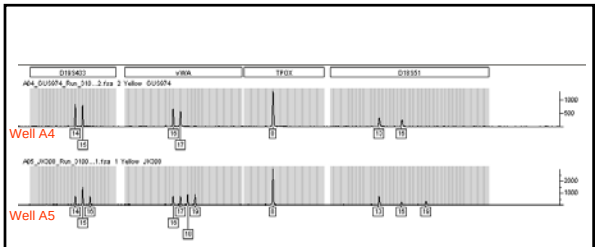


STR Locus	Likely "true" JK308 genotype
D8S1179	13,13
D21S11	29,31.2
D7S820	8,8
CSF1PO	11,13

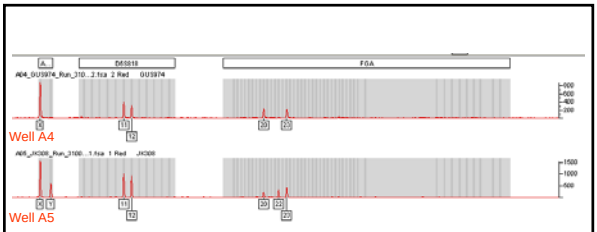
Mixed Sample



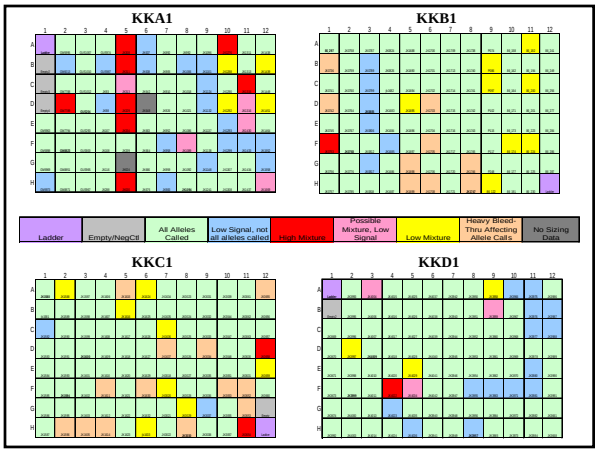
STR Locus	Likely "true" Well A5 genotype
D3S1358	14,17
TH01	8,9.3
D13S317	8,10
D16S539	11,12
D2S1338	17,23



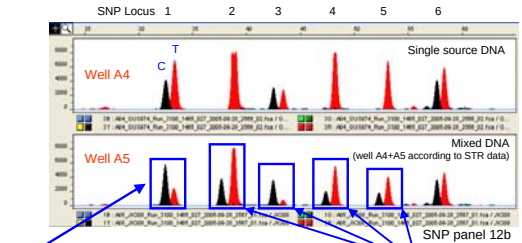
STR Locus	Likely "true" JK308 genotype
D19S433	15,16
VWA	18,19
TPOX	8,8
D18S51	13,19



STR Locus	Likely "true" JK308 genotype
amelogenin	X,Y
D5S818	11,12
FGA	22,23 or 22,22



Challenges Created for SNP Data Evaluation with Mixed Samples

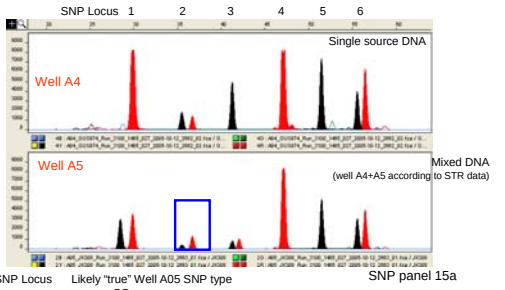


Reversal of a "normal" heterozygote peak height pattern

SNP Locus	Likely "true" Well A05 SNP type
1	CC
2	CC or CT
3	CC
4	CC or CT
5	CC or CT
6	CT

Unusual heterozygote peak height ratios

SNP Panel 15a



SNP panel 15a

SNP Locus	Likely "true" Well A05 SNP type
1	CC
2	**TT**
3	TT
4	TT
5	CC
6	CT

Mixture in well A5 would probably go undetected with this 6plex SNP panel (except for heterozygote peak imbalance at a single locus)

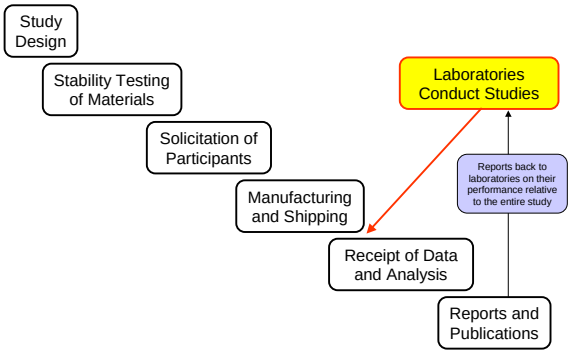
Lessons Learned

- Quantitate all received samples prior to typing
- Run autosomal STRs to confirm that samples are from a single source and are unique
- Resolving mixed samples is time consuming and results are sometimes still ambiguous (SNPs more so than STRs)
- Use caution when running a new assay on samples of unknown quality
- By following this strategy we could have saved time and expense

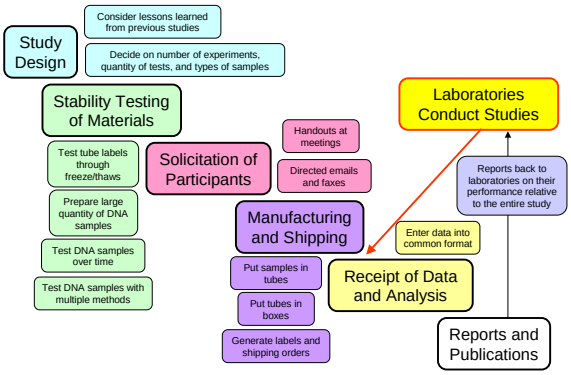
Outline

- STR loci and kits
- Real Time PCR for DNA quantitation
- SNP markers
- Case example
- Interlaboratory Studies
- NIST Standard Reference Materials (SRMs)
- miniSTRs
- Sample Quality Control

Process for Interlaboratory Study

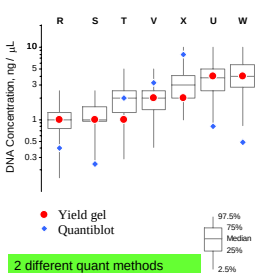


Process for Interlaboratory Study



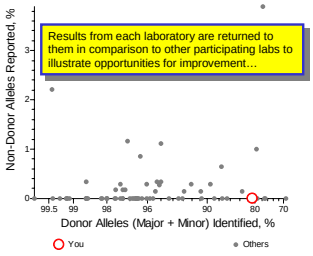
Individual Performance in an Interlaboratory Study

DNA Quantitation



2 different quant methods gave different results; this lab followed the Quantiblot results

Accuracy in STR Typing



See Kline, M.C., *et al.* (2003) *Anal. Chem.* 75: 2463-2469

Outline

- STR loci and kits
- Real Time PCR for DNA quantitation
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- NIST Standard Reference Materials (SRMs)
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- Sample Quality Control

NIST Standard Reference Materials (SRMs)

SRM 2390 - DNA Profiling Standard
Meets RFLP Needs

NIST SRMs are used to help calibrate forensic genotyping laboratories

SRM 2392 - Mitochondrial DNA Standard
Cell Lines and Cloned HV1 Plasmid

SRM 2393 – mtDNA heteroplasmy

SRM 2395 – Y chromosome DNA standards

STRBase

Short Tandem Repeat DNA Internet Database

. . .working with industry to develop and apply technology, measurements and standards

Recent Additions

- **Forensic SNP Information** (will be official site for ISFG SNP information) .../SNP.htm
- **NIST publications** and presentations as pdf files .../NISTpub.htm

We Regularly Update

- Reference List
- Variant Alleles
- Addresses for Scientists
- Links to Other Web Sites
- Y-STR Information

We will continue to add downloadable PowerPoint files that can be used for training purposes

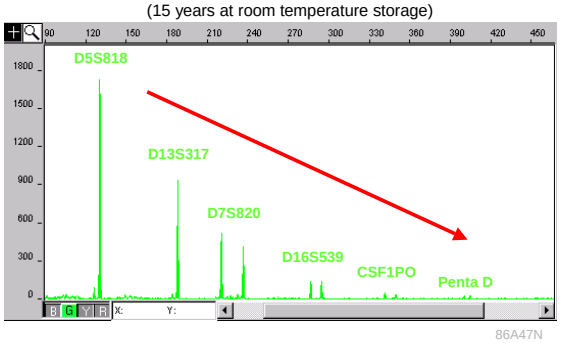
http://www.cstl.nist.gov/biotech/strbase

Outline

- STR loci and kits
- Real Time PCR for DNA quantitation
- SNP markers
- Case example
- Interlaboratory Studies
- NIST Standard Reference Materials (SRMs)
- miniSTRs
- Sample Quality Control

PowerPlex 16 Result on Aged Blood Stain

(15 years at room temperature storage)



Degraded DNA – loss of larger alleles – partial profiles

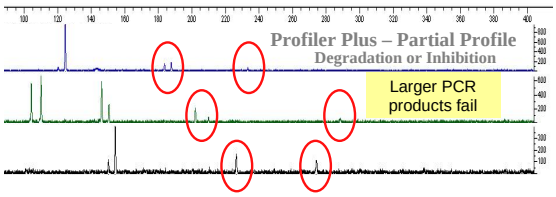
World Trade Center Towers

(Sept 11, 2001)

Wreckage at Ground Zero

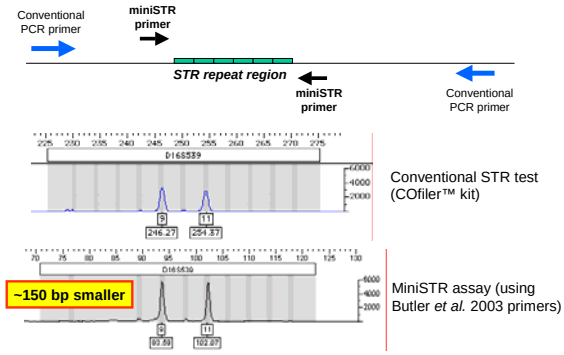
Highly degraded DNA was recovered

World Trade Center – Phase I Summary



	12,392	Bone samples processed
	3,405	Full profiles (13 STR loci)
Over 6800 profiles	2,143	High partial profiles (≥7 STR loci)
	2,670	Low partial profiles (<7 STR loci)
	4,174	No loci

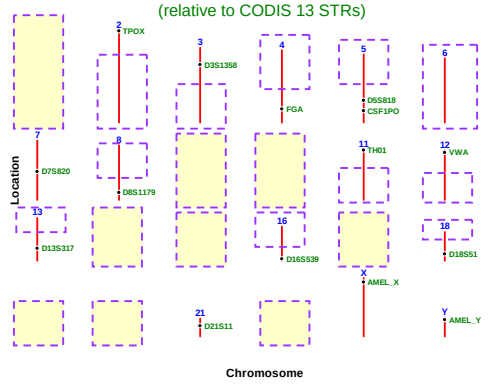
A miniSTR is a reduced size STR amplicon that enables higher recovery of information from degraded DNA samples



Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 7.2, ©Elsevier Science/Academic Press

Potential Locations for New miniSTR Loci

(relative to CODIS 13 STRs)



Characterization of New miniSTR Loci

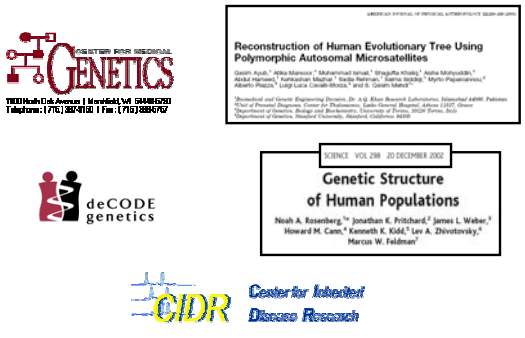
"Computer Work"

Candidate STR marker selection (e.g. Marshfield Clinic Center of Medical Genetics) → Pull down sequence data from the web (e.g. NCBI) → Identify Chromosome Location (e.g. Human BLAT Search) → Screen for PCR Primers (e.g. Primer3) → Test primers for Multiplex-ability (e.g. AutoDimer - NIST)

"Laboratory Work"

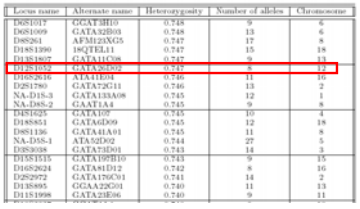
Test Markers on Population samples → Sequence homozygotes to determine allele sizes → Build Macros for Genotyping → Construct Allelic Ladders

Candidate STR marker selection



Characterization of New miniSTR Loci

Rosenberg *et al.* 2002 – 1062 samples; 377 STRs; diverse populations

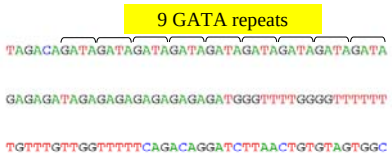


Focus on:

High Heterozygosity
Small # of Alleles
Tetranucleotide Repeats

Identification of PCR Primers

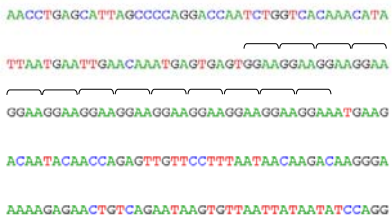
PCR Primer Design



PCR Primer Design

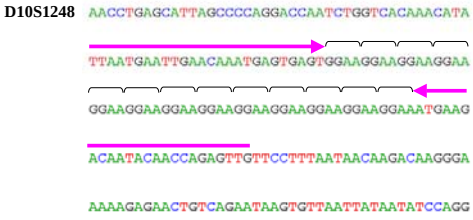
REJECT!

PCR Primer Design



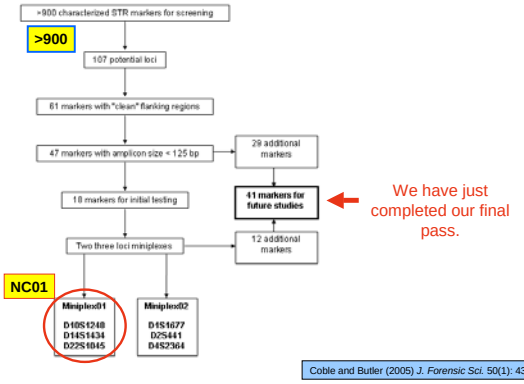
13 GGAA Repeats

PCR Primer Design

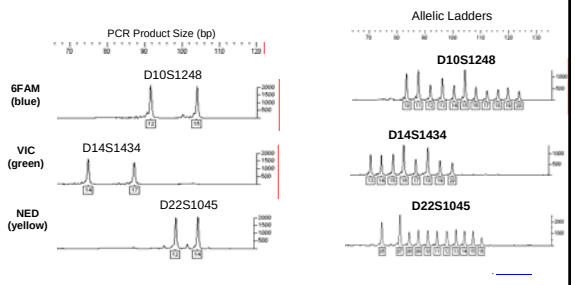


102 bp Amplicon

Initial Testing Results with Potential miniSTR Loci

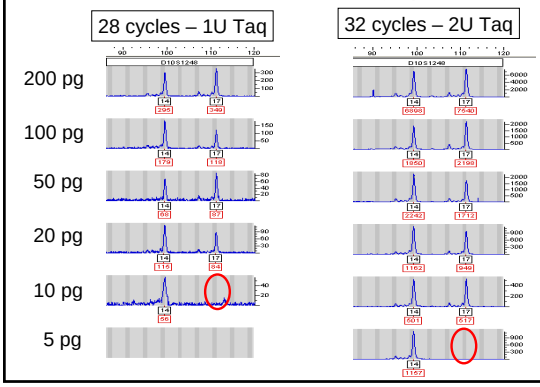


Miniplex "NC01"



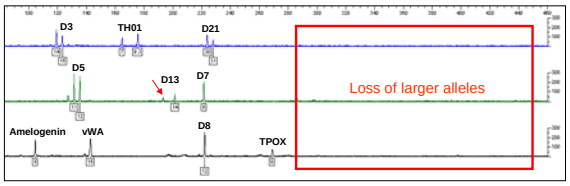
Coble and Butler (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA *J. Forensic Sci.* 50(1): 43-53

miniSTR Assay Sensitivity (D10S1248)



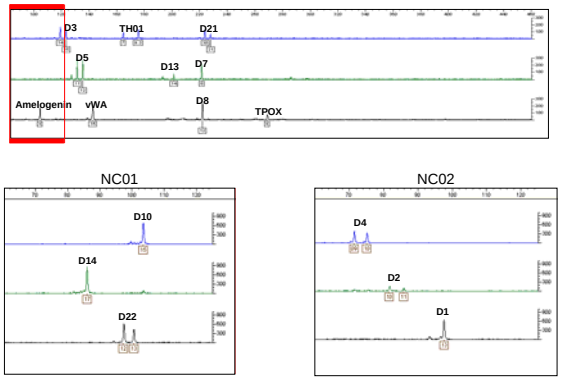
Sensitivity - Degraded DNA from an OU Bone Sample

PP16



10 pg/μL (30pg input DNA), 32 cycles, 2U Taq

Sensitivity - Degraded DNA from an OU Bone Sample



Global Impact of NC miniSTRs

FSI (2006) **156(2):** 242-244

Short communication

The evolution of DNA databases—Recommendations for new European STR loci

Peter Gill [a,*], Lyn Fereday [b], Niels Morling [c], Peter M. Schneider [d]

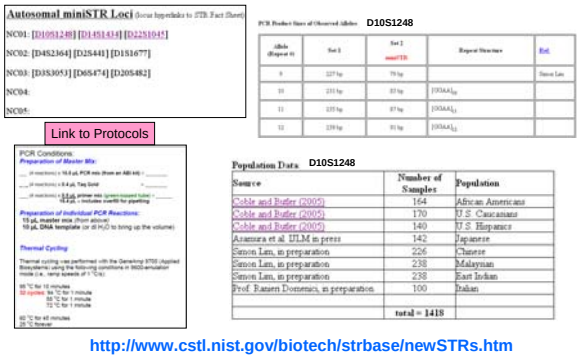
[a] Forensic Science Service, Birmingham, UK
[b] Forensic Science Service, London, UK
[c] Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark
[d] Institute of Legal Medicine, University of Cologne, Germany

Received 25 May 2005; accepted 26 May 2005

...recommended that existing multiplexes are re-engineered to enable small amplicon detection, and that three new mini-STR loci with alleles <130 bp (D10S1248, D14S1434 and D22S1045) are adopted as universal. This will increase the number of European standard Interpol loci from 7 to 10.

(D14 has been replaced with D2S441 from NC02)

Characterization of New miniSTRs



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

Outline

- STR loci and kits
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- miniSTRs
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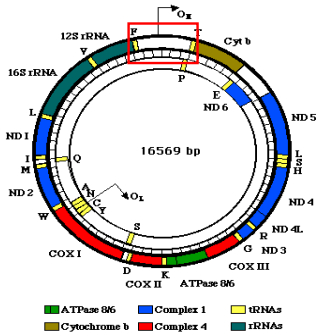
The Mitochondrial Genome



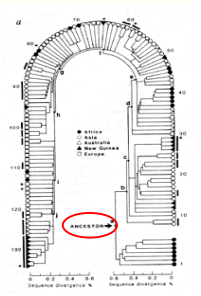
Control Region (D-loop)
~1100 bp – non-coding

Coding region
13 polypeptides
22 tRNAs
2 rRNAs

CRS – Anderson *et al.* (1981)
rCRS – Andrews *et al.* (1999)

http://www.mitomap.org/

mtDNA as a Genetic Marker

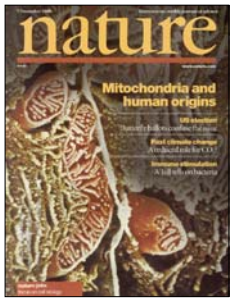


Cann *et al.* 1987

RFLP analysis of 134 mtDNA types from 148 individuals ~ 370 different restriction sites per individual.

"Out of Africa"

mtDNA as a Genetic Marker

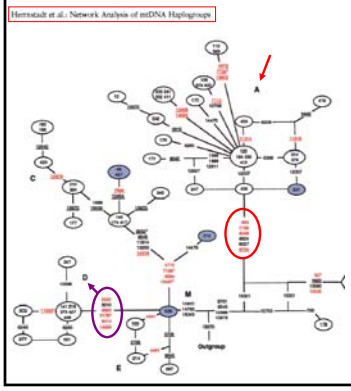


Ingman *et al.* (2000)

53 entire genome sequences from diverse global populations.

Confirmation for OOA.

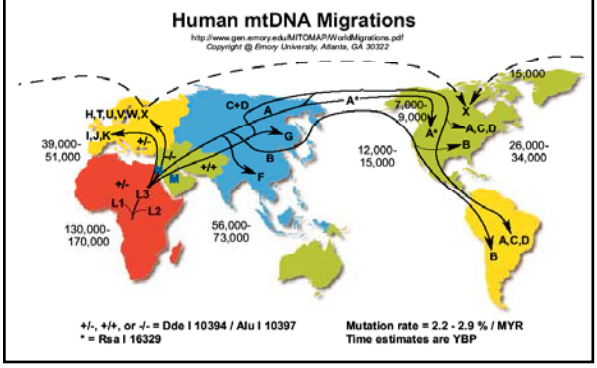
Mitochondrial Haplogroups



Haplogroup - A group of related haplotypes.

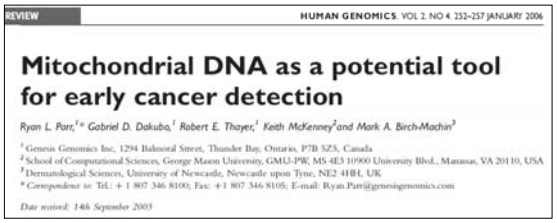
Each haplogroup cluster is defined by a set of specific, shared polymorphisms.

mtDNA Haplogroups (RFLP)



mtDNA as a Forensic Tool

Cases that have utilized mtDNA testing



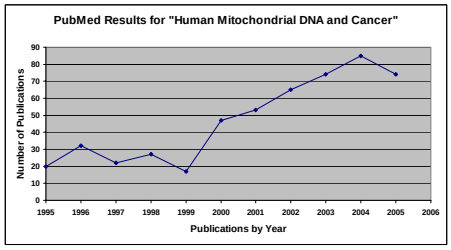
REVIEW HUMAN GENOMICS. VOL 2. NO 4. 252–257 JANUARY 2006

Mitochondrial DNA as a potential tool for early cancer detection

Ryan L. Parr,[1]* Gabriel D. Dakubo,[1] Robert E. Thayer,[1] Keith McKenney[2] and Mark A. Birch-Machin[3]

[1] Genesis Genomics Inc, 1294 Balmoral Street, Thunder Bay, Ontario, P7B 5Z5, Canada
[2] School of Computational Sciences, George Mason University, GMU-PW, MS 4E3 10900 University Blvd., Manassas, VA 20110, USA
[3] Dermatological Sciences, University of Newcastle, Newcastle upon Tyne, NE2 4HH, UK
* Correspondence to: Tel.: + 1 807 346 8100; Fax: +1 807 346 8105; E-mail: Ryan.Parr@genesisgenomics.com

Date received: 14th September 2005



Quality Control of mtDNA Data Using Phylogenetics

- NIST – Early Detection Research Network (EDRN) test site for cancer biomarker evaluation.
- mtDNA as a cancer biomarker.
- Samples from JHU (Patient lung tumor tissue and blood).
- WERB – internal review of manuscripts

Quality Control of mtDNA Data Using Phylogenetics

Blood vs. Tumor from patient 09 had 39 mutational differences!

Position	rCRS	Blood	Tumor
72	T	C	T
6524	T	T	C
9540	T	T	C
10398	A	A	G
12705	C	C	T
15904	C	T	C
16223	C	C	T
16265	A	A	T
16298	T	C	T

Quality Control of mtDNA Data Using Phylogenetics

Blood vs. Tumor from patient 09 had 39 mutational differences!

W. European (haplogroup V)

Position	rCRS	Blood	Tumor
72	T	C	T
6524	T	T	C
9540	T	T	C
10398	A	A	G
12705	C	C	T
15904	C	T	C
16223	C	C	T
16265	A	A	T
16298	T	C	T

African (haplogroup L3)

My Analysis

1) A sample switch occurred.

2) 8 instances of 100% complete mutational reversions affecting only haplogroup V diagnostic mutations AND a simultaneous gain of 31 completely homoplasmic mutations at only the diagnostic polymorphisms associated with the African haplogroup L3.

Decision

1) "IT'S POSSIBLE"

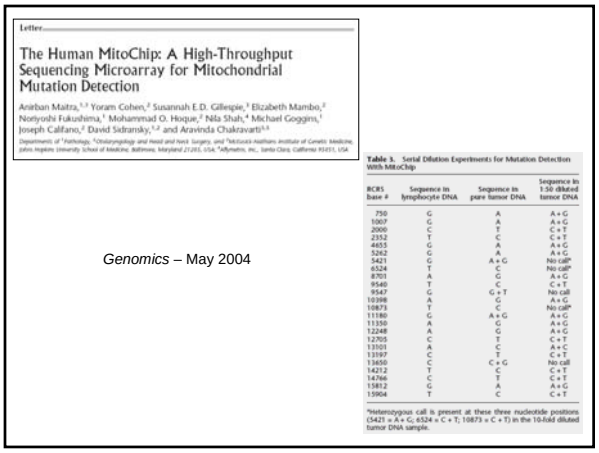
✓ 2) 8 instances of 100% complete mutational reversions affecting only haplogroup V diagnostic mutations AND a simultaneous gain of 31 completely homoplasmic mutations at only the diagnostic polymorphisms associated with the African haplogroup L3.

STR Analysis of Blood and Tumor

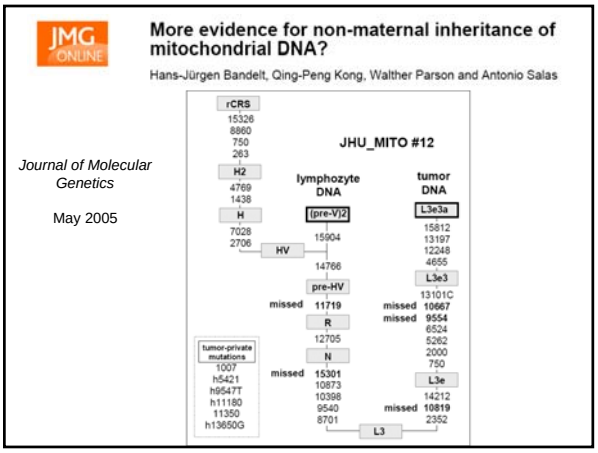
STR Marker	Blood 09	Tumor 09
Amelogenin	X, X	X, Y
D3S1358	16, 17	15, 16
TH01	9, 9	7, 7
D21S11	29, 30	28, 28
D18S51	15, 19	18, 20
Penta E	11, 15	11, 16
D5S818	11, 12	11, 13
D13S317	11, 12	11, 12
D7S820	11, 14	10, 12
D16S539	11, 11	10, 11
CSF1PO	11, 12	10, 11
Penta D	9, 14	9, 14
vWA	17, 17	15, 19
D8S1179	10, 14	13, 14
TPOX	9, 11	8, 11
FGA	22, 23	24, 24

STR Analysis of Blood and Tumor

STR Marker	Blood 09	Tumor 09
Amelogenin	X, X	X, Y
D3S1358	16, 17	15, 16
TH01	9, 9	7, 7
D21S11	29, 30	28, 28
D18S51	15, 19	18, 20
Penta E	11, 15	11, 16
D5S818	11, 12	11, 13
D13S317	11, 12	11, 12
D7S820	11, 14	10, 12
D16S539	11, 11	10, 11
CSF1PO	11, 12	10, 11
Penta D	9, 14	9, 14
vWA	17, 17	15, 19
D8S1179	10, 14	13, 14
TPOX	9, 11	8, 11
FGA	22, 23	24, 24


The Human MitoChip: A High-Throughput Sequencing Microarray for Mitochondrial Mutation Detection

Genomics – May 2004


More evidence for non-maternal inheritance of mitochondrial DNA?

Hans-Jürgen Bandelt, Qing-Peng Kong, Walther Parson and Antonio Salas

Journal of Molecular Genetics

May 2005

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

A call for mtDNA data quality control in forensic science

Yong-Gang Yao[a,1], Claudio M. Bravi[b], Hans-Jürgen Bandelt[c,*]

FBI Forensic mtDNA database - USA.AFR.000942

HV1	HV2
16126-16187-16189-16223-16264 16270-16278-16293-16311-16519	73-249d-263-290d-291d 309.1C-315.1C-489

A call for mtDNA data quality control in forensic science

Yong-Gang Yao[a,1], Claudio M. Bravi[b], Hans-Jürgen Bandelt[c,*]

FBI Forensic mtDNA database - USA.AFR.000942

HV1	HV2
16126-16187-16189-16223-16264 16270-16278-16293-16311-16519	73-249d-263-290d-291d 309.1C-315.1C-489

African haplogroup L1b

Asian haplogroup C1

"Artificial Recombination"

Lessons Learned

- Bandelt et al. (2004), Yao et al. (2004), Salas et al. (2005) – practical suggestions for improved quality control and methods to identify errors in data using the well-established mtDNA phylogeny.
- Increased interactions with experts in the mtDNA world (aDNA, population genetics, etc…)
- Sample quality – LCN, degradation can lead to erroneous results (e.g. artifacts, contamination) – need for improved protocols (controls) and established methods for analyses.

NIST Human Identity Project Team

John Butler
(Project Leader)

Margaret Kline

Pete Vallone

Mike Coble

Dave Duewer
Anal. Chem. Division

Jan Redman

Amy Decker

Becky Hill

Chris DeAngelis
computer programmer

Funding: **Interagency Agreement 2003-IJ-R-029 between National Institute of Justice (NIJ) and NIST Office of Law Enforcement Standards (OLES)**