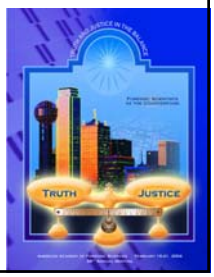
SNPs and Strips:

Approaches to Rapid Screening of mtDNA types

John Butler

AAFS Workshop:
Forensic Human Mitochondrial DNA Analysis
February 16, 2004



Presentation Outline

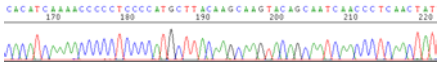
Advantages to Screening Methods

SNPs
- Uses for mtSNPs
- Methodologies for SNP Typing
- SNP Assay Design for SNaPshot

Strips
- SSO Probe History and Chemistry
- Results with Roche LINEAR ARRAYs

Disadvantages to Sequencing

- Expensive
  - Primarily due to intensive labor in data analysis
- Error possibilities with more data to review
- Most information is not used



Review forward and reverse sequences across 610 bases only to report…

263G, 315.1C **Most common type: found in ~7% of Caucasians…**

Advantages to Screening Methods

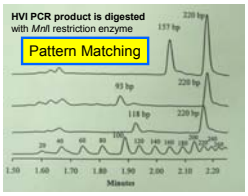
- Rapid results
- Aids in exclusion of non-matching samples
- Less labor intensive
- Usually less expensive
- Permits more labs to get involved in mtDNA

Screening assays are essentially a presumptive test prior to final confirmatory DNA sequencing.

Sequencing is necessary to certify that every position matches between a question and a known sample.

Two Examples of mtDNA Screening Methods

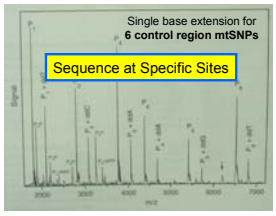
Butler *et al.* (1998) *Electrophoresis* 19:119-124
mtDNA typing with PCR-RFLP and CE-LIF



3 different mtDNA sequences can be distinguished from one another

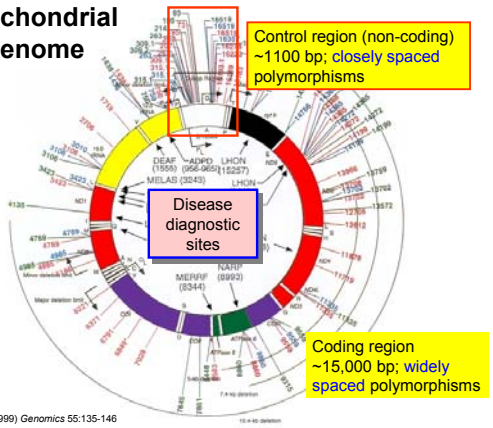
DNA sizes (bp) after *MnlI* digestion
220-35-**157**-28-3
220-35-39-**93**-3-22-28-3
220-35-39-**118**-28-3

Li, Butler, *et al.* (1999) *Electrophoresis* 20:1258-1265
mtDNA SNP typing with TOF mass spectrometry



HV1	HV2
H**16069**	L00**146**
H**16311**	H00**152**
	L00**195**
	H00**247**

Mitochondrial Genome



Levin *et al* (1999) *Genomics* 55:135-146

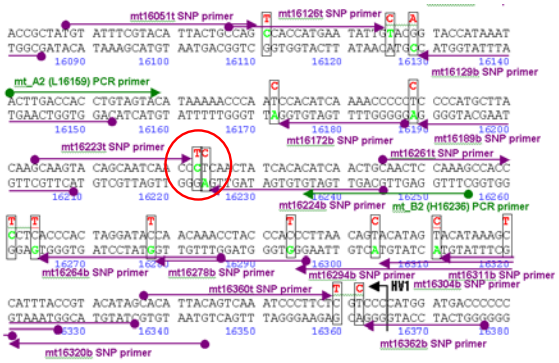
Control Region (16024-576) SNPs

- 1,122 nucleotide positions; typically only 610 bases analyzed (HVI: 16024-16365; HVII: 73-340)
- Challenges with typing closely spaced SNPs
  - Probes are disrupted by neighboring polymorphism(s)

Coding Region (577-16023) SNPs

- 15,446 nucleotide positions
- Challenges with typing widely spaced SNPs
  - Multiplex PCR required
- Polymorphisms may have medical significance

Closely Spaced Polymorphisms Make Assay Design Challenging for Control Region Mitochondrial SNPs

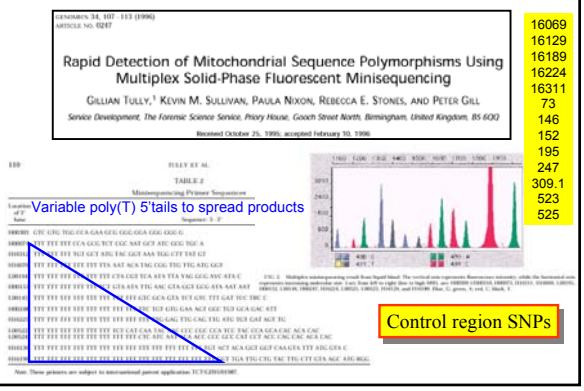


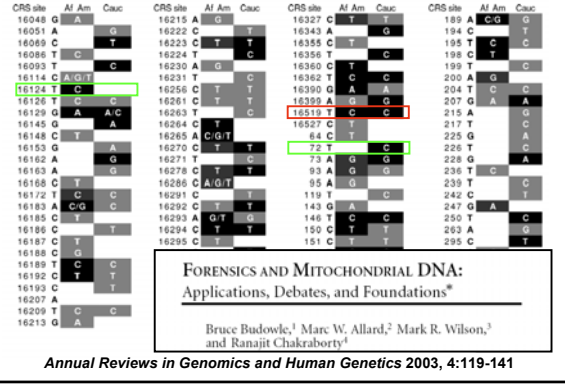
SNPs

Use of mtSNPs

- Rapid screen with informative control region SNPs
  - Minisequencing
  - Tully *et al.* (1996) *Genomics* 34: 107-113
  - Budowle *et al.* (2003) *Annu. Rev. Genomics Hum. Genet.* 4:119-141
- Quality control for control region sequence data with informative coding region SNPs
  - Coding region SNPs used to classify haplogroup
  - Brandstätter *et al.* (2003) *Int. J. Legal Med.* 117: 291-298
  - Allard *et al.* (2002) *J. Forensic Sci.* 47(6): 1215-1223
- Aid resolution of most common HV1/HV2 sequence types with coding region SNPs to improve forensic discrimination
  - Full mtGenome sequencing to find optimal SNP markers
  - Parsons and Coble (2001) *Croat. Med. J.* 42(3): 304-309
  - Coble *et al.* (2004) *Int. J. Legal Med., in press*
  - Vallone *et al.* (2004) *Int. J. Legal Med., in press*

Early Multiplex SNP Detection Work





Use of Haplogroup Defining mtSNPs

Int J Legal Med (2003) 117 : 291–298
DOI 10.1007/s00414-003-0395-2

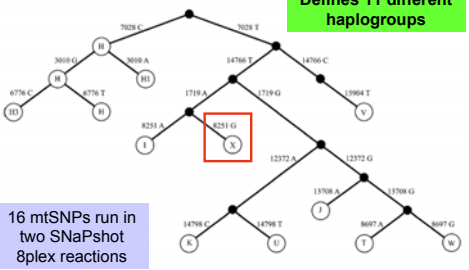
ORIGINAL ARTICLE

Anita Brandstätter · Thomas J. Parsons · Walther Parson

Rapid screening of mtDNA coding region SNPs for the identification of west European Caucasian haplogroups

Defines 11 different haplogroups

G709A
G1719A
A1811G
G3010A
T6365C
T6776C
C7028T
G8251A
G8697A
G9055A
A11251G
G12372A
G13708A
C14766T
T14798C
C15904T



16 mtSNPs run in two SNaPshot 8plex reactions

Haplogroups Defined by Control Region mtSNPs in SWGDAM Caucasian Samples (n = 1771)

Allard *et al.* (2002) *J. Forensic Sci.* 47(6):1215-1223

- **H** (46%): 73A
- **U** (15.6%): 16270T
- **T** (10.5%): 16126C, 16294T
- **J** (10%): 16069T, 16126C, 295T
- **K** (8.9%): 16224C, 16311C
- **I** (2%): 16223T, 199C, 204C, 250C
- **V** (1.9%): 16298C, 72C
- **W** (1.9%): 16223T, 189G, 195C, 204C, 207A
- **X** (1.6%): 16189C, 16223T, 16278T, 195C
- **M** (1.9%): 16223T, 16298C

If a **G is observed at 8251**, then the sample can be classified as a member of **haplogroup X** so the following control region SNPs should be expected: 16189C, 16223T, 16278T, 195C

Efforts with Coding Region Sequencing
applied to human identity testing

- Tzen *et al.* (2001) *Forensic Sci. Int.* 120:204-209
  - Nucleotide positions 8389 to 8865 (portions of ATP8, ATP6)
  - 119 Chinese individuals
- Andreasson *et al.* (2002) *Biotechniques* 32:124-133
  - Pyrosequencing for 11 coding region reactions
  - 190 Swedish individuals
- Lee *et al.* (2002) *Int. J. Legal Med.* 116:74-78
  - mtCyt B
  - 98 Korean individuals
- Lutz-Bonengel *et al.* (2003) *Int. J. Legal Med.* 117:133-142
  - mtATP6, mtATP8, mtND4
  - 109 German individuals
- Poetsch *et al.* (2003) *Mitochondrion* 3:133-137
  - Nucleotide positions 8306 to 9021 (portions of tRNA K, ATP8, ATP6)
  - 180 German individuals
- Coble *et al.* (2004) *Int. J. Legal Med., in press*
  - **241 complete mtGenomes from 18 most common Caucasian HV1/HV2 types**

How many SNPs found in each study?

Recent Efforts with Whole mtGenome Analysis

- Ingman *et al.* (2000) *Nature* 408:708-713
  - **53 mtGenomes** from diverse worldwide origin
  - GenBank AF346963-AF347015
- Maca-Meyer et al. (2001) BMC Genetics 2:13
  - **33 mtGenomes** from diverse worldwide origin
  - GenBank AF381981-AF382013
- Herrnstadt *et al.* (2002) *Am. J. Hum. Genet.* 70:1152-1171
  - **560 mtGenomes (coding region only)** from major African, Asian, and European origins
  - See MitoKor website for sequences; http://www.mitokor.com
- Kong *et al.* (2003) *Am. J. Hum. Genet.* 73:671-676
  - **48 mtGenomes** from East Asian lineages
  - GenBank AY255133-AY255180
- Ingman et al. (2003) *Genome Res.* 13:1600-1606
  - **52 mtGenomes** from Australian and New Guinean Aborigines and Polynesians
  - GenBank AY289051-AY289102
- Coble *et al.* (2004) *Int. J. Legal Med., in press*
  - **241 mtGenomes** from most common Caucasian types
  - GenBank AY495090-AY495330

68 PCR rxn
136 seq rxn
Herrnstadt *et al.* (2002)

Approximately 1,000 full mtGenomes have been sequenced

Strategies for Whole mtGenome Analysis

58 PCR rxn
116 seq rxn
Levin *et al.* (1999)

24 PCR rxn
48 seq rxn
Rieder *et al.* (1998)
Ingman *et al.* (2000)

18 PCR rxn
36 seq rxn
Aldridge *et al.* (2003)

12 PCR rxn
95 seq rxn
Coble *et al.* (2004)

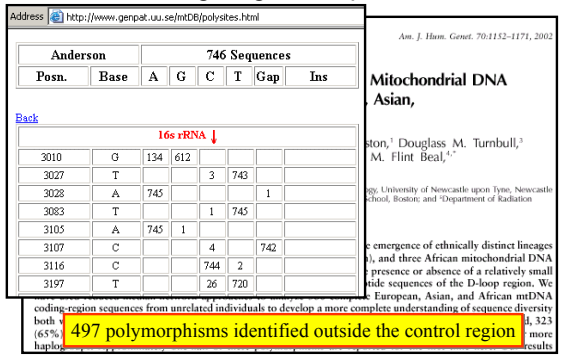
32 PCR rxn
64 seq rxn
Maca-Meyer *et al.* (2001)

15 PCR rxn
47 seq rxn
Kong *et al.* (2003)

Publication of 560 Complete mtDNA Coding Region Sequences

Address http://www.genpat.uu.se/mtDB/polysites.html

Anderson		746 Sequences					
Posn.	Base	A	G	C	T	Gap	Ins
Back							
		16s rRNA ↓					
3010	G	134	612				
3027	T			3	743		
3028	A	745				1	
3083	T			1	745		
3105	A	745	1				
3107	C			4		742	
3116	C			744	2		
3197	T			26	720		

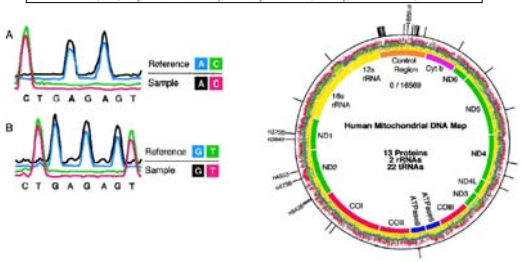


Blazej, *et al.* (2003) *Genome Res.* 13:287-293

Polymorphism Ratio Sequencing: A New Approach for Single Nucleotide Polymorphism Discovery and Genotyping

Robert G. Blazej,[1] Brian M. Paegel,[2] and Richard A. Mathies[1,2,3]

[1]University of California, Berkeley/University of California, San Francisco Joint Bioengineering Graduate Group, Berkeley, California 94720, USA; [2]Department of Chemistry, University of California, Berkeley, California 94720, USA



30 minutes to sequence an entire mtGenome compared against another sample

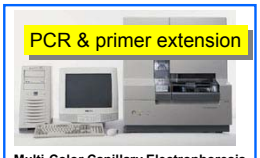
Methodologies for SNP Typing

High-tech
- **SNaPshot (minisequencing)**
- Luminex 100 allele-specific hybridization
- Pyrosequencing
- TaqMan
- Primer extension with time-of-flight mass spectrometry
- TagArray (SNPstream UHT)
- Affymetrix hybridization chip

Low tech
- **Reverse dot blot (LINEAR ARRAYs)**
- PCR-RFLP
- Allele-specific PCR

See Budowle *et al.* (2004) *Forensic Sci. Rev.* 16:21-36 for a review of some SNP typing technologies

SNP Typing Instrumentation at NIST



Multi-Color Capillary Electrophoresis (ABI 310 or 3100)

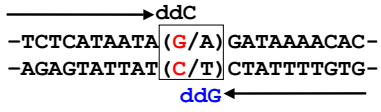
Luminex 100 Flow Cytometer

Time-of-Flight Mass Spectrometer

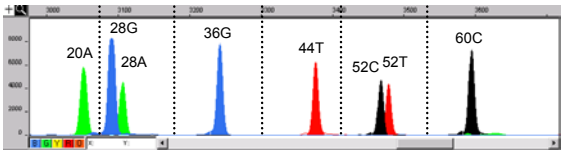
ABI 7000 SDS

SNP Extension Primer Design

- Must anneal to DNA template with 3'end of primer next to SNP site
- Can anneal to either top strand or bottom strand
- Should have uniform annealing temperature (by lengthening 5'end of SNP primer)
- Should not form significant hairpins or dimers with other SNP or PCR primers



Detection of SNPs with ABI 310/3100



SNaPshot™ CEPH Control Reaction

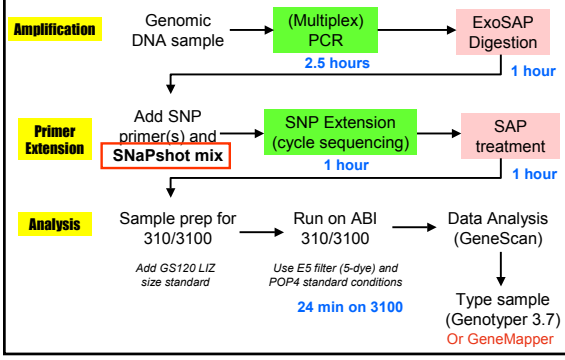
20 nucleotides — ddA
36 nucleotides — ddG
44 nucleotides — ddT
60 nucleotides — ddC

Multiplexing possible by use of different length primers

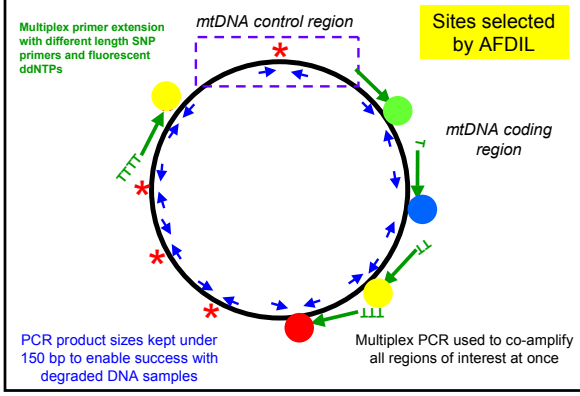
Poly(T) tail or non-nucleotide linker to aid separation

Priming site

Protocol with SNaPshot™ "Kit"



Coding Region mtSNP 11plex Assay



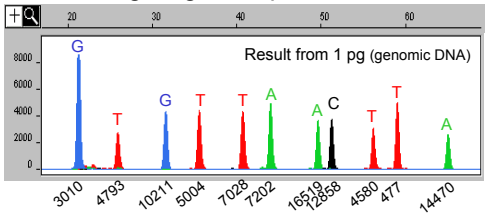
The use of "tailed" SNP primers allows for multiplexing in the SNaPshot assay

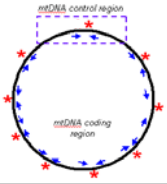
Sequences for 11 SNP primers

TGTTGGATCAGGACATCCC	19/19
TTTTTCAGAAGTGAAAGGGGGC	18/22
TTTTTTTTTACTAAGAAGAATTTTATGGA	20/30
TTTTTTTTTTTTTAGACCCAGCTACGCAAAATC	20/34
TTTTTTTTTTTTTTTTTGACACGTACTACGTTGTAGC	20/38
TTTTTTTTTTTTTTTTTTTTCCACAACACTTTCTCGGCCT	20/42
TTTTTTTTTTTTTTTTTTTTTTTGTGGGCTATTTAGGCTTTATG	22/46
TTTTTTTTTTTTTTTTTTTTTTTTTTGCAGCCATTCAAGCAATCCTATA	23/50
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTGGTTAGAACTGGAATAAAAGCTAG	25/54
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCCCTCCCACTCCCATACTAC	20/58
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGGGAATGATGGTTGTCTTTGG	21/62

Template binding sequence – black
Tailed sequence for fragment separation - red

mtDNA Coding Region 11plex SNaPshot 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

Strips

Roche LINEAR ARRAYS
(a.k.a. reverse dot blots)

Commercial kit so SNP selection and assay design is already done for you…

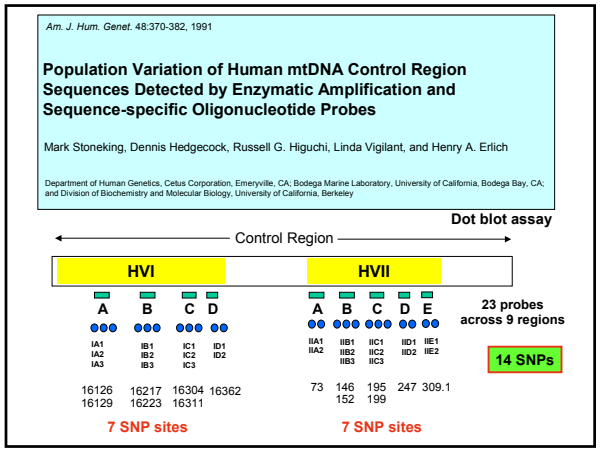
Mito "Strips"

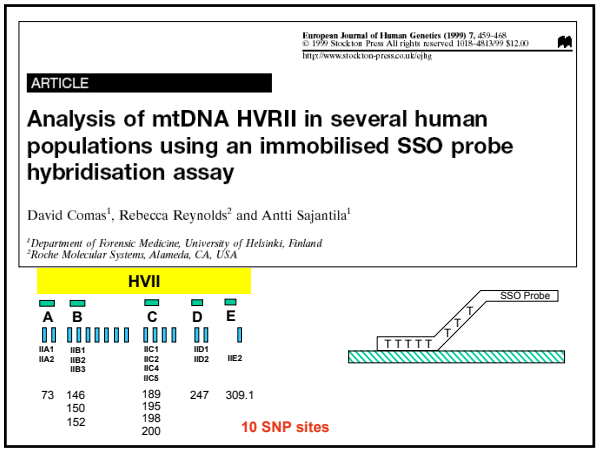
- Roche Applied Science (Indianapolis, IN) recently released a mtDNA typing kit
- LINEAR ARRAY Mitochondrial DNA HVI/HVII Region-Sequence Typing Kit
- Cat. No. 03 527 867 001
- Cost $1500 for 50 reactions
- NIST was involved in beta-testing and performed a population study with these LINEAR ARRAYs

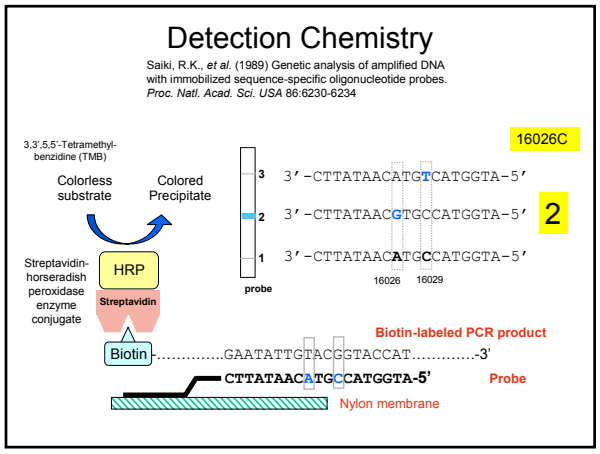
Previous Publications on mtDNA Typing Assays with SSO Probes (dot blot, reverse dot blot, linear arrays)

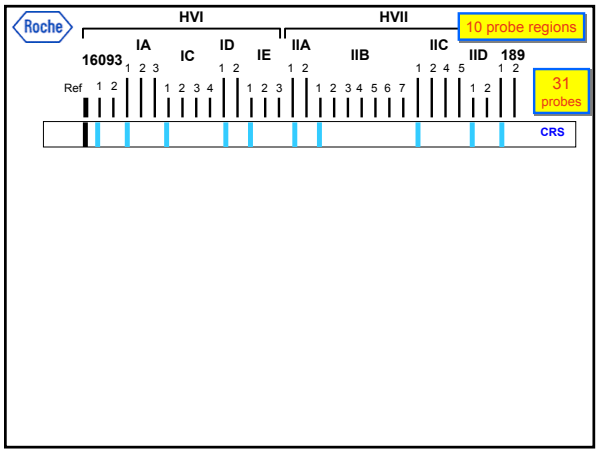
- Stoneking *et al.* (1991) Population variation of human mtDNA control region sequences detected by enzymatic amplification and sequence-specific oligonucleotide probes. *Am. J. Hum. Genet.* 48:370-382
- Skowasch, K., *et al.* (1994) Development of PCR-based reverse dot-blot typing system for the control region of mtDNA. *Proceedings of the Fifth International Symposium on Human Identification*, Madison, WI: Promega, p. 127.
- Comas, D., *et al.* (1999) Eur. J. Hum. Genet. 7:459-468
- Calloway, C.D., *et al.* (2000) *Am. J. Hum. Genet.* 66:1384-1397
- Reynolds, R., *et al.* (2000) *J. Forensic Sci.* 45(6):1210-1231
- Gabriel, M.N., *et al.* (2001) *Croatian Medical Journal* 42(3):328-335
- Gabriel, M.N., et al. (2003) *Croatian Medical Journal* 44(3):293-298
- Calloway, C., et al. (2003) Validation of the LINEAR ARRAY Mitochondrial DNA HVI/HVII Region-Sequence Typing kit. *Proceedings of the 14th International Symposium on Human Identification*.
- Calloway, C., et al. (2003) Applications of the LINEAR ARRAY Mitochondrial DNA HVI/HVII Region-Sequence Typing kit. *Proceedings of the 14th International Symposium on Human Identification*.
- Kline, M.C., et al. (2003) Semi-automation of mtDNA arrays: results from 666 population samples and comparisons. *Proceedings of the 14th International Symposium on Human Identification*.

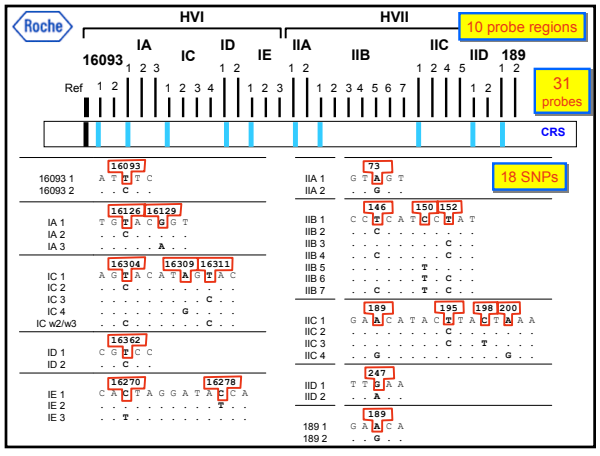
Terry Melton population studies…











Lessons Learned with LINEAR ARRAYS

- pH is important to wash solutions
  - If above protocol's pH 7.4, then the blue color will not develop correctly and signal will be lost
- Quantification value obtained on Agilent is not equivalent to yield gel information provided by Roche
  - May need to make adjustments—do a sensitivity titration in your lab

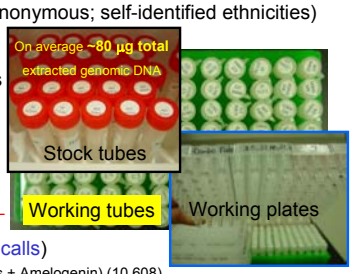
NIST U.S. Population Samples

As of 06/2003 666 males (anonymous; self-identified ethnicities)

286 Caucasians
252 African Americans
128 Hispanics

Whole blood received from Interstate Blood Bank (Memphis, TN)

Working tubes/plates 1 ng/uL



To date: (~50,000 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 SNPs 50 markers on sub-set of samples (11,498)

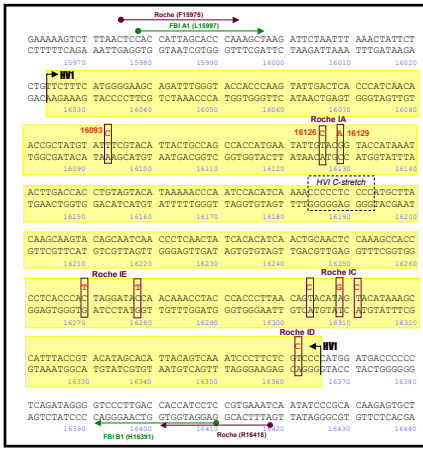
Samples supplied to **OhioU** for miniSTR typing and **AFDIL** for whole mtGenome sequencing

PCR Amplification

- Protocol calls for 5 pg input DNA (based on nuclear DNA measurement)
- We used 1 ng DNA instead and reduced PCR cycle number from 34 (protocol) to 28
- Thermal cycling on GeneAmp 9700:
  - 94 ºC for 14 minutes
  - **28 cycles**: 92 ºC for 15s, 59 ºC for 30s, and 72 º C for 30s
  - 72 ºC for 10 minutes
  - hold at 10 ºC
- 50 uL PCR volume (protocol) with duplex amplification

HVI primers amplify a **444 bp PCR product**:
Forward (L15975-15993) 5'-biotin-CTCCACCATTAGCACCCAA-3'
Reverse (H16418-16401) 5'-biotin-ATTTCACGGAGGATGGTG-3'

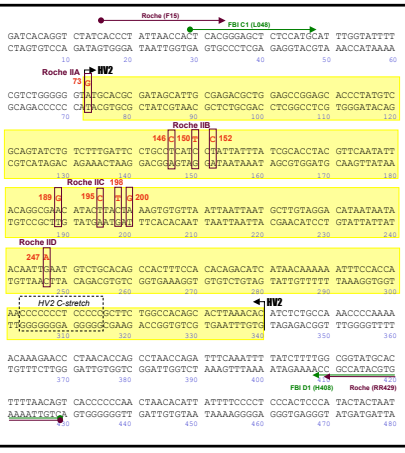
HVII primers amplify a **416 bp PCR product**:
Forward (L15-34) 5'-biotin-CACCCTATTAACCACTCACG-3'
Reverse (H429-410) 5'-biotin-CTGTTAAAAGTGCATACCGC-3'



Hypervariable Region I
16024-16365
342 bp examined

SSO Probes
16093
16126
16129
16270
16278
16304
16309
16311
16362

Only 9 sites examined

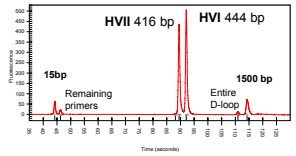


Hypervariable Region II
73-340
268 bp examined

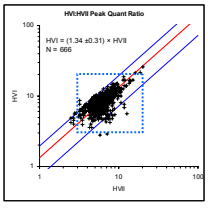
SSO Probes
73
146
150
152
189
195
198
200
247

Only 9 sites examined

Analysis of HVI/HVII PCR Products



Agilent 2100 Bioanalyzer sized and quantified HVI/HVII products



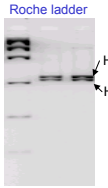
Usually 2-20 ng/uL obtained for each amplicon

Aimed for 50 ng on each mito strip

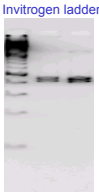
12 samples can be run in ~45 minutes

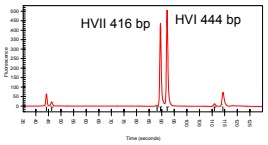
Gel Results

Roche ladder

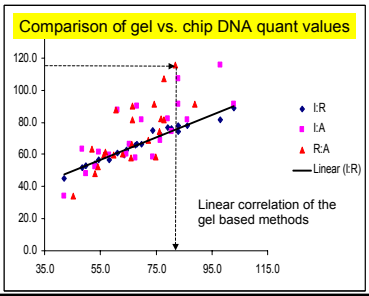


Invitrogen ladder



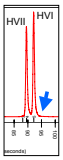


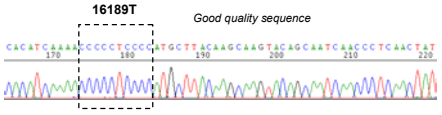
Comparison of gel vs. chip DNA quant values

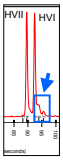


Linear correlation of the gel based methods

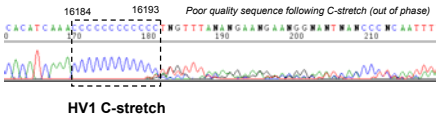
Analysis of HVI/HVII PCR Products







The Agilent electropherogram also gives an indication of the HVI "C-stretch" by the presence of extra peaks (see Butler *et. al.* (1998) *Electrophoresis* 19, 119-124).



Automated Washing and Color Development

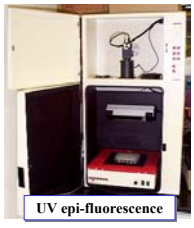
Tecan Profiblot – processes sample through wash steps

24 strips per run
~2 hours per run
2 or 3 runs easily performed per day

Step	File	Time	Temp	Solution
1	Temp		55 °C	
2	Disp		55 °C	Wash
3	Pause		55 °C	
4	Inc	15 min	55 °C	
5	Asp		55 °C	
6	Disp		55 °C	Wash
7	Asp		55 °C	
8	Disp		55 °C	SA-HRP Conjugate
9	Inc	5 min	55 °C	
10	Asp		55 °C	
11	Disp		55 °C	Wash
12	Asp		55 °C	
13	Disp		55 °C	Wash
14	Inc	12 min	55 °C	
15	Asp		55 °C	

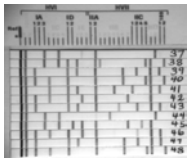
16	Cool		25 °C	
17	Disp		25 °C	Wash
18	Asp		25 °C	
19	Disp		25 °C	Citrate
20	Inc	5 min	25 °C	
21	Asp		25 °C	
22	Disp		25 °C	Color Dev
23	Inc	15 min	25 °C	
24	Asp		25 °C	
25	Disp		25 °C	DI Water
26	Asp		25 °C	
27	Disp		25 °C	DI Water
28	Inc	5 min	25 °C	
29	Asp		25 °C	
30	Disp		25 °C	DI Water
31	End		25 °C	

Digital Recording of LINEAR ARRAYS



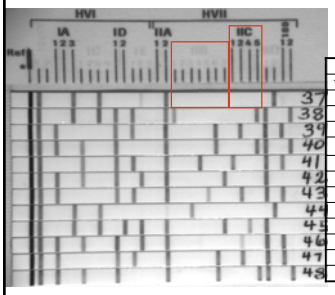
UV epi-fluorescence

GeneGnome (Syngene)



Yellow light used with filters so blue color lines could be archived as black and white images

Data Interpretation for LINEAR ARRAYs



Analysis of probe results is still manual!

	HVI					HVII			
16093	A	C	D	E	A	B	C	D	189
1	3	3	0	w2	2	0	0	2	0
1	3	3	1	1	2	1	5	1	2
1	1	0	1	1	2	6	2	1	1
1	1	1	1	0	2	6	5	1	2
1	0	1	1	1	2	4	1	1	1
1	1	1	1	2	2	7	0	1	0
1	1	0	1	1	2	5	2	1	1
1	3	3	0	0	w2	3	0	2	0
1	3	3	2	2	2	7	4	1	0
1	1	1	1	2	2	0	w2	1	1
1	1	0	1	1	2	6	2	1	1
1	1	1	1	0	2	6	5	1	2

Typing results from 50 ng of each PCR product

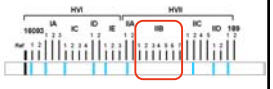
"Blank" Calls on LINEAR ARRAYs

We observed 640 "blanks" (**9.6% of calls**) on 346 different individuals (**52% of samples typed**).

*Different individuals typing as a blank for the same probe region could have different substitutions but for the purposes of data analysis the blanks are considered to represent the same variant (see Melton *et al.* (2001) *J. Forensic Sci.* 46(1):46-52)

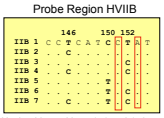
SSO Probe Region	Number Observed	Frequency	Budowle et al. (1998) Cau, AA, His
16093	23	3.5%	
HVIA	33	5.0%	3, 9, 3%
HVIC	76	11.4%	3, 20, 10%
HVID	33	5.0%	7, 17, 4%
HVIE	60	9.0%	
HVIIA	3	0.5%	0, 0, 0%
HVIIB	96	14.4%	16, 70, 55%
HVIIC	122	18.3%	11, 47, 13%
HVIID	42	6.3%	5, 5, 18%
189	152	22.8%	

Blanks expected based on full sequence analysis of 1393 individuals



PCR product fails to hybridize to any probe in region due to additional polymorphisms in the probe region that prevent hybridization

Probe Region HVIIB



Nucleotide positions 151 and 153 are common variants in African Americans

Summary of Our Population Typing with Roche mtDNA LINEAR ARRAYS

Typing frequencies for 666 NIST population samples

#*	Freq	% Types	% People
1	185	65.6	27.8
2	46	16.3	13.8
3	18	6.4	8.1
4	4	1.4	2.4
5	3	1.1	2.3
6	4	1.4	3.6
7	1	0.4	1.1
8	9	3.2	10.8
9	2	0.7	2.7
10	4	1.4	6.0
11	1	0.4	1.7
12	1	0.4	1.8
18	1	0.4	2.7
23	1	0.4	3.5
28	1	0.4	4.2
51	1	0.4	7.7

- 282 different types
- 185 were unique (occurred only once)
- 51 samples had "Most Common Type"

"Most Common Type" evaluated further with mtDNA coding region SNP assay

HV1/HV2 Anderson (CRS) except 263G, 315.1C
Haplogroup H

All Roche mtDNA LINEAR ARRAYS: 1111111111

51

site	rCRS	1	1	1	1	2	2	3	4	4	5	12	15
3010	G	**A**	G	G	G	**A**	G	G	G	G	G	G	**A**
4793	A	A	A	A	A	A	A	A	A	**G**	A	A	A
10211	C	C	C	C	C	C	C	C	C	C	C	C	C
5004	T	T	**C**	T	T	T	T	T	T	T	T	T	T
7028	C	C	C	C	**T**	C	**T**	C	T	C	C	C	C
7202	A	A	A	A	A	A	A	A	A	A	A	A	A
16519	T	T	T	**C**	T	**C**	**C**	T	T	**C**	**C**	**C**	**C**
12858	C	C	C	C	C	C	C	C	C	C	C	C	C
4580	G	G	G	G	G	G	**A**	G	**A**	G	G	G	G
477	T	T	T	T	T	**C**	T	T	T	T	T	T	T
14470	T	T	T	**A**	T	T	T	T	T	T	T	T	T

Improved resolution of mtDNA samples with coding region SNPs

12 types were observed
4 types were unique

Comparison of Other U.S. Population Data with SSO Probes

Population	N	#types	diversity	Most Common Type	MCT frequency
Caucasian	922	226	0.964	11111111	15.4%
African Am	805	251	0.983	12112021	6.8%
Hispanic	555	170	0.963	12122011	11.7%
Total	2282	502	0.998	11111111	**7.2%**

8 regions, 21 probes, 13 SNPs

Melton *et al.* (2001) *J. Forensic Sci.* 46(1): 46-52

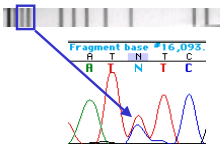
Population	N	#types	diversity	Most Common Type	MCT frequency
Caucasian	286	116	0.960	1111111111	16.4%
African Am	252	129	0.977	1141224211	10.7%
Hispanic	128	74	0.954	1102120111	16.4%
Total	666	282	0.985	1111111111	**7.7%**

10 regions, 31 probes, 18 SNPs

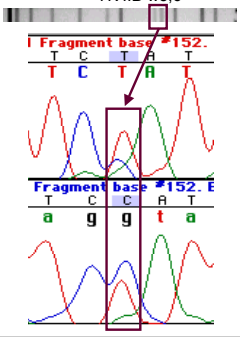
Kline *et al.* (2003) NIST population study

Heteroplasmy Detection

Accurate Detection of Heteroplasmy at position **16093**



Position 152
HVIIB w5,6

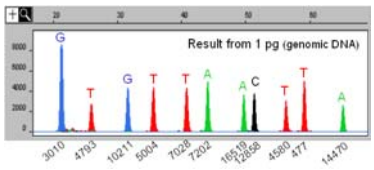


Observed 7 Times in 666 samples

AA	Cauc
HVIIC 2/4 (n=1) 152	HVID 1/2 (n=1) 16362
16093 1/2 (n=1)	HVIIB 3/4 (n=2) 146
	HVIIB 5/6 (n=1) 152
	189 1/2 (n=1)

Sites Observed: 16093, 16362, 146, 152, 189

NIST mtDNA Work



Coding Region mtSNP 11plex (minisequencing assay)

Developed with AFDIL to resolve mtDNA most common types

Int. J. Legal Med., in press

Roche Linear Arrays (probes for HVI/HVII)

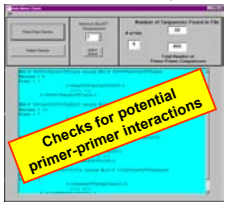
manuscript in preparation

Beta-test/Population Study

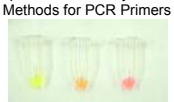
Technology Development Efforts

*Centered around **multiplex PCR**…*

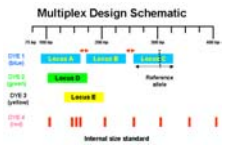
Created Custom Primer Design Software



Implemented Quality Control Methods for PCR Primers

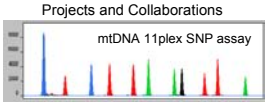


Standardized Assay Design Formats



Schoske *et al.* (2003) *Anal. Bioanal. Chem.* 375:333-343

Demonstrated Success with Multiple Projects and Collaborations



Y-STR 20plex, cat STR 12plex

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NIST Project Team:
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Pete Vallone
Margaret Kline
Jan Redman
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