Considerations Regarding Forensic DNA Typing and Future Directions

Professor Bruce Budowle
Executive Director of the Institute of Applied Genetics
Department of Forensic and Investigative Genetics
University of North Texas Health Science Center
Fort Worth, Texas 76107

Technology Works

- Generally speaking this statement is true
- Mitigating Factors
  - Pushing the envelope
  - Uncertainty/Risk
  - Bias
  - Reliability
  - Validation
- Humans are involved!!!

Methodology

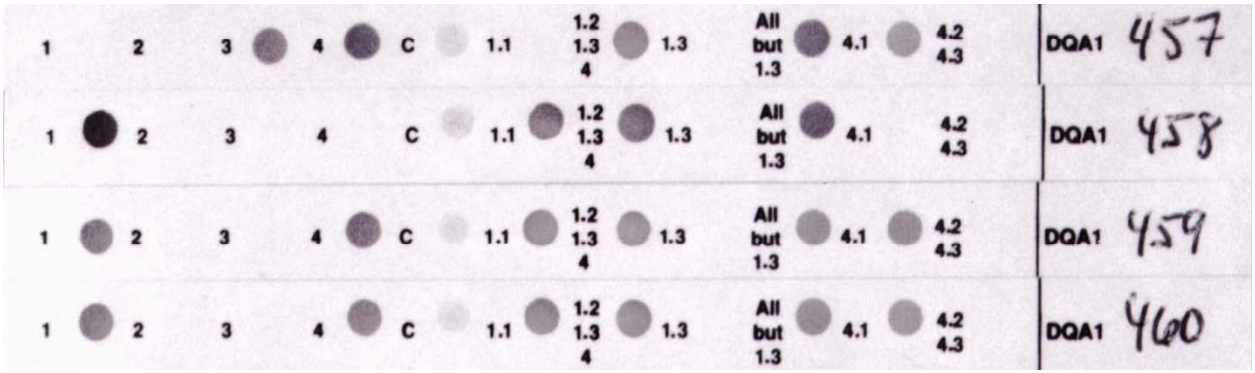
Forensic Concerns

- Bad method done poorly
- Bad method done well
- Good method done poorly
- Good method done well,
  but not accepted in legal system

Historical Example of Method Issues

- Methods can be thought to be reliable
- But sufficient validation studies must be carried out
- Results of validation studies should not be ignored
- HLA-DQA1 and Polymarker

HLA-DQA1 and PM Loci Dot Blots

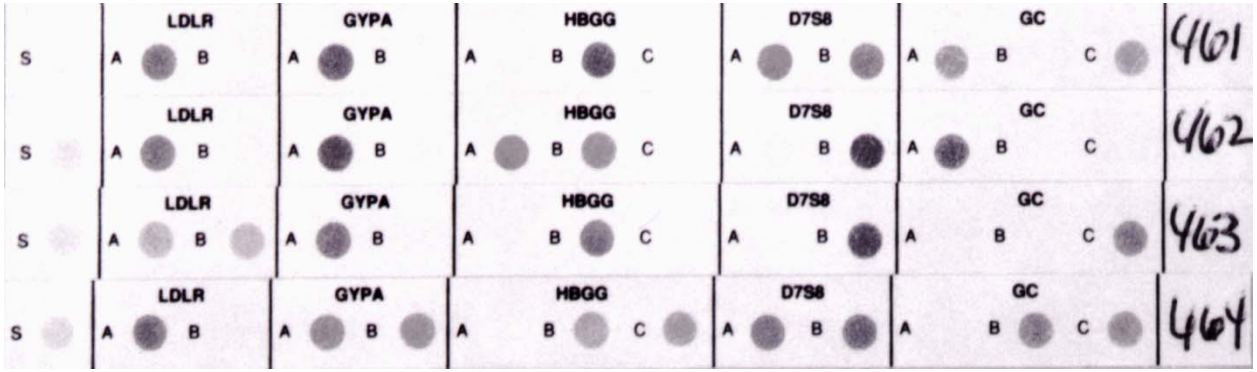


Control

Victim K

Suspect K

Evidence Q



Control

Victim K

Suspect K

Evidence Q

Errors in Typing Results

- Thermocycler temperature performance affected reliability
  - Four more GC residues in allele 1 than alleles 4,3, and 2 of DQA1
  - If the denaturing temp is not high enough allele 1 may not denature
  - Causing allele dropout
- Note: manufacturer laboratory scientists were not using outer wells of thermocycler
- Real cases with discrepancies inconsistent with data

Comey, C.T., Jung, J.M., and Budowle, B.: Use of formamide to improve PCR amplification of HLA-DQA1 sequences. BioTechniques 10(1):60-61, 1991

Errors in Typing Results

- Amplicon denatured for hybridization to immobilized probes
  - Selective loss of GC B and HLA DQA1 4.1 probe signals
- Primers can re-anneal and extend if the samples is not immediately hybridized
- Blocks the allele variant for hybridization
- Causing allele dropout
- Real cases with discordant DQA1/PM and STR results

Grow M, Phillips V, Reynolds R.: Post-amplification primer extension of heat-denatured AmpliType PCR products: effects on typing results. J Forensic Sci. 41(3):497-502, 1996

Validation

- Requisite!
- Without proper validation the limits are not defined
- Performing validation and ignoring results is unacceptable

Human Failings

- Mistakes will be made by humans with any system
- But some human failings are inexcusable
- FBI misidentification of latent print in Madrid bombing case
- SE33 variants
- One report describes electrophoretic SE33 anomalies
- Another report does not observe it
  - Sampling
  - Not aware and thus looking for it
  - Poorly calibrated instrument

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Concordance and population studies along with stutter and peak height ratio analysis for the PowerPlex® ESX 17 and ESI 17 Systems

Carolyn R. Hill [a,*], David L. Duewer [a], Margaret C. Kline [a], Cynthia J. Sprecher [b], Robert S. McLaren [b], Dawn R. Rabbach [b], Benjamin E. Krenke [b], Martin G. Ensenberger [b], Patricia M. Fulmer [b], Douglas R. Storts [b], John M. Butler [a]

[a] National Institute of Standards and Technology, Chemical Science and Technology Laboratory, Gaithersburg, MD 20899-8312, USA

Table 4
Discordant results for samples in this study. Null or different alleles due to an insertion or deletion outside of the primer binding site are in bold and underlined.

Locus	PP-ESX17	PP-ESI17	Identifiler	PP16	MiniFiler	NIST-NC01	NIST-23plex	PP-SE33
D1S1656	**15.3**, 15.3	14, 15.3	*a*	*a*	*a*	*a*	*a*	*a*
D3S1358	14, 17	14, 17	14, **14**	14, 17	*a*	*a*	*a*	*a*
D16S539	**12**, 12	12, 13	12, 13	12, 13	12, 13	*a*	*a*	*a*
D18S51	13, 15	13, 15	**15**, 15	13, 15	13, 15	*a*	*a*	*a*
D19S433	13, 14	13, 14	**14**, 14	*a*	*a*	*a*	*a*	*a*
D19S433	13, 14.2	13, 14.2	**14.2**, 14.2	*a*	*a*	*a*	*a*	*a*
D22S1045[b]	**17**, 17	15, 17	*a*	*a*	*a*	15, 17	15, 17	*a*
D22S1045[b]	**17**, 17	15, 17	*a*	*a*	*a*	15,17	15,17	*a*
D22S1045[b]	**17**, 17	15, 17	*a*	*a*	*a*	15,17	15,17	*a*
D22S1045[b]	**16**, 16	15, 16	*a*	*a*	*a*	15,16	15,16	*a*
SE33	26.2, 27.2	26.2, 27.2	*a*	*a*	*a*	*a*	*a*	26.2, **26.2**
SE33	20, 28.3	20, 28.3	*a*	*a*	*a*	*a*	*a*	20, **29.2**
SE33	24.2, 28.2	24.2, 28.2	*a*	*a*	*a*	*a*	*a*	**28.2**, 28.2
SE33	21.2, 26.2	21.2, 26.2	*a*	*a*	*a*	*a*	*a*	21.2, **21.2**
SE33	24.2, 25.2	24.2, 25.2	*a*	*a*	*a*	*a*	*a*	24.2, **24.2**
SE33	19, **19**	19, 25.2	*a*	*a*	*a*	*a*	*a*	19, 25.2

a—The compared kit does not provide results for this locus.
b—After inclusion of an additional D22S1045 forward primer to correct the null allele, these samples are not discordant in the commercial PP-ESX17 kit.

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Identification and secondary structure analysis of a region affecting electrophoretic mobility of the STR locus SE33

Dennis Y. Wang, Robert L. Green, Robert E. Lagacé, Nicola J. Oldroyd, Lori K. Hennessy, Julio J. Mulero *

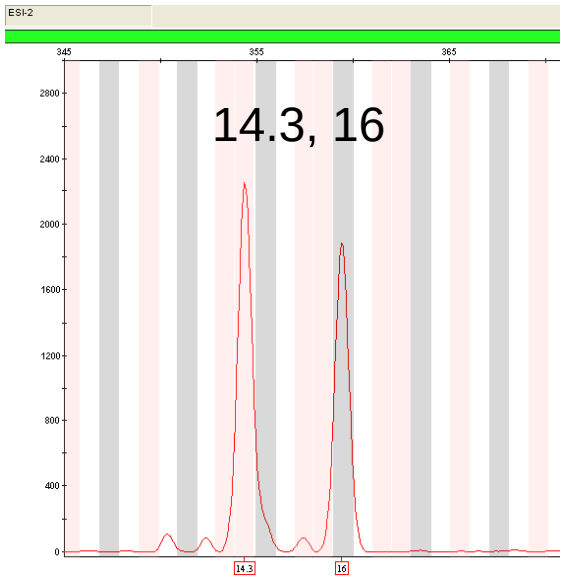
Life Technologies, 850 Lincoln Centre Drive, Foster City, CA 94404, United States

Table 1
Discordant SE33 samples from the population study. The discordant alleles due to a mobility shift or allele dropout when compared to the SEfiler Plus™ kit are shown in bold and underlined. The kits used in the study were SEfiler Plus™, NGM SElect™, SE33 experimental primers, and the Promega ESX-17 and ESI-17 kits. Electropherograms for samples IBB297 and IBB298 are shown in Figs. 2 and 3 respectively. AA, African American; C, Caucasian.

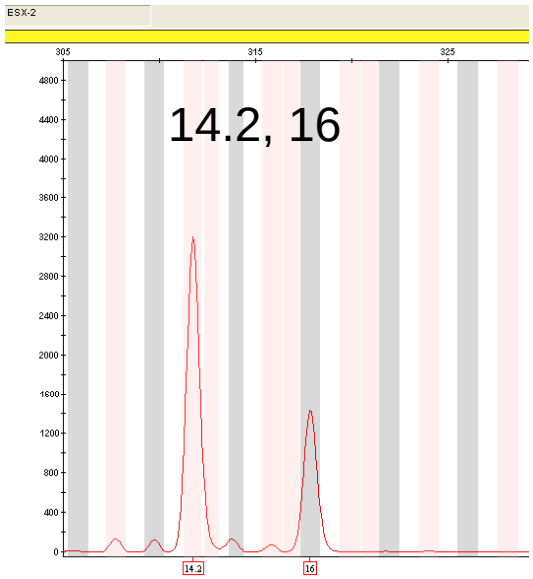
	Ethnicity (sex)	Sample ID	SEfP	NGM SElect	Experimental	ESX-17	ESI-17	Genetic Variation
(1)	AA (Male)	IBB039	17, 20	17, 20	**17.1**, 20	17, 20	**17.1**, 20	1
(2)	AA (Male)	IBB052	18, 20.2	18, 20.2	18, **20.3**	18, 20.2	18, **20.3**	1
(3)	AA (Fem)	IBB114	18, 24.2	18, 24.2	18, **24.3**	18, 24.2	18, 24.3	1
(4)	AA (Male)	IBB115	21, 22.2	21, 22.2	21, **22.3**	21, 22.2	21, **22.3**	1
(5)	AA (Male)	IBB121	20, 21.2	20, 21.2	20, **21.3**	20, 21.2	20, **21.3**	1
(6)	AA (Male)	IBB135	23.2, 28.2	23.2, 28.2	**23.3**, 28.2	23.2, 28.2	**23.3**, 28.2	1
(7)	AA (Male)	IBB160	13.2, 19	13.2, 19	**13.3**, 19	13.2, 19	**13.3**, 19	1
(8)	AA (Fem)	IBB187	14, 21.2	14, 21.2	14, **21.3**	14, 21.2	14, **21.3**	1
(9)	AA (Fem)	IBB196	17, 21.2	17, 21.2	17, **21.3**	17, 21.2	17, **21.3**	1
(10)	AA (Male)	IBB198	15, 20.2	15, 20.2	15, **20.3**	15, 20.2	15, **20.3**	1
(11)	AA (Male)	IBB233	17, 20.2	17, 20.2	17, **20.3**	17, 20.2	17, **20.3**	1
(12)	AA (Fem)	IBB253	20.2, 21	20.2, 21	**20.3**, 21	20.2, 21	**20.3**, 21	1
(13)	AA (Fem)	IBB262	13.2, 27.2	13.2, 27.2	**13.3**, 27.2	13.2, 27.2	**13.3**, 27.2	1
(14)	AA (Male)	IBB297	20.2, 21	20.2, 21	**20.3**, 21	20.2, 21	**20.3**, 21	1
(15)	AA (Male)	IBB658	17, 18.2	17, 18.2	17, **18.3**	17, 18.2	17, **18.3**	1
(16)	AA (Male)	IBB153	19, 25.2	19, 25.2	19, **25.3**	19, **19**	19, **25.3**	2
(17)	AA (Male)	IBB298	16, 18	16, 18	16, **18.1**	16, **16**	16, **18.1**	2
(18)	C (Fem)	IBB509	26.2, 30.2	26.2, 30.2	**26.3**, 30.2	30.2, **30.2**	**26.3**, 30.2	2
(19)	AA (Male)	IBB145	20, 22.2	20, 22.2	**20.1**, 22.2	22.2, **22.2**	20.1, 22.2	3

1) G/A_{18} SNP in experimental amplicon sequence (Fig. 4B).
2) C/T_{10} SNP in experimental amplicon sequence (Fig. 4B).
3) G/A_{11} SNP in experimental amplicon sequence (Fig. 4B).

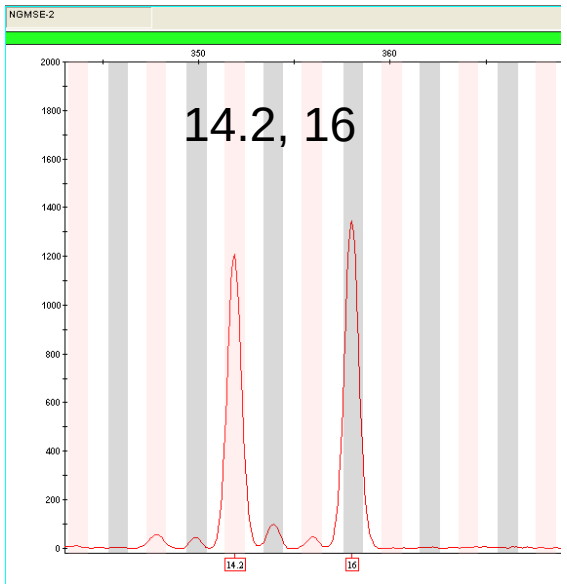
SE33 Type Discordance



ESI-17



ESX-17



NGM SElect

The ESI-17 kit results yielded a discordant SE33-14.3 allele

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Variants observed for STR locus SE33: A concordance study

Carey Davis [a], Jianye Ge [a], Jonathan King [a], Naseem Malik [b], Volker Weirich [c], Arthur J. Eisenberg [a], Bruce Budowle [a,*]

[a] Department of Forensic and Investigative Genetics, Institute of Investigative Genetics, Department of Forensic and Investigative Genetics, University of North Texas Health Science Center, 3500 Camp Bowie Blvd, Fort Worth, TX 76107, United States
[b] Universität Bern, Forensische Molekularbiologie, Institut für Rechtsmedizin, Bern, Switzerland
[c] Landeskriminalamt Mecklenburg-Vorpommern, Rampe, Germany

Table 1
Discordant SE33 alleles. The discordant alleles are in bold and underlined. The kits used in this study were NGM SElect™, ESX 17, and ESI 17. African ancestry (A); Caucasian ancestry (C) and Unknown ancestry (U).

Sample	Ethnicity	Sex	ESI-17	ESX-17	NGM SElect	SNP
1	A	Male	18, **19.3**	N/A	18, 19.2	G/A_{18}
2	A	Male	**14.3**, 18	14.2, 18	14.2, 18	G/A_{18}
3	C	Male	**27.3**, 30.2	27.2, 30.2	27.2, 30.2	G/A_{18}
4	C	Male	**18.1**, 23.2	18, 23.2	18, 23.2	C/T_{19}
5	A	Male	18, **22.3**	18, 22.2	18, 22.2	G/A_{18}
6	A	Male	17, **23.3**	17, 23.2	17, 23.2	G/A_{18}
7	A	Male	**22.3**, 26.2	22.2, 26.2	22.2, 26.2	G/A_{18}
8	A	Male	**13.3**, 20	13.2, 20	13.2, 20	G/A_{18}
9	A	Male	**14.3**, 17	14.2, 17	14.2, 17	G/A_{18}
10	A	Male	**12.3**, 21	12.2, 21	12.2, 21	G/A_{18}
11	C	Male	**14.1**, 16	14, 16	14, 16	C/T_{10}
12	A	Male	18, **21.3**	18, 21.2	18, 21.2	N/A
13	U	Male	**18.1**, 29.2	18, 29.2	18, 29.2	N/A
14	A	Male	**14.3**, 29	14.2, 29	14.2, 29	N/A
15	A	Male	**19.3**, 20	19.2, 20	19.2, 20	N/A
16	A	Male	18, **23.3**	18, 23.2	18, 23.2	N/A
17	C	Male	16, **16**	16, 19	16, 19	None

Sequencing Results Show Shift Due to SNP (not indel)

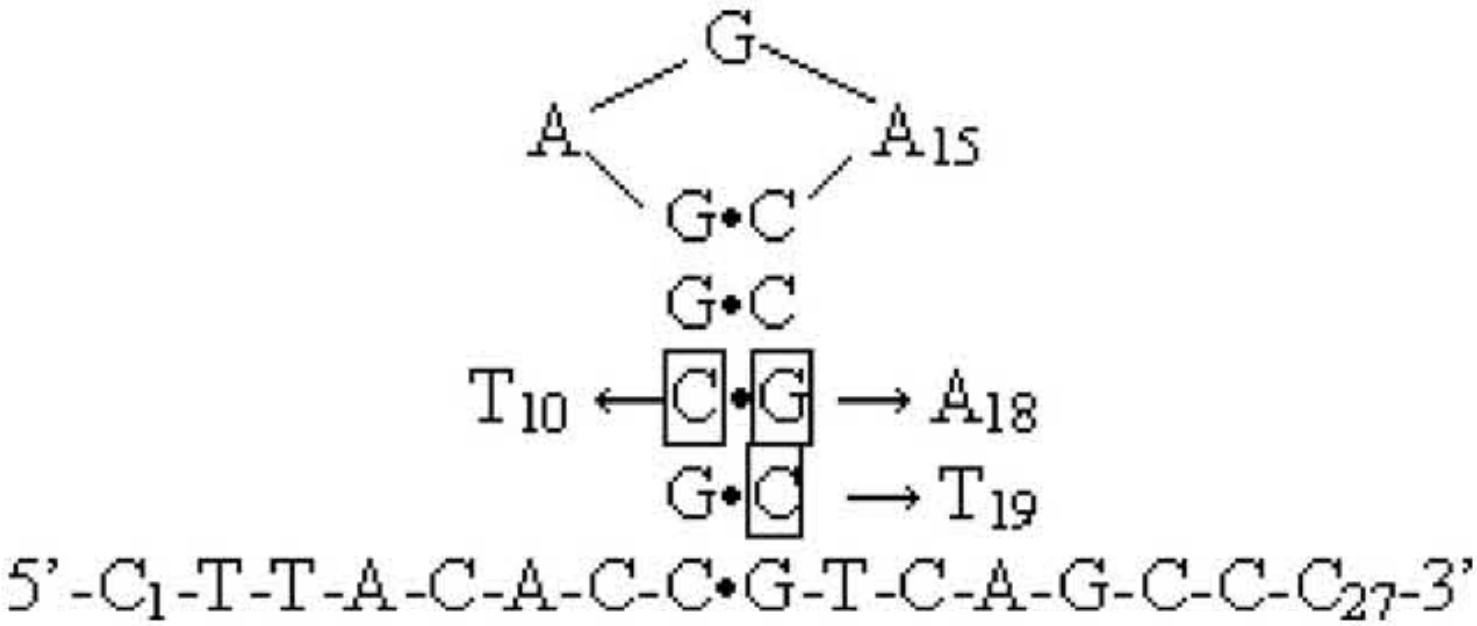


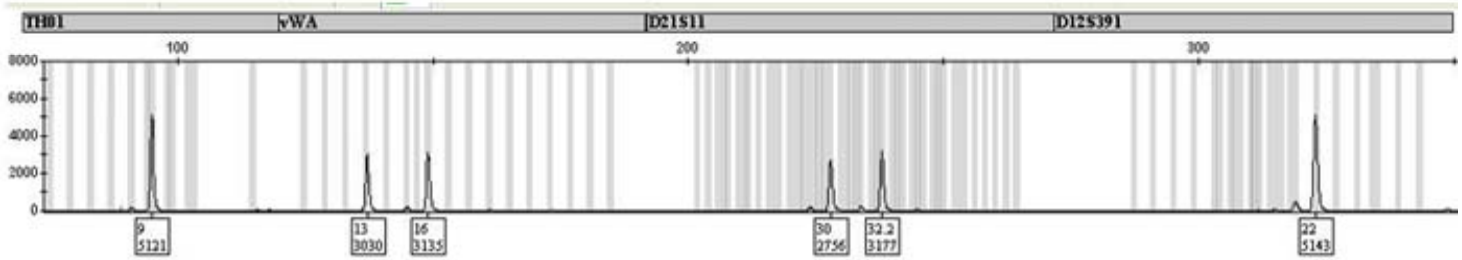
Fig. 2. The new SNP C/T_{19}. The most stable secondary structure for the sequence encompassing the polymorphic region (using MFOLD computer model). The sequence is annotated with the variant SNPs found in the polymorphic region. The free energy value for the new variant is $\Delta G = -2.44$ kcal/mol as compared to the wild type $\Delta G = -5.79$ kcal/mol.

Initial Low-Copy Number (LCN) Work

- Early work on "touch samples":
  - van Oorschot, R. A. and Jones, M. K. (1997) *Nature*. 387(6635): 767
  - Findlay, I., et al (1997) *Nature*. 389(6651): 555-556
- Application to routine limited quantity casework:
  - Gill, P., et al (2000) *Forensic Sci. Int.* 112(1): 17-40
  - Whitaker, J. P., et al (2001) *Forensic Sci. Int.* 123(2-3): 215-223
  - Gill, P. (2001) *Croatian Medical Journal* 42(3): 229-32
- Note that Touch Samples do not necessarily equate to LCN samples

Comparison of STR Results with Different Amounts of DNA

1ng Standard Result



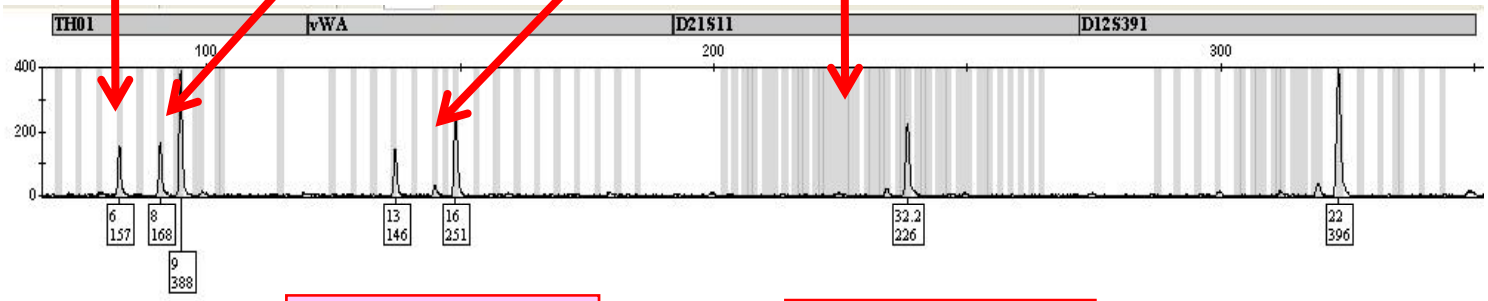
33pg LT-DNA: 2 reps

Heterozygote peak imbalance (57%)

Allele Drop In

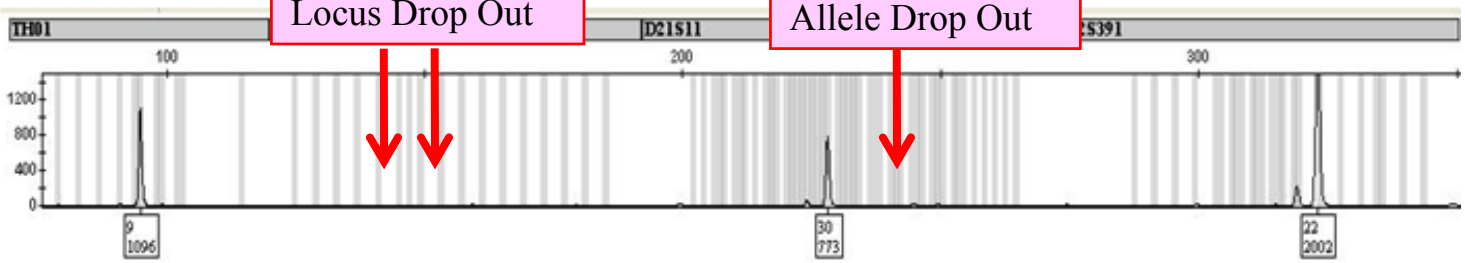
Increased Stutter (43%)

Allele Drop Out



Locus Drop Out

Allele Drop Out



Risk

Risk

- A scientist might say
  - "I am willing to take the risk…"
- But who is really at risk?
  - The scientist?
  - Suspects, Victims, Families, Society??

Bias in Law

DNA is from suspect

H_p

DNA is from an unknown person

H_d

- Asymmetry of the law - a thousand guilty go free vs one wrongly accused innocent person!
- What about the victim?

Forensic Science & Bias

- Database searches can tolerate false positives more so than false negatives
  - Can resolve with follow up
  - Investigative leads
  - Incumbent on scientist to convey uncertainty
- Casework tolerates false exclusions more so than false inclusions
  - Bias in law

Forensic Science & Bias

- A DNA threshold is a biased tool!
  - Because we are concerned about false inclusions/associations
- Set thresholds sufficiently high to greatly reduce the chance of false inclusions
  - Data below threshold become inconclusive
  - and importantly still can be used for exculpatory purposes

Forensic Science & Bias

- Driven by the degree of risk that should be taken
- What if the scientists do not convey the risk or uncertainty?
- Is that a serious concern or should we turn a blind eye?

OFFICE OF CHIEF MEDICAL EXAMINER
Charles S. Hirsch, M.D., *Chief Medical Examiner*

DEPARTMENT OF FORENSIC BIOLOGY
Mechthild Prinz, Ph.D., *Director*
Howard J. Baum, Ph.D., *Deputy Director*
421E. 26th St., New York, NY 10016
Tel: 212.323.1200 · Fax: 212.323.1590 · TTY: 212.447.2086 · E-mail: dnalab@ocme.nyc.gov

New Evolving DNA Reporting Approach

ADDITIONAL REPORT

No statistics

For previous results, evidence received, and disposition, see report dated October 18, 2007.

SUMMARY OF RESULTS:

The suspect, [redacted] is included as a contributor to the mixtures detected on stain 1A from seat belt "E11" and swab "ES15" from "steering wheel–right", and he cannot be excluded as a contributor to the mixtures detected on swabs "ES3" from "gear shift" and "ES6" from "brake pedal", from the report dated October 19, 2007 from case:

FB number	victim's name	report dates
[redacted]		July 18, 2007, October 17, 2007, and October 19, 2007

Cannot Exclude Interpretation

- Most of the DNA alleles seen in the DNA profile of XXX are seen in the mixture of DNA found on the sample listed below. Since the absence of the alleles can be explained, **he cannot be excluded** as a possible contributor of the mixture.

Cannot Exclude Interpretation

- No statistics are provided with this statement
- So no risk or uncertainty is conveyed
- We will visit later the bias and allele drop out statistical issues with such interpretations

Relevance

- Some scientists have said
  - "Relevance is for the court to decide"
- Is it up to the court to decide?
- Or are there situations where the scientist should not absolve himself/herself from considering relevance?
- Examples such as the Knox case demonstrate that this simple statement is insufficient for addressing the role of the scientist
- Perhaps it is not so black and white

Amanda Knox Case
The Knife

Selected because it looked cleaner than other knives

Does Evidence Support the Hypothesis?

- Or better posed
  - Is there an alternate hypothesis/interpretation of the findings?
- Should alternate hypotheses be considered?

- We need to develop training in this regard!

Other Tests Were Performed!

- Sample Screening
  - Identification of tissue source
    - Blood
    - Semen
    - Saliva
  - Time, labor, cost
  - DNA decision tree
    - Quality/Quantity of Body Fluid

Presumptive Test

- Sample B from the handle of the knife yielded a negative result for the presumptive tetramethyl benzidine (TMB) test.
  - Extremely sensitive
  - Blood can be diluted 100,000 -1,000,000 times.
- Knife was collected only 12 days after the crime
- Hemoglobin is fairly stable molecule
- Peculiar and difficult to reconcile that the TMB was negative

Alternate Hypothesis

- Extremely unlikely to have been able to wash away all traces of hemoglobin and preferentially leave behind solely DNA
- General plausible explanations for the presence of DNA on items
  - Contamination
  - Primary and secondary transfer
  - A person's DNA will be found on his/her items in his/her home, place of work, and other places
  - DNA also can be picked up by others and passed on to other items
- Evidence does not support that DNA on knife was from blood

What should have been done?

- Consider relevance!
  - Background DNA
    - Collect other knives and utensils in drawer
    - Test for presence of DNA
- Incumbent on scientists to consider alternate hypotheses, especially if they are probable
- Understand consequences of low level DNA typing
- Education

Not Unique to This Case

A Case Example

LEAD STORY: King walks free after murder case dropped

Deceased woman –

- Prosecution hypothesis: offender is male and punched her in the face in committing the offence
- Swabs taken from her face, both cheeks
- Y STR (male) analysis of left and right cheek swabs
- There were two men of interest, A and B - at different times

CLEARED: Zion King is relieved to be a free man again

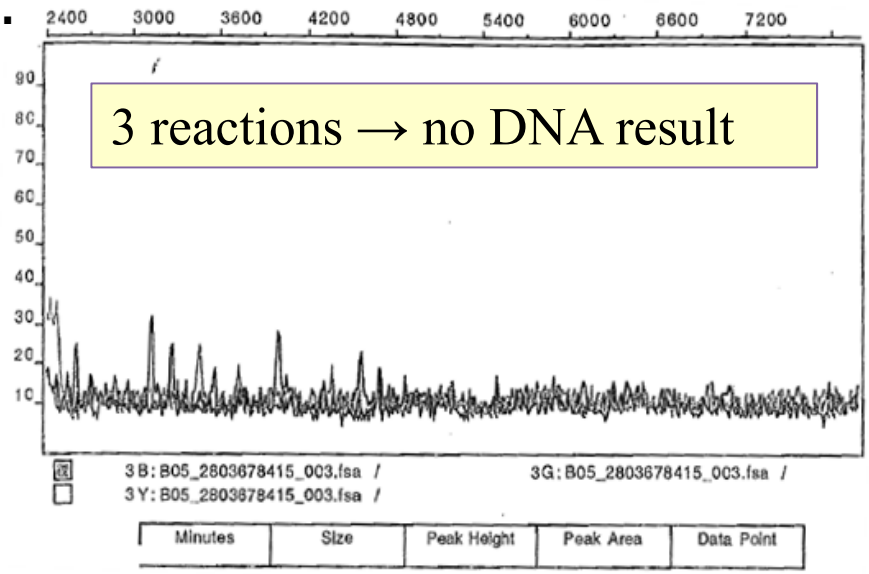
Yesterday in the High Court in Napier, Justice Denis Clifford granted a defence application led by co-counsel Peter Williams, QC, to dismiss the charge after Crown prosecutor Russell Collins conceded the case against King was not strong enough.

Mr Collins said it was "unsafe" for the Crown to offer its evidence as a reliable basis for a jury to reach a verdict.

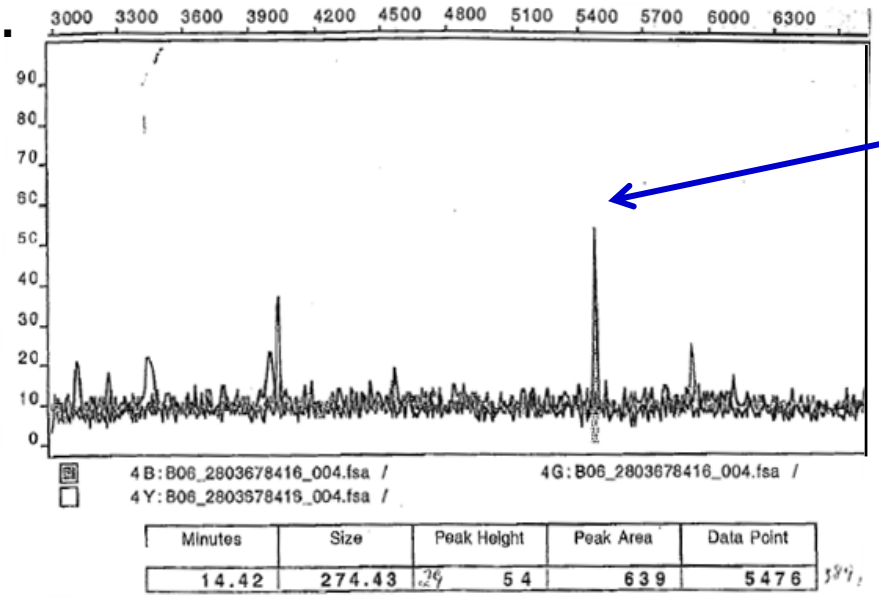
From Hawkes Bay Today, 9 February 2010

Left Cheek Swab

A.



B.

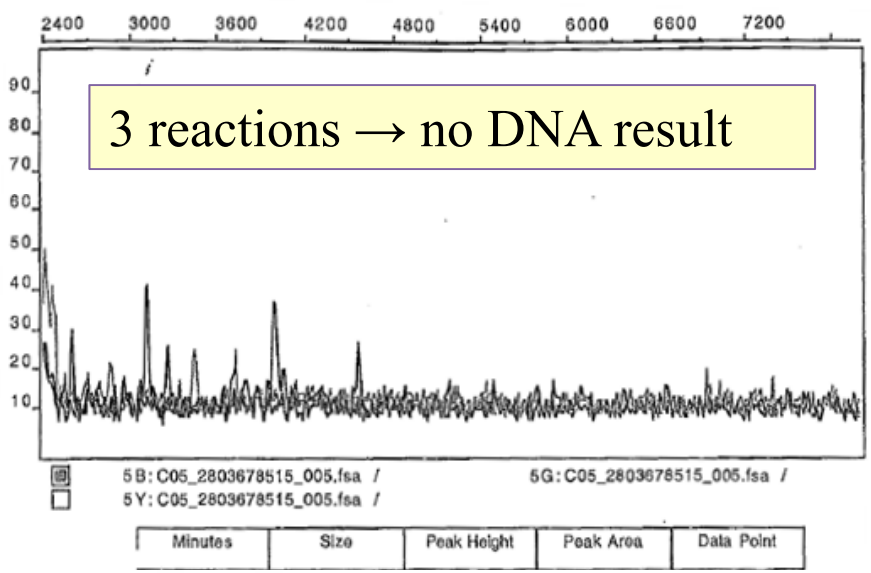


Peak 54RFU

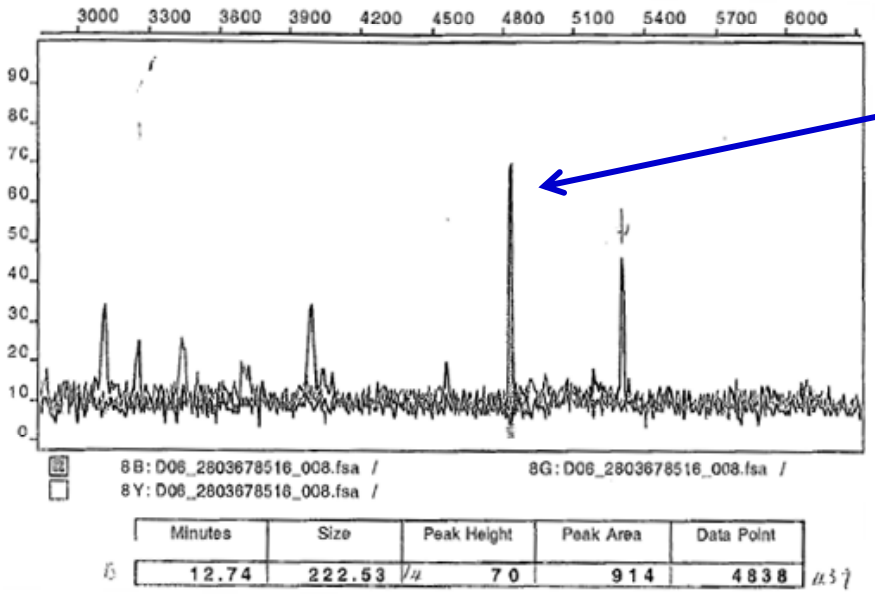
1 Peak (out of 12 loci)

Right Cheek Swab

A.



B.



Peak 70RFU

1 Peak (out of 12 loci)

Case Results

- Two total peaks, observed at two different loci, were seen in ***only one*** of four replicates
  - Consensus profile approach requires alleles to be replicated
  - These peaks should not have been reported as alleles
  - These peaks should not have been used for inclusionary or exclusionary purpose
- No peaks at the other loci were detected
- Peaks had very low heights of 54rfu and 70rfu (threshold values range from 50 – 250rfu)
- Violates "Published" Rule and yet was reported!

Bias

- A scientist might say
  - "I am not biased, I am objective and trained to be so"
- However, we are all biased
- Then the scientist says
  - " There is no bias because I detected all the alleles before looking at the reference samples"
- Is that a correct assessment?

Recall Cannot Exclude…

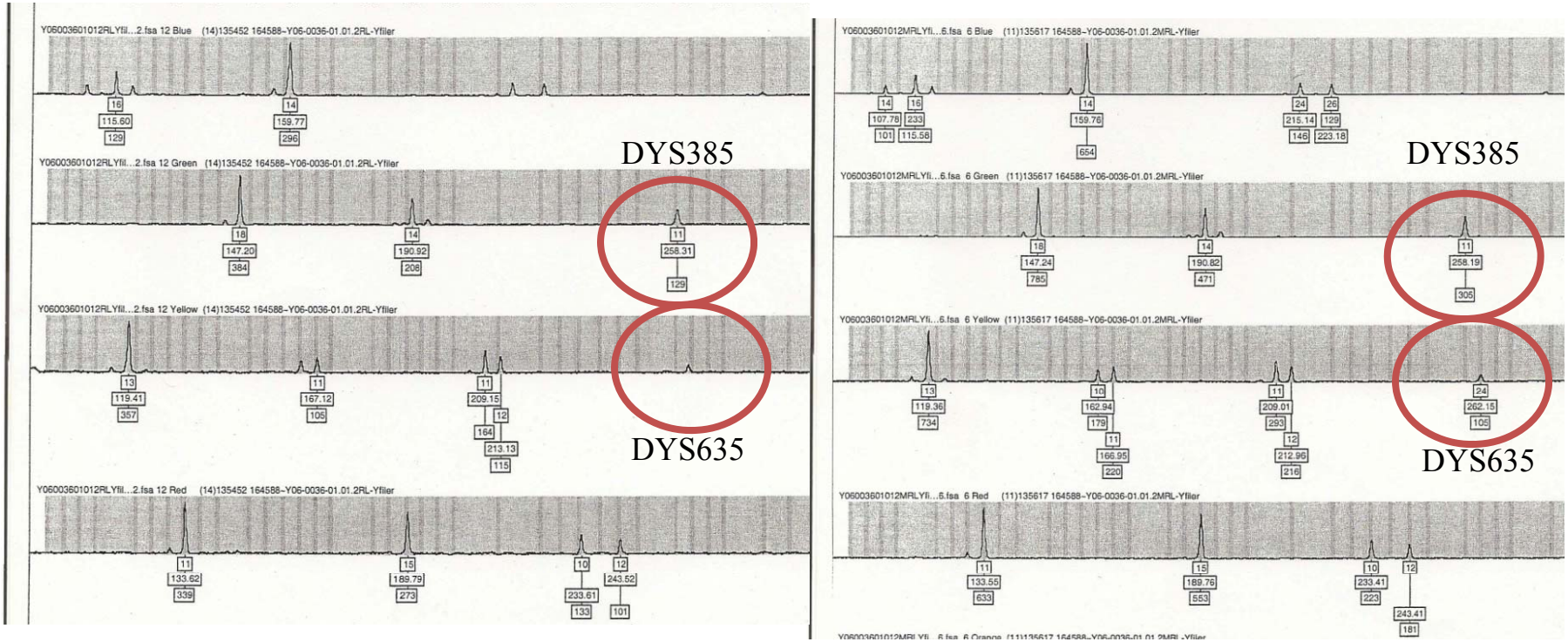
- Most of the DNA alleles seen in the DNA profile of XXX are seen in the mixture of DNA found on the sample listed below. Since the absence of the alleles can be explained, **he cannot be excluded** as a possible contributor of the mixture.

Bias Example

Loci	D21S11	FGA	TH01	vWA	D8S1179	D18S51	D3S1358	D19S433	D16S539	D2S1338
E	27,29,30.2,32.2	19,22,24,26	6,7,9.3	14,17,19	10,12,14,16	8,10,11,12	14,15,17,18	12,14,16	9,11,12	17,20,23,25
S1	29,30.2	19,22	9.3	17,19	**13,15**	10	**15,16**	14,16	9,11	20,25
E	27,29,30.2,32.2	19,22,24,26	6,7,9.3	14,17,19	10,12,14,16	8,10,11,12	14,15,17,18	12,14,16	9,11,12	17,20,23,25
S 2	**28,31.2**	19,22	9.3	**15,18**	12,14	10	15,17	14,16	9,11	20,25
E	27,29,30.2,32.2	19,22,24,26	6,7,9.3	14,17,19	10,12,14,16	8,10,11,12	14,15,17,18	12,14,16	9,11,12	17,20,23,25
S 3	29,30.2	**23,27**	9.3	17,19	12,14	10	15,17	**13,15**	9,11	20,25

- These 3 suspects are all included but the loci with potential drop-out change
- "Sliding window drop-out"

Bias - LCN and Y STRs



Specimen #	DYS456	DYS389 I	DYS390	DYS389 II	DYS458	DYS19	DYS385 a/b
	NR	NR	NR	NR	NR	NR	NR
	(14)16*	14	24,26	NR	18	14*	11
	17	14	26	31	18	15	11,18

Specimen #	DYS393	DYS391	DYS439	DYS635	DYS392	H4	DYS437	DYS438	DYS448
	NR	NR	NR	NR	NR	NR	NR	NR	NR
	13*	10,11	11,12	24	NR	11	15	10,12	NR
	13	11	11	24	11	11	15	10	20

NR = no result ( ) = weaker allele * = possible additional allele(s) present below threshold

DYS456	DYS389 I	DYS390	DYS389 II	DYS458	DYS19	DYS385a/b	DYS393	DYS391	DYS439	DYS635	DYS392	H4	DYS437	DYS438	DYS448
NS	14	24	NR	18	NS	NS	NS	10	11	24	NR	11	15	10	NR
NS	14	24	NR	18	NS	NS	NS	10	11	24	NR	11	15	12	NR
NS	14	24	NR	18	NS	NS	NS	10	12	24	NR	11	15	10	NR
NS	14	24	NR	18	NS	NS	NS	10	12	24	NR	11	15	12	NR
NS	14	24	NR	18	NS	NS	NS	11	11	24	NR	11	15	10	NR
NS	14	24	NR	18	NS	NS	NS	11	11	24	NR	11	15	12	NR
NS	14	24	NR	18	NS	NS	NS	11	12	24	NR	11	15	10	NR
NS	14	24	NR	18	NS	NS	NS	11	12	24	NR	11	15	12	NR
NS	14	26	NR	18	NS	NS	NS	10	11	24	NR	11	15	10	NR
NS	14	26	NR	18	NS	NS	NS	10	11	24	NR	11	15	12	NR
NS	14	26	NR	18	NS	NS	NS	10	12	24	NR	11	15	10	NR
NS	14	26	NR	18	NS	NS	NS	10	12	24	NR	11	15	12	NR
NS	14	26	NR	18	NS	NS	NS	11	11	24	NR	11	15	10	NR
NS	14	26	NR	18	NS	NS	NS	11	11	24	NR	11	15	12	NR
NS	14	26	NR	18	NS	NS	NS	11	12	24	NR	11	15	10	NR
NS	14	26	NR	18	NS	NS	NS	11	12	24	NR	11	15	12	NR

NR = no result
NS = not searched in database

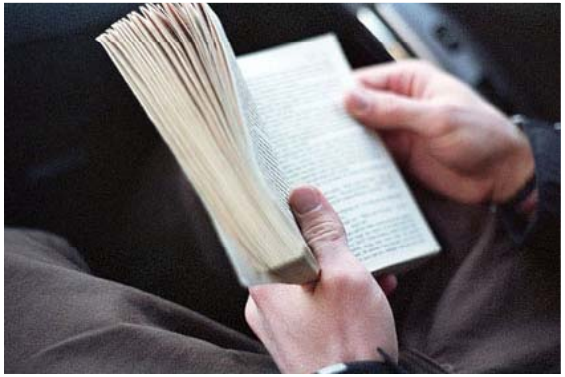
Interpretation

- Already addressed, but
- A scientist may say
  - “I have a set of defined guidelines and therefore my interpretation of results is reliable”
- What are the protocols?

LCN Transfer Studies

- Secondary transfer studies have thus far concentrated mainly on DNA originating from the epithelial cells of hands
  - Wash hands, shake, evaluate transfer
  - Not realistic

- Saliva is a rich source of DNA that is commonly transferred during normal day-to-day activities:
  - Placing a pen in mouth while studying
  - Licking a thumb before turning a page



http://2.bp.blogspot.com/-WMX1y8dgW48/TVsTlwedvHI/AAAAAAAALMc/seMImN6OUiI/s1600/man_reading_book.jpg

http://4.bp.blogspot.com/-ZcLFSOAbKTA/TefOjIVIMhI/AAAAAAACDjo/fQhvP3A1EZU/s400/Sucking-on-pens.jpg

Saliva Study

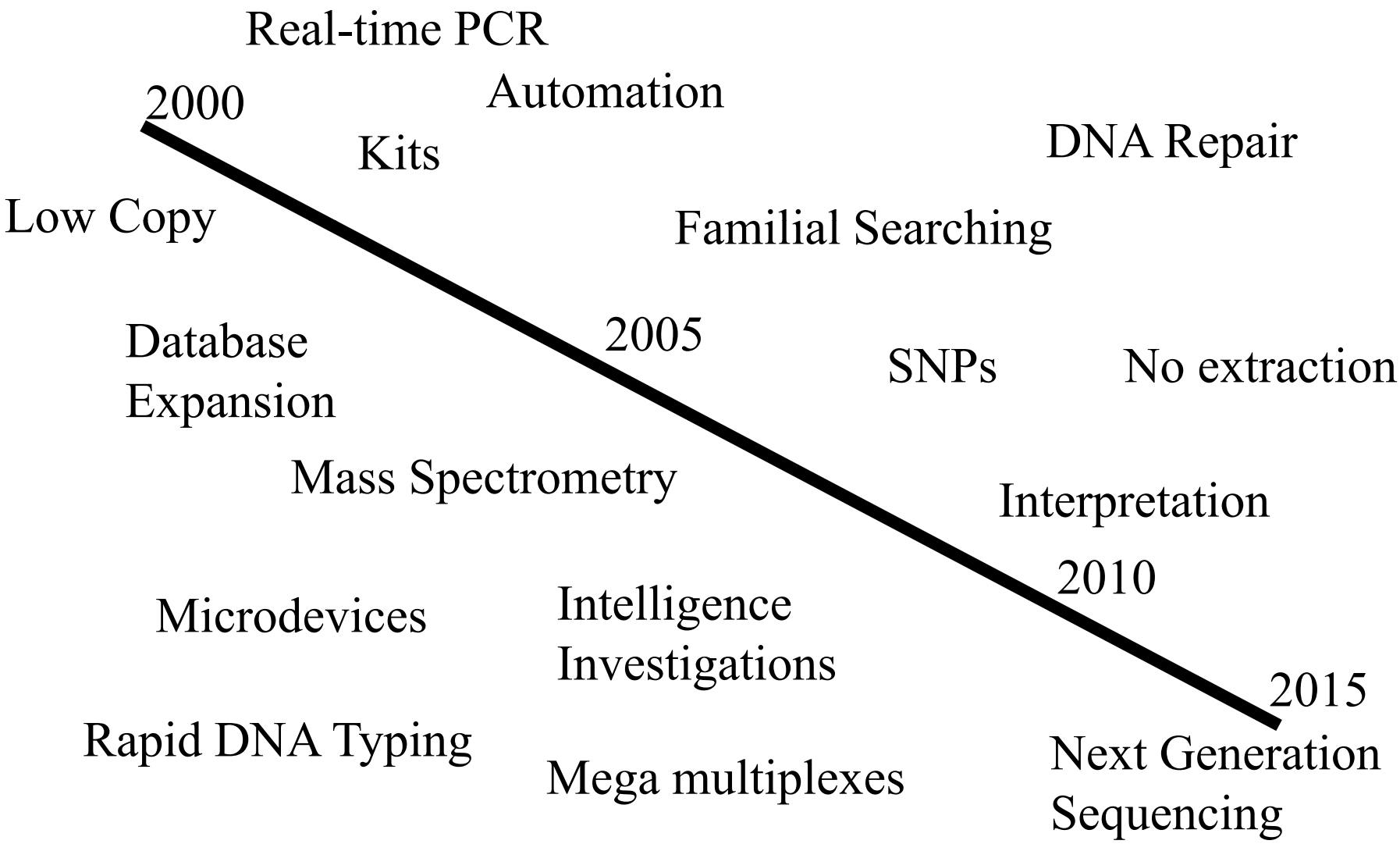
- Study conducted under the hypothesis that saliva, which is rich in DNA, can be a more prevalent source of genetic material during transfer events than hand epithelial cells
  - Saliva-based DNA transfer can result in higher levels of deposited DNA than previously observed by transfer studies
  - The profile of the initial depositor can be more prevalent in secondary transfer samples than previously observed by transfer studies

Bottom Line

- Hand washing studies conclude that last person in transfer line tends to be the dominant profile
- Good shedders and Bad shedders
- Saliva studies show that primary donor can be dominant profile
- **No value to shedder status!**
- Saliva traces make everyone a good shedder
- Impacts relevance!

FORENSIC DNA TIMELINE

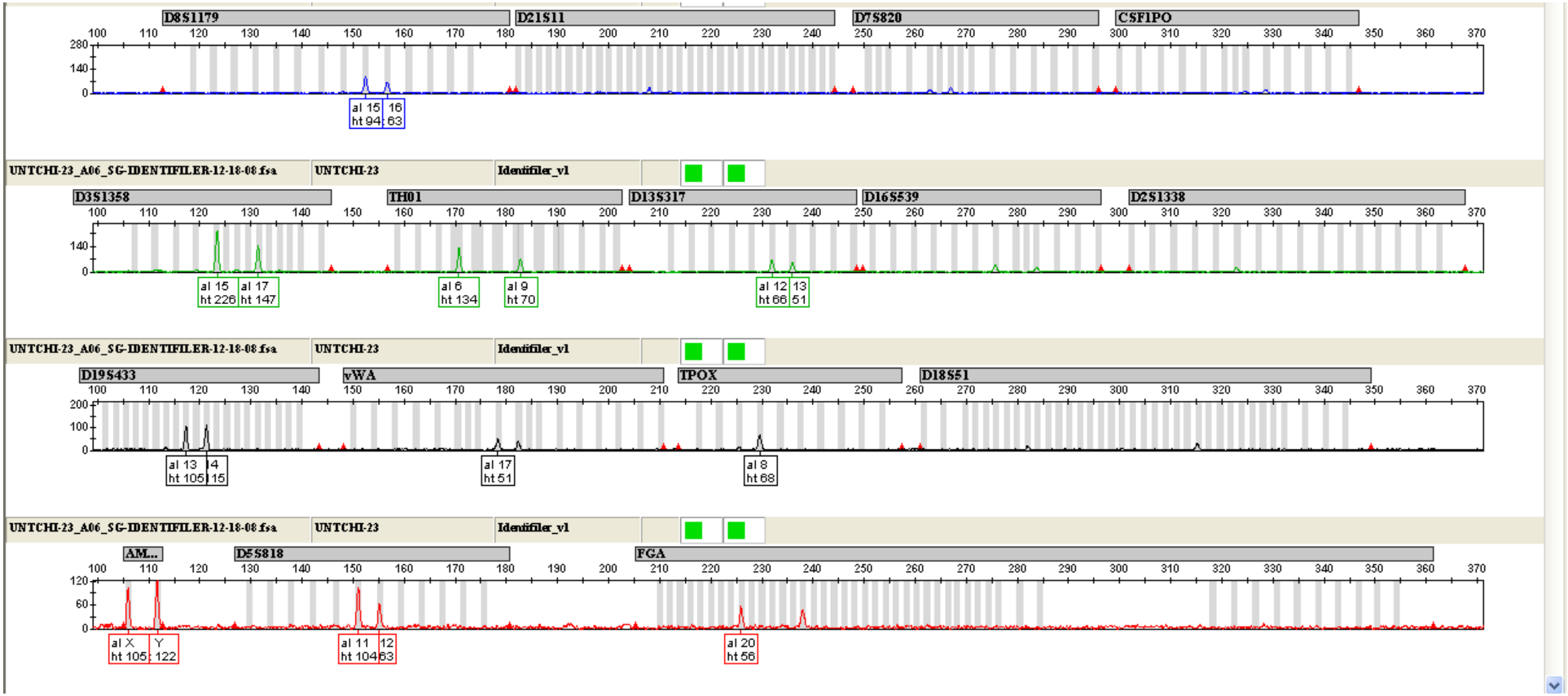
21st Century



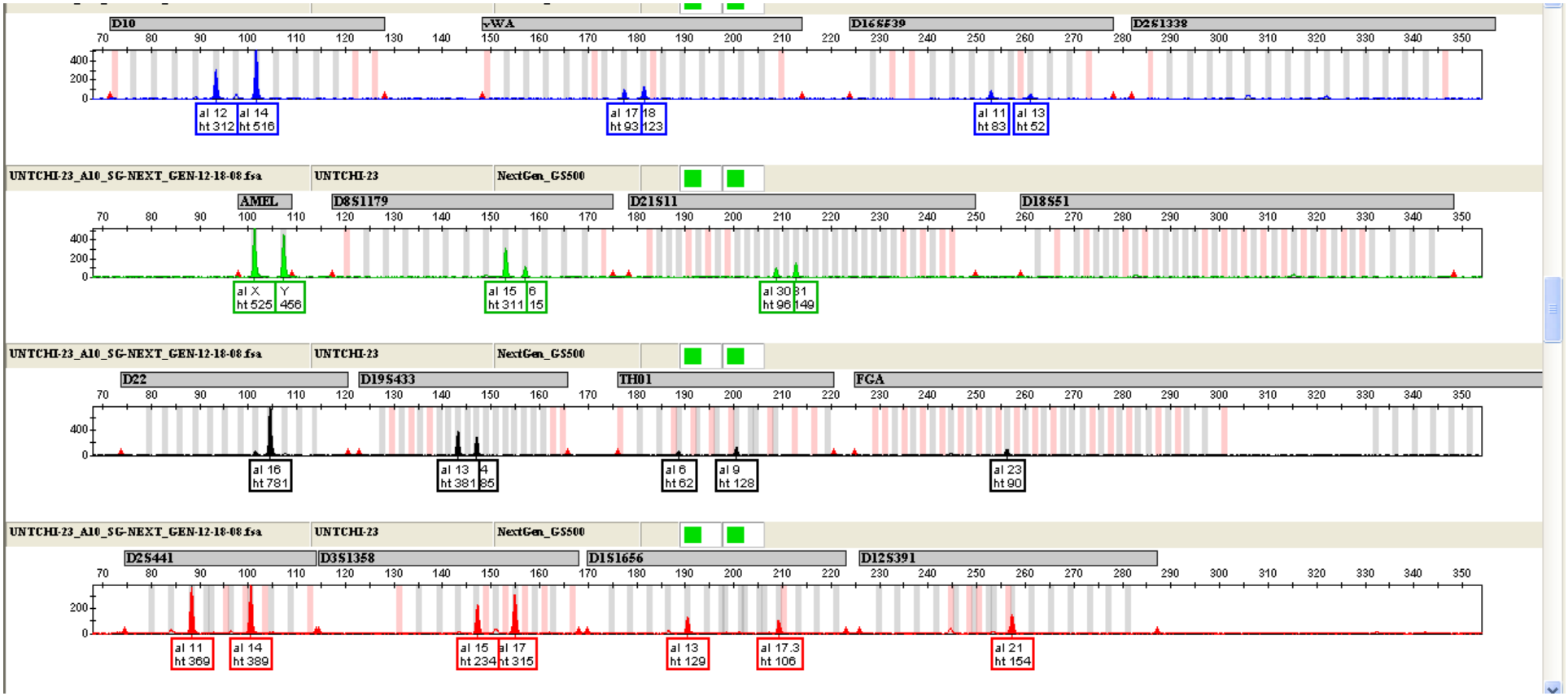
Kits

- Standard loci
- Quality tested products
- Took burden off analyst
- Greater shared experiences

Bone Sample Amplified with Identifiler



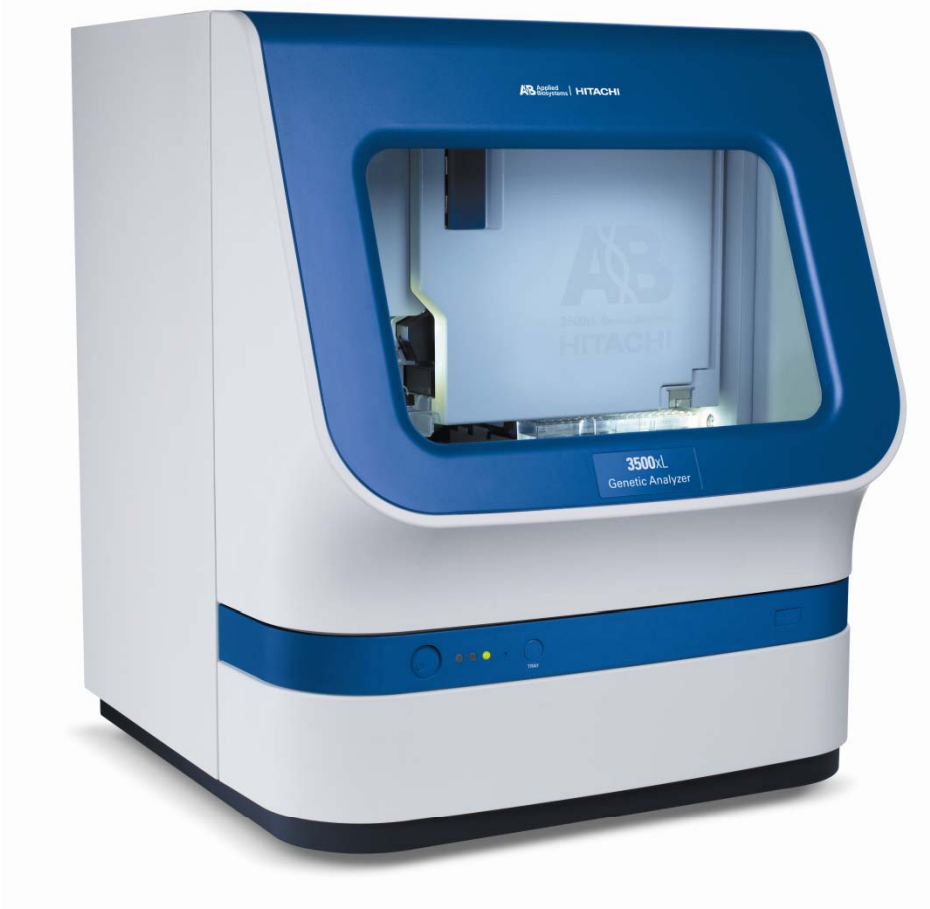
Bone Sample Amplified with Next-Generation Multiplex STR System



Enhancing Databases

More Markers
More Kits

3500
Laser Design



Smaller, Single Excitation Line Solid State Laser

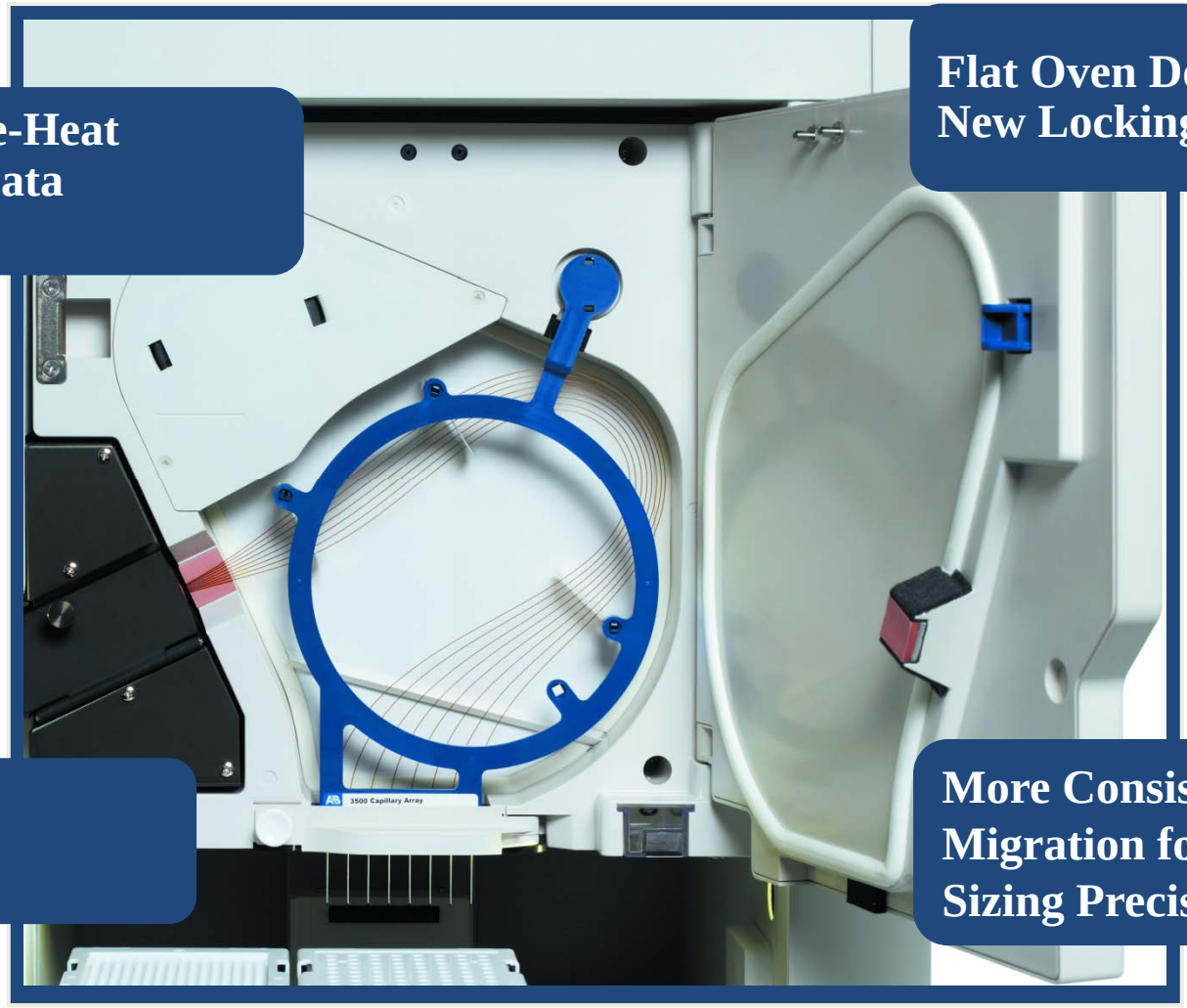
Minimal Heat Output

Standard Voltage Plug

Improved Temperature Control System

Optional Pre-Heat Control in Data Collection

Flat Oven Door Seal and New Locking Mechanism



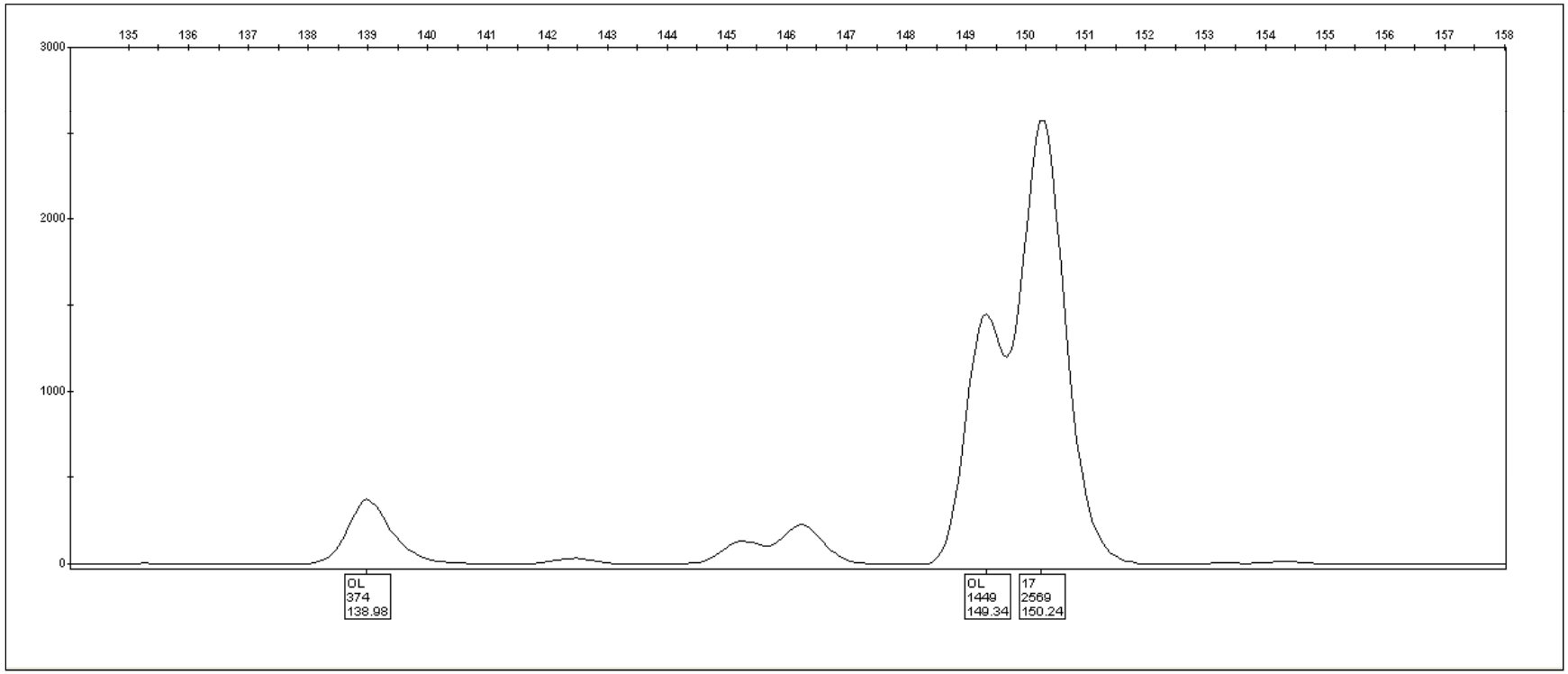
Smaller Oven

More Consistent Migration for Better Sizing Precision

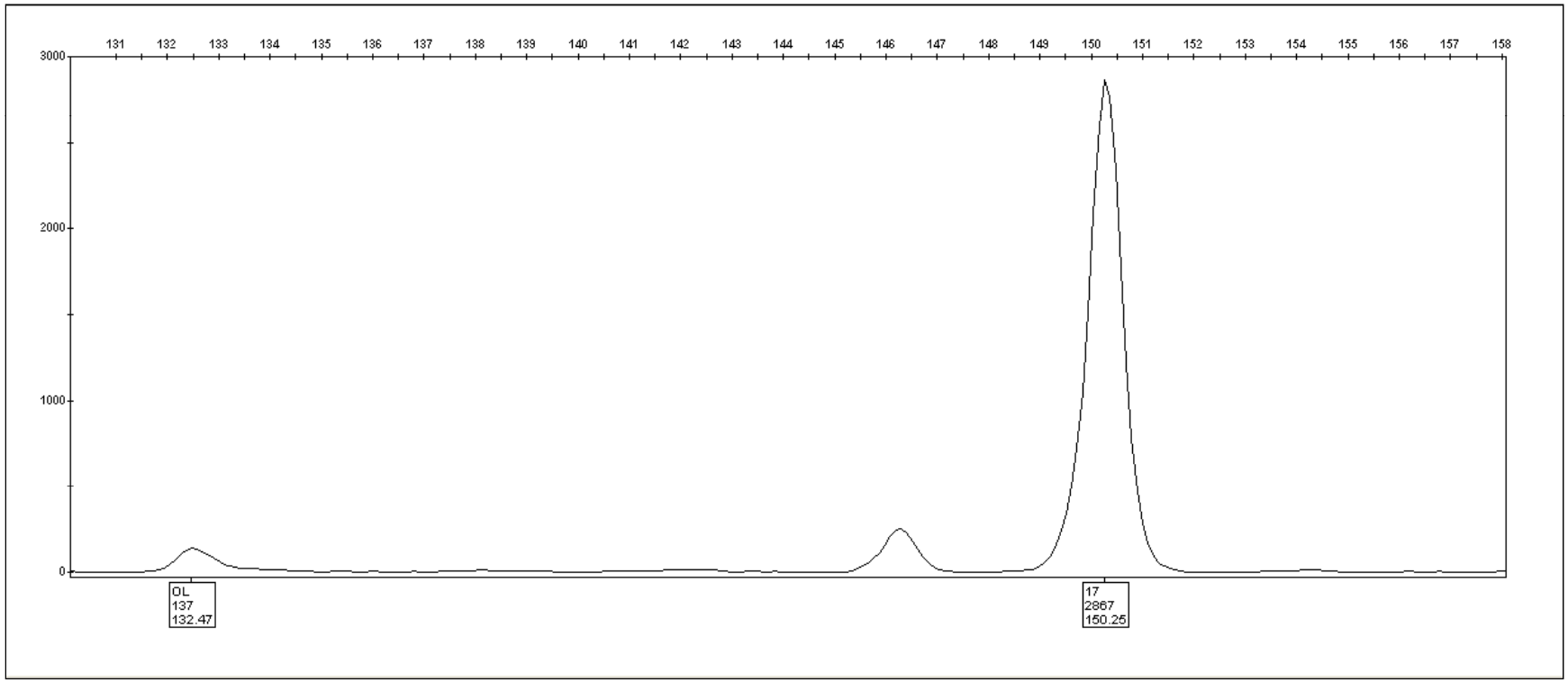
McLaren et al, FSI Genetics 2008

- Split peak artifact due to post-PCR reannealing of the unlabeled, unincorporated vWA primer to the 3′-end of the tetramethylrhodamine (TMR)-labeled strand of the vWA amplicon
- Occurs in the capillary post-electrokinetic injection
- Split peak is eliminated by incorporation into the loading cocktail of a sacrificial hybridization sequence (SHS) oligonucleotide that is complementary to the vWA primer

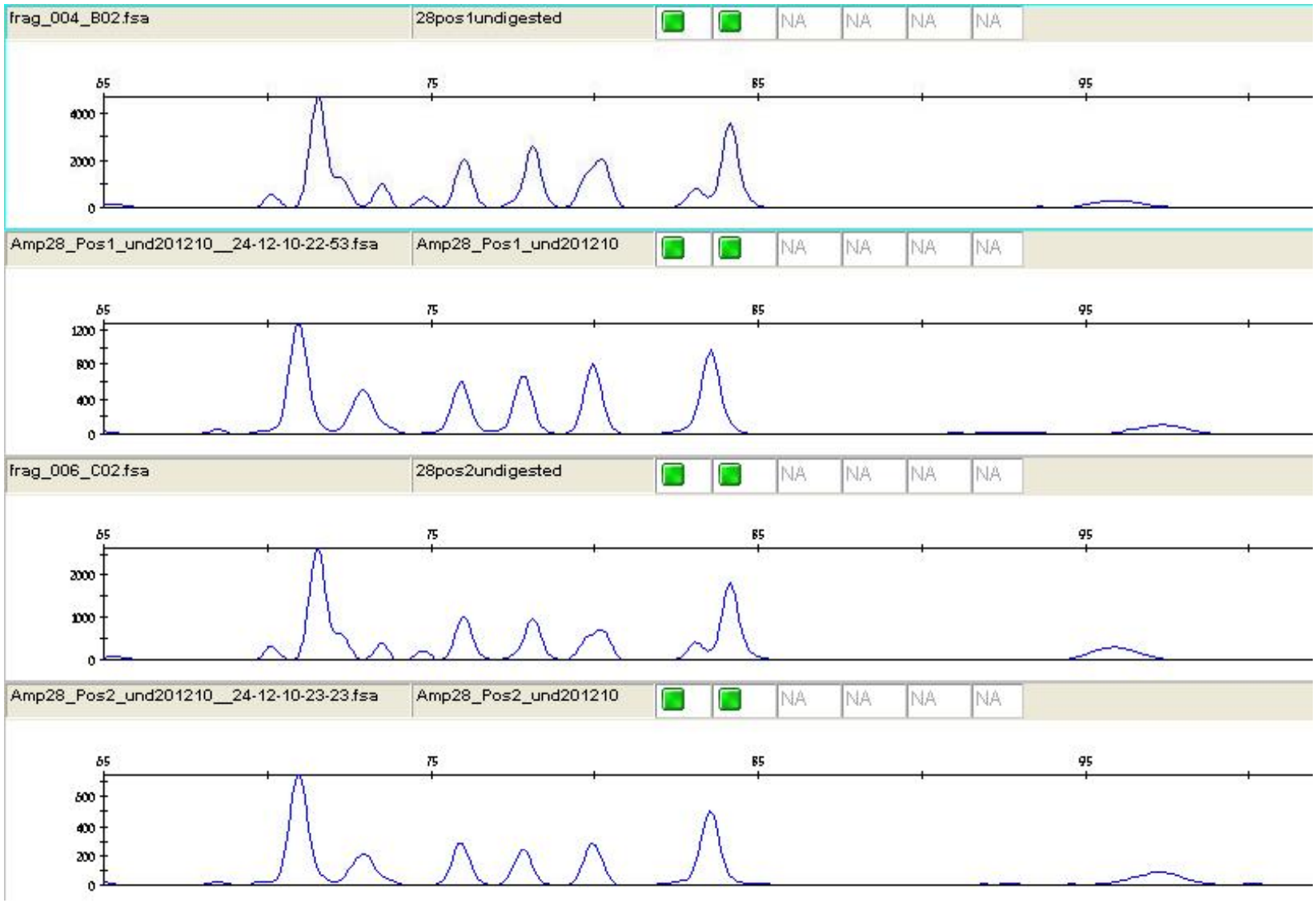
Heating Issues
“Split Peak” at vWA Locus - 3130



Same Sample - 310



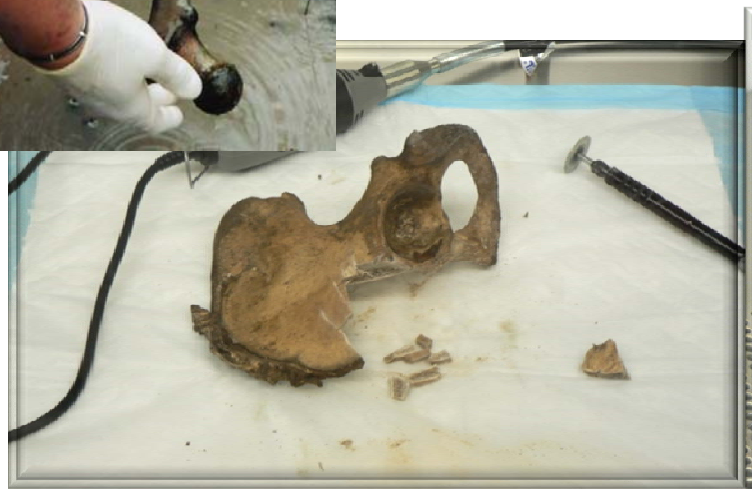
Same Sample – 3100/3500

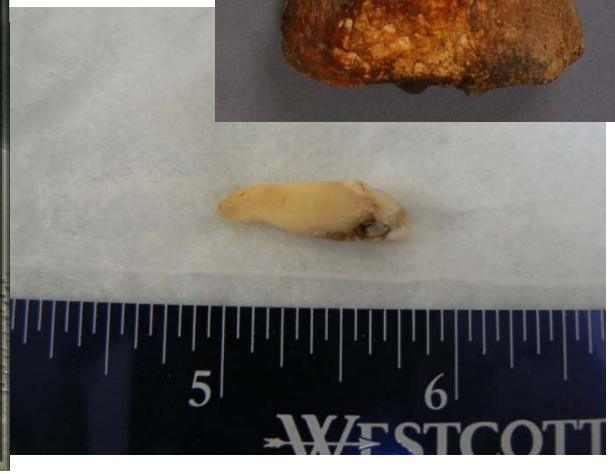


Rapid DNA Typing

- Rapidly identify individuals by DNA typing
- Military, forensic, homeland security, and intelligence community
- Self-contained turnkey system
- Swab in --- Result out
- 90 minutes to 2 hours
- Informed identification decisions regarding arrest, detention, or release of suspects, and eventually as it matures analyze crime scene evidence.
- NetBio, IntegenX, MicroLab Diagnostics
- Bottom line – makes DNA typing an actionable tool for investigative leads

Analysis of Difficult Samples



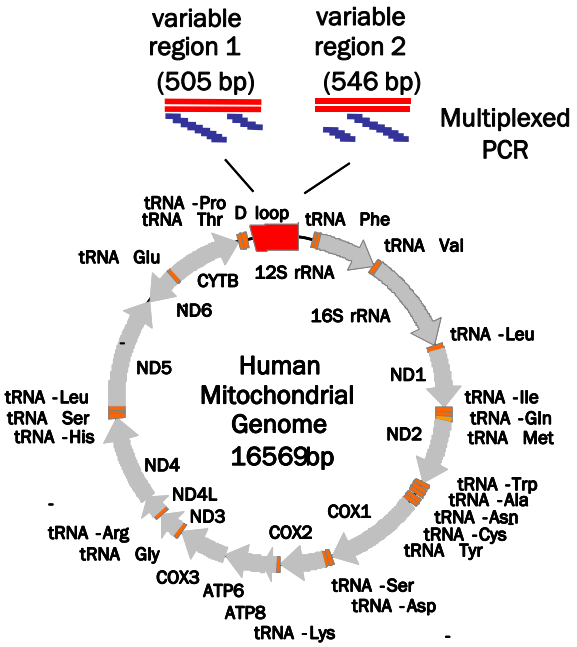


mtDNA is the most successful marker

Mass Spectrometry Advantages

- No labeling
- Mass accuracy
- multiplexing
- Quantitation - Mixture interpretation
- Automation
- Cost – e.g., mtDNA

PLEX-ID ANALYSIS STRATEGY

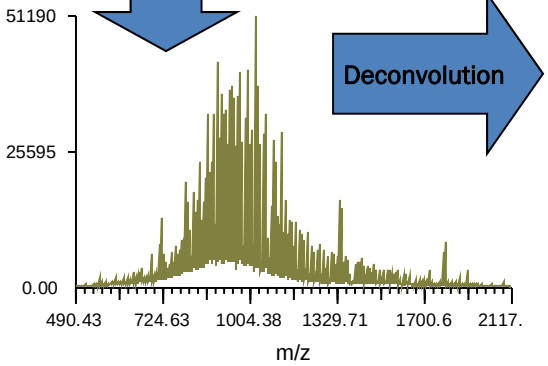


PCR amplification of multiple targets

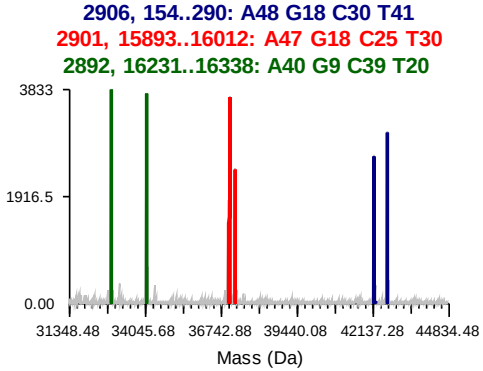
PCR Product(s)

Automated desalting on the PLEX-ID

ESI-MS

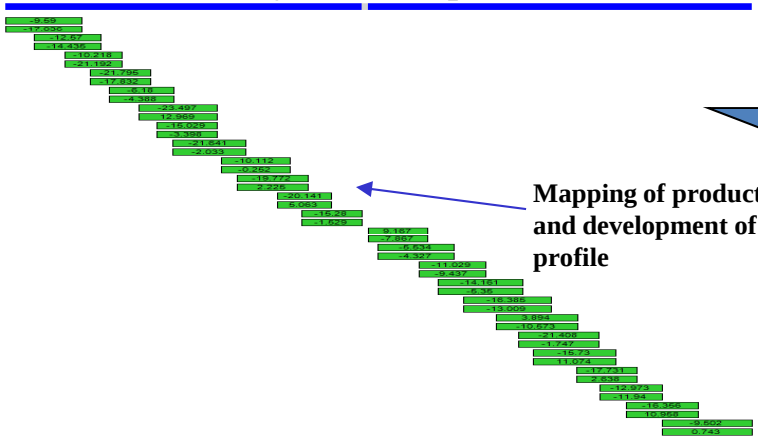


Deconvolution



ESI-MS analysis of complex mixture

Data Processing



Mapping of products to a coordinate map and development of base composition profile

Compare sample and reference base composition profiles

Database search

Concordance?

Assess expected match frequency

Mass Spectrometry

Base composition

Sample 1 --- A-24, G-30, C-18, T-28

Sample 2 --- A-23, G-31, C-18, T-28

A to G transition

PLEX-ID: Advances and Applications

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Analytical Biochemistry 344 (2005) 53–69
ANALYTICAL BIOCHEMISTRY
www.elsevier.com/locate/yabio

Base composition analysis of human mitochondrial DNA using electrospray ionization mass spectrometry: A novel tool for the identification and differentiation of humans

Thomas A. Hall [a], Bruce Budowle [b], Yun Jiang [a], Lawrence Blyn [a], Mark Eshoo [a], Kristin A. Sannes-Lowery [a], Rangarajan Sampath [a], Jared J. Drader [a], James C. Hannis [a], Patina Harrell [a], Vivek Samant [a], Neill White [a], David J. Ecker [a], Steven A. Hofstadler [a,*]

Forensic Science International: Genetics xxx (2012) xxx–xxx
Contents lists available at SciVerse ScienceDirect
Forensic Science International: Genetics
journal homepage: www.elsevier.com/locate/fsig

Automated analysis of sequence polymorphism in STR alleles by PCR and direct electrospray ionization mass spectrometry

John V. Planz [a,*], Kristen A. Sannes-Lowery [b], David D. Duncan [b], Sheri Manalili [b], Bruce Budowle [a], Ranajit Chakraborty [a], Steven A. Hofstadler [b], Thomas A. Hall [b]

[a] University of North Texas Health Science Center at Fort Worth, TX, USA
[b] Ibis Biosciences, Carlsbad, CA, USA

Forensic Science International: Genetics Supplement Series 2 (2009) 527–528
Contents lists available at ScienceDirect
Forensic Science International: Genetics Supplement Series
journal homepage: www.elsevier.com/locate/FSIGSS

Research article

Validation of mass spectrometry analysis of mitochondrial DNA

Bruce Budowle [a,b,*], Arthur J. Eisenberg [a,b], Suzanne Gonzalez [a,b], John V. Planz [a,b], Kristin A. Sannes-Lowery [c], Thomas A. Hall [c], Jessica E. Paulsen [c], Steven A. Hofstadler [c]

[a] Department of Forensic and Investigative Genetics, University of North Texas Health Science Center, Ft Worth, TX 76107, USA
[b] Institute of Investigative Genetics, University of North Texas Health Science Center, Ft Worth, TX 76107, USA
[c] Ibis Biosciences, Inc., a wholly-owned subsidiary of Abbott Molecular, Carlsbad, CA 92008, USA

Base Composition Profiling of Human Mitochondrial DNA Using Polymerase Chain Reaction and Direct Automated Electrospray Ionization Mass Spectrometry

Thomas A. Hall,[†] Kristin A. Sannes-Lowery,[†] Leslie D. McCurdy,[‡] Constance Fisher,[‡] Theodore Anderson,[§] Almira Henthorne,[†] Lora Gioeni,[‡] Bruce Budowle,[∥] and Steven A. Hofstadler*[,†]

Ibis Biosciences, subsidiary of Abbott Molecular, Inc., Carlsbad, California 92008, Federal Bureau of Investigation, Quantico, Virginia 22135, Armed Forces DNA Identification Laboratory, Rockville, Maryland 20850, and Department of Forensic and Investigative Genetics, Institute of Investigative Genetics, University of North Texas Health Science Center, Fort Worth, Texas 76107

Forensic Science International: Genetics Supplement Series 2 (2009) 524–526
Contents lists available at ScienceDirect
Forensic Science International: Genetics Supplement Series
journal homepage: www.elsevier.com/locate/FSIGSS

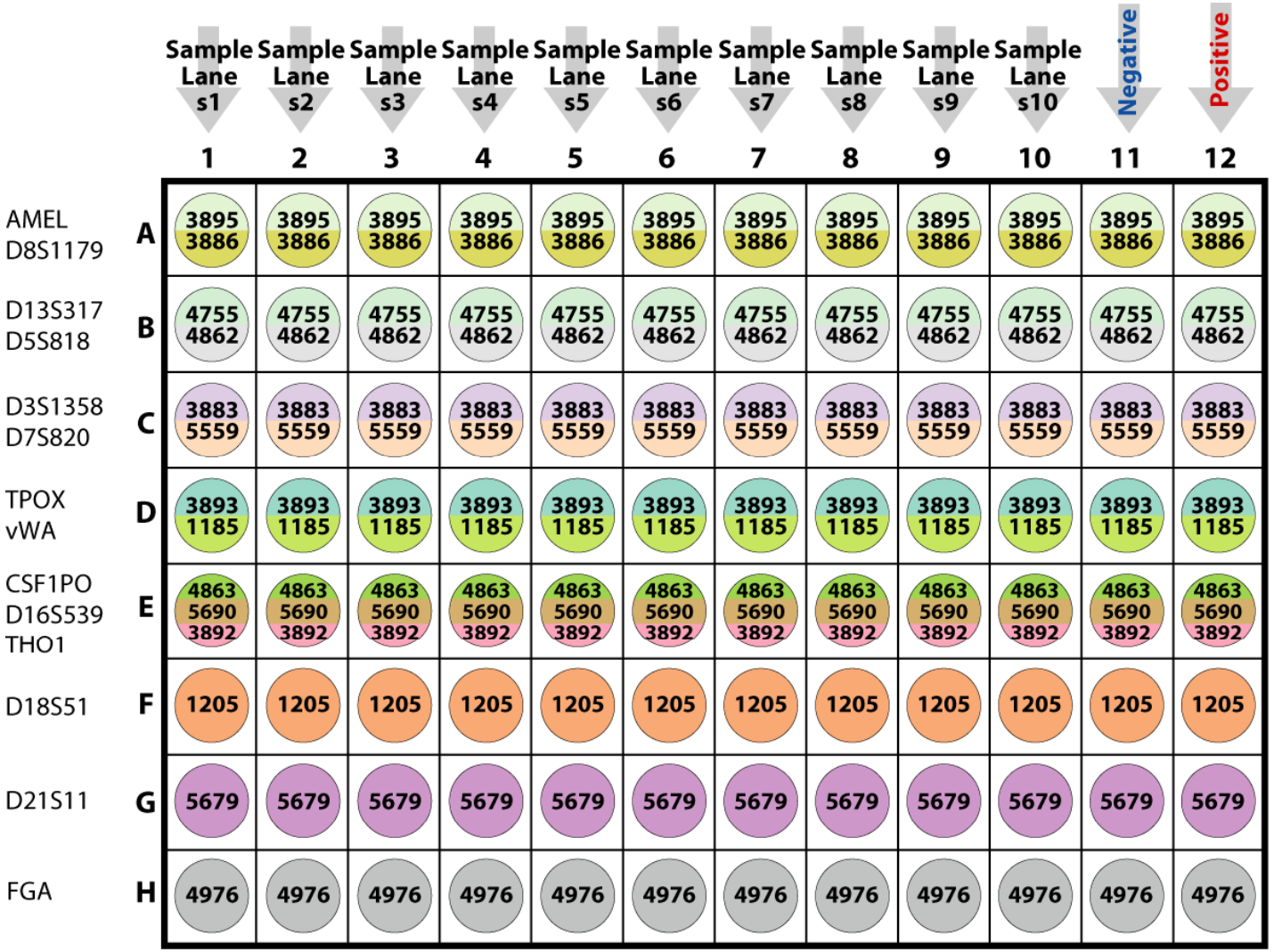
Research article

Analysis of DNA forensic markers using high throughput mass spectrometry

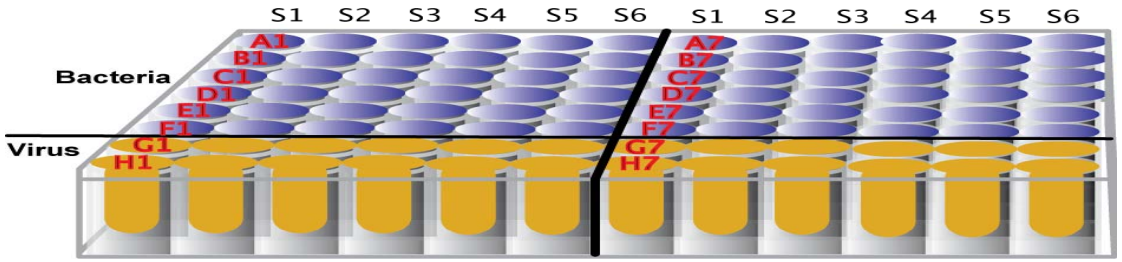
Steven A. Hofstadler [a,*], Thomas A. Hall [a], Kristin A. Sannes-Lowery [a], Sheri Manalili [a], Jessica E. Paulsen [a], Leslie D. McCurdy [b], Lora Gioeni [b], Thuy Penella [b], Arthur J. Eisenberg [c,d], John V. Planz [c,d], Bruce Budowle [c,d]

[a] Ibis Biosciences Inc., A Subsidiary of Abbott Molecular Inc., 1891 Rutherford Road, Carlsbad, CA 92008, USA
[b] Federal Bureau of Investigation, Quantico, VA 22135, USA
[c] Department of Forensic and Investigative Genetics, University of North Texas Health Science Center, Fort Worth, TX 76107, USA
[d] Institute of Investigative Genetics, University of North Texas Health Science Center, Fort Worth, TX 76107, USA

PLEX-ID STR CODIS CORE LOCI V2.0 ASSAY FORMAT



Biodefense Kit



Copies	CALIBRANT	Grouping	pp code	Target
150	BWMPLXCAL: BA CAL	A1	BCT352	*Bacillus anthracis*
			BCT2339	*Yersinia pestis*
			BCT1076	*Clostridium botulinum*
150	BWMPLXCAL: FT CAL	B1	BCT355	*Bacillus anthracis*
			BCT2328	*Francisella tularensis*
150	BWMPLXCAL: COX CAL	C1	BCT2381	*Bacillus anthracis-pX01*
			BCT1079	*Coxiella burnetii*
			BCT1084	*Ricksettia prowazekii*
150	BWMPLXCAL: BRUC CAL	D1	BCT1111	*Brucella melintensis*
			BCT1105	*Shigella Flexneri*
150	BWMPLXCAL: BURK CAL	E1	BCT1070	*Burkholderia mallei*
			BCT2323	*Vibrio cholera*
			BCT1106	*Shigella Flexneri*
150	BWMPLXCAL: VC CAL	F1	BCT2332	*Francisella tularensis*
			BCT2927	*Vibrio cholera*
			BCT2326	*Yersinia pestis*
150	CALIBRANT_POX-PCR_BLUNT_V002	G1	VIR985	Orthopoxvirus
			VIR966	Alphavirus
300	CALIBRANT_FLUA_mix_1.0	H1	VIR1266	Influenza
			VIR979	Orthopoxvirus

Grouping	pp code	Target	CALIBRANT	Copies
A7	BCT1083	*Ricksettia prowazekii*	BWMPLXCAL: RICK CAL	150
	BCT2012	*Vibrio cholera*		
B7	BCT358	Yersinia pestis/E. coli O	BWMPLXCAL	150
	BCT1071	*Burkholderia mallei*		
C7	BCT1080	*Coxiella burnetii*		
	BCT1075	*Clostridium botulinum*	CBOTCAL: CB CAL	150
D7	BCT2379	*Bacillus anthracis-pX02*	Calibrant_Bacillus_anthracis	150
E7	BCT1112	Brucella melintensis	Calibrant_BACCLADES1	150
F7	BCT2337	Yersinia pestis	Calibrant_BACCLADES1	150
G7	VIR858	Filovirus	CALIBRANT_FILO_PCR_BLUNT_V004	300
	VIR2798	Influenza		
H7	VIR2499	Alphavirus	CALIBRANT_ALPHA-PVIR01PLUS	300
	VIR853	Filovirus		

SNP Assay

Multiplex	Locus	Primer Pair
A	rs13182883	4678
	rs1058083	4932
	rs1821380	4548
	rs214955	4564
	rs7704770	4574
B	rs7205345	4567
	rs987640	4561
	rs985492	4577
	rs1478829	4559
	rs10488710	4538
C	rs6444724	4553
	rs1554472	4570
	rs279844	4539
	rs1410059	4566
	rs2073383	4698
D	rs1523537	4680
	rs13134862	4565
	rs560681	4618
	rs9951171	4683
	rs6811238	5189
E	rs445251	4546
	rs2272998	4560
	rs6591147	4576
	rs3780962	4563
	rs321198	4568
F	rs2503107	4572
	rs1019029	4556
	rs1358856	4547
	rs12997453	5570
	rs740598	4545
G	rs7229946	4550
	rs315791	4627
	rs2567608	4682
	rs447818	4937
	rs1109037	4634
H	rs338882	4958
	rs13218440	4687
	rs1336071	4554
	rs10092491	4544
	rs7520386	4713

rs1058083 + rs13182883 + rs1821380 + rs214955 + rs7704770

rs10488710 + rs1478829 + rs7205345 + rs985492 + rs987640

rs1410059 + rs1554472 + rs2073383 + rs279844 + rs6444724

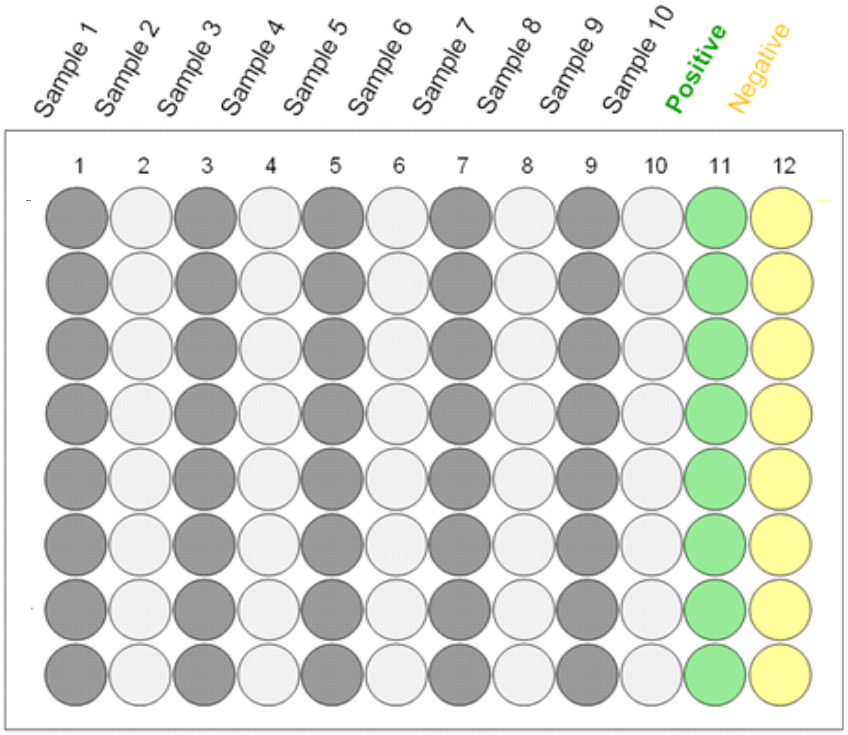
rs13134862 + rs1523537 + rs560681 + rs6811238 + rs9951171

rs2272998 + rs321198 + rs3780962 + rs445251 + rs6591147

rs1019029 + rs12997453 + rs1358856 + rs2503107 + rs740598

rs1109037 + rs2567608 + rs315791 + rs447818 + rs7229946

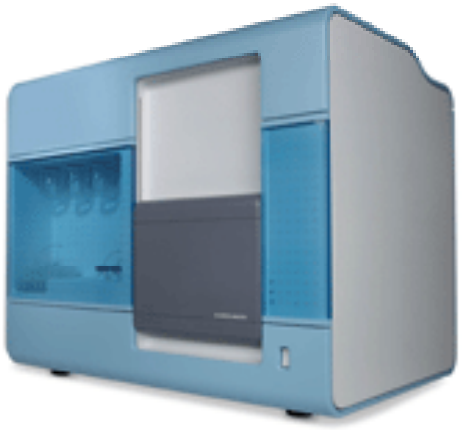
rs10092491 + rs13218440 + rs1336071 + rs338882 + rs7520386



Next Generation Sequencing Platforms

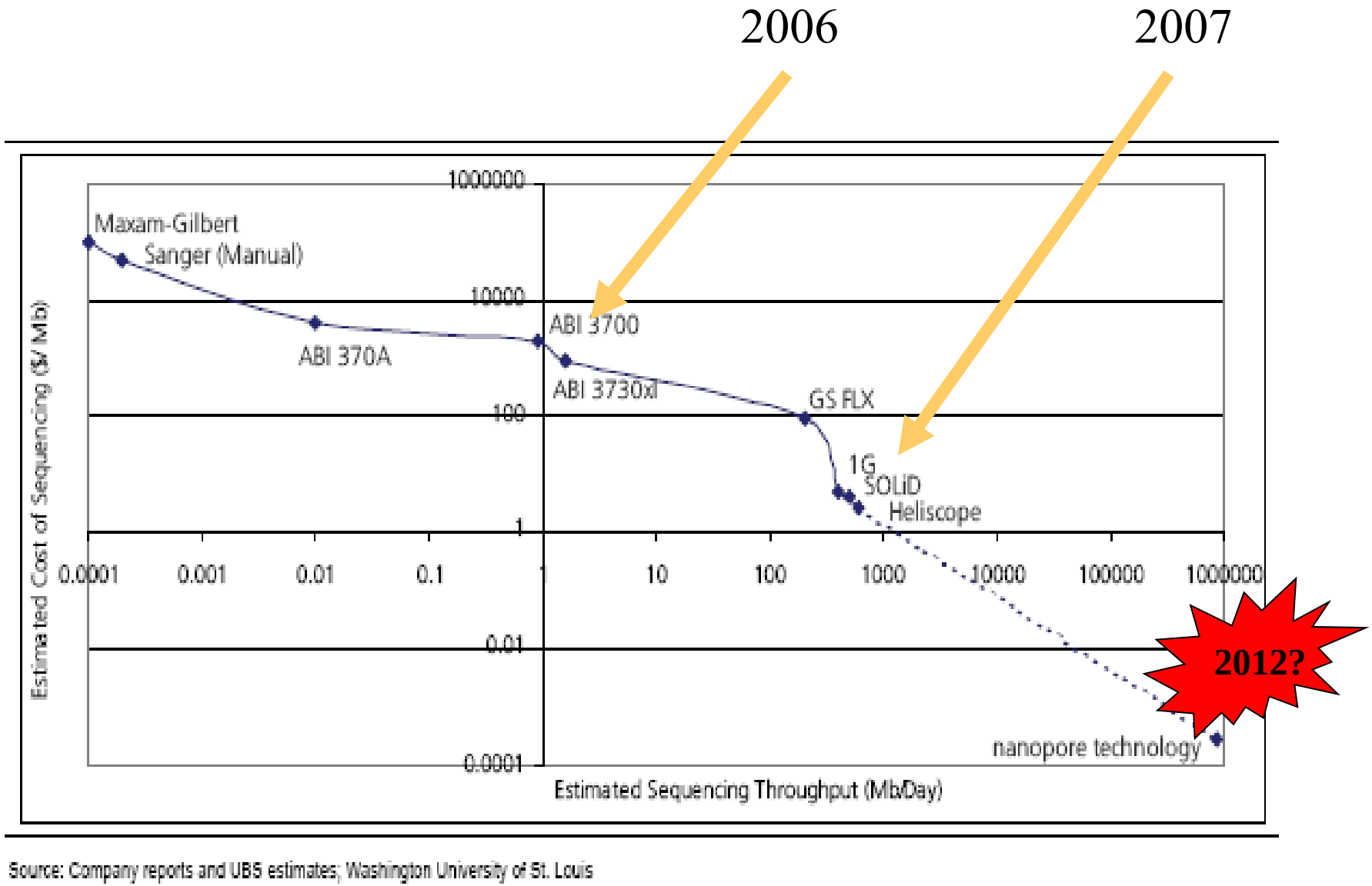
Roche 454

Applied Biosystems SOLiD



Illumina Genome Analyzer

Ion Torrent



Source: Company reports and UBS estimates; Washington University of St. Louis

SOLiD™ Workflow

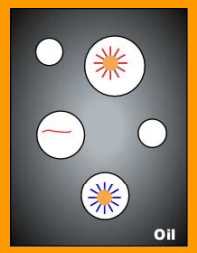
Application specific sample preparation

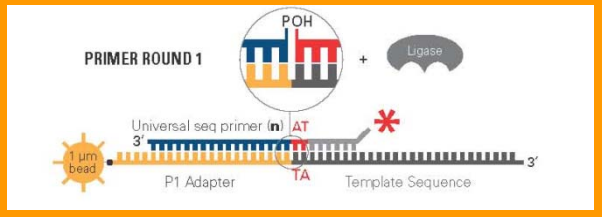
Application specific Data analysis

Emulsion PCR & substrate preparation

Sequencing chemistry

Imaging and analysis





B. anthracis SNPs

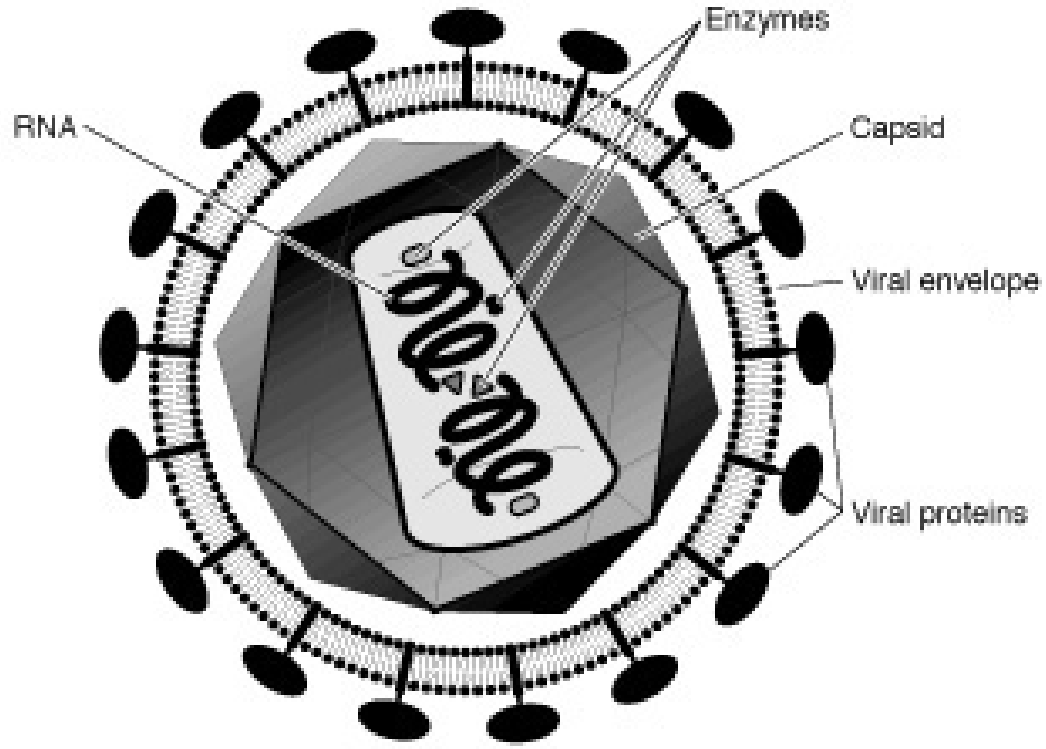
(ambiguities with allelic asymmetry filtered)

	A0032	A0324	A0377	A2012
Chromosome				
SNPs	324	331	434	0
ambiguities	11*	7*	13*	1
total calls	335	338	447	1
pXO1				
SNPs	18	23	26	0
ambiguities	0	1	0	0
total calls	18	24	26	0
pXO2				
SNPs	10	8	11	0
ambiguities	0	0	0	0
total calls	10	8	11	0

* Four shared ambiguities in imperfect repeat region

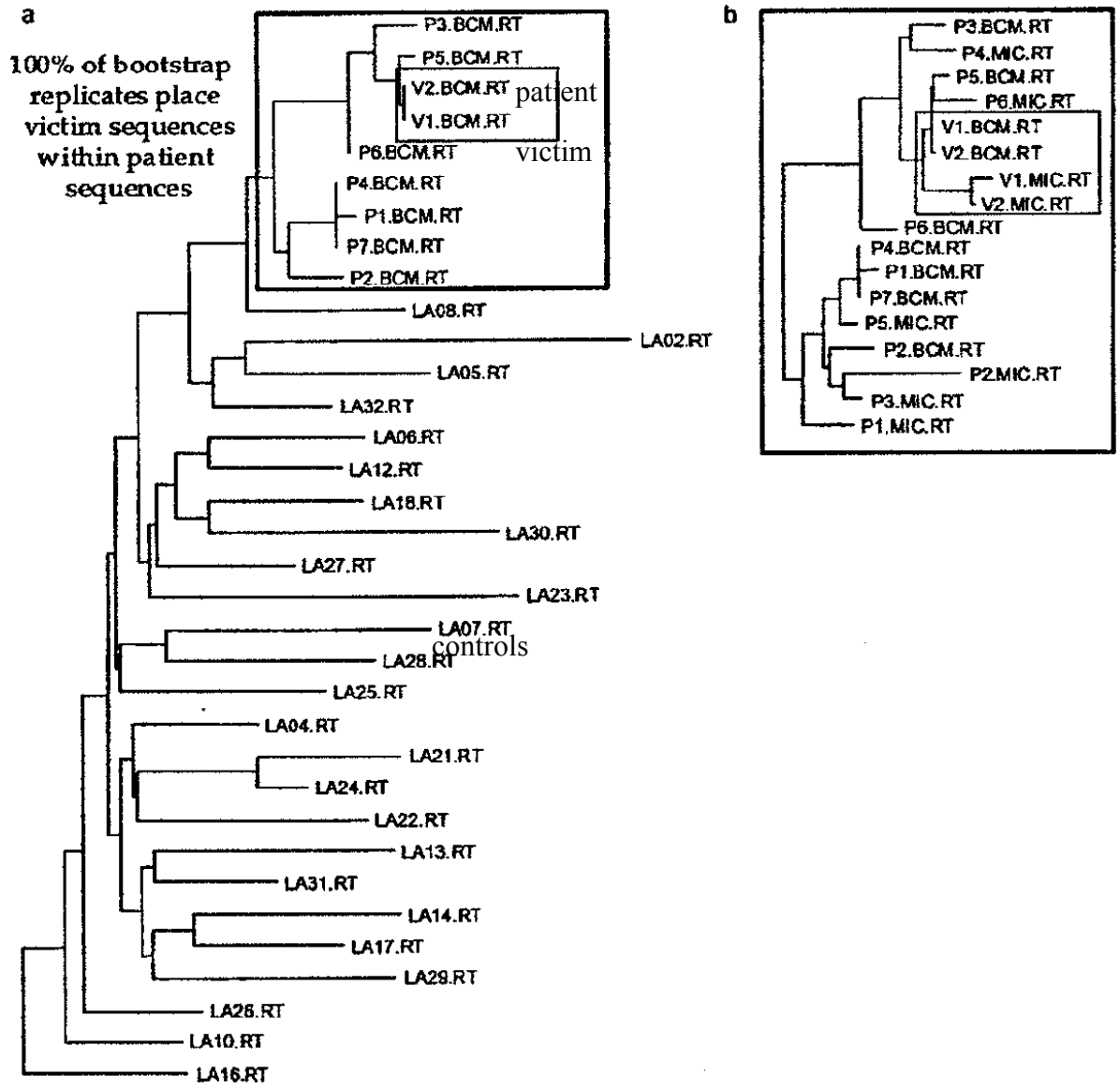
Use of strain identification in sexual assault and child molestation

Molecular Evidence of HIV-1 Transmission in Criminal Cases



Structure of Human Immunodeficiency Virus (HIV)

RT region



Victim sequences embedded in patient sequences

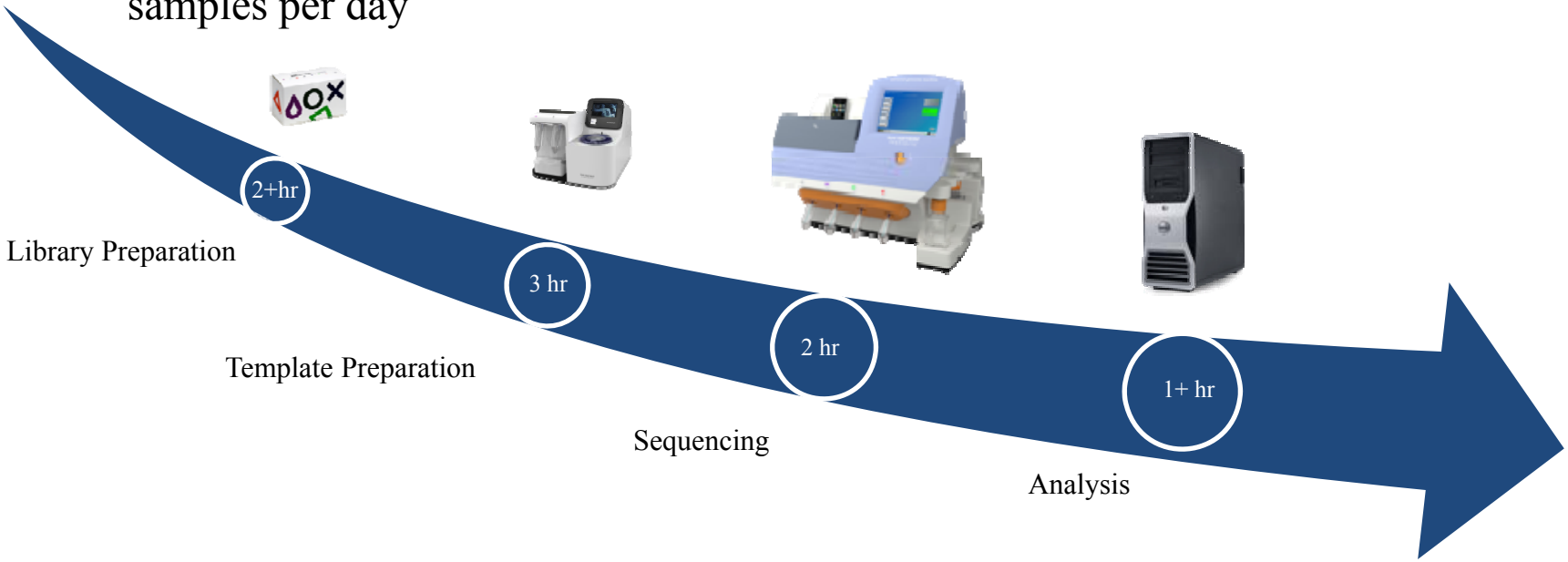
Dr. Schmidt was found guilty of second degree attempted murder and is serving a 50 year sentence

- The admissibility of the conclusion that the HIV samples were closely related was challenged on appeal
- Use of DNA evidence is well-established in Louisiana, but its use to establish similarities between viral infections was without precedent (Note: no statistical strength)
- The appeal was rejected by the Louisiana State Supreme Court in 2000
- The case was then appealed to the United States Supreme Court, and the appeal was rejected March, 2002

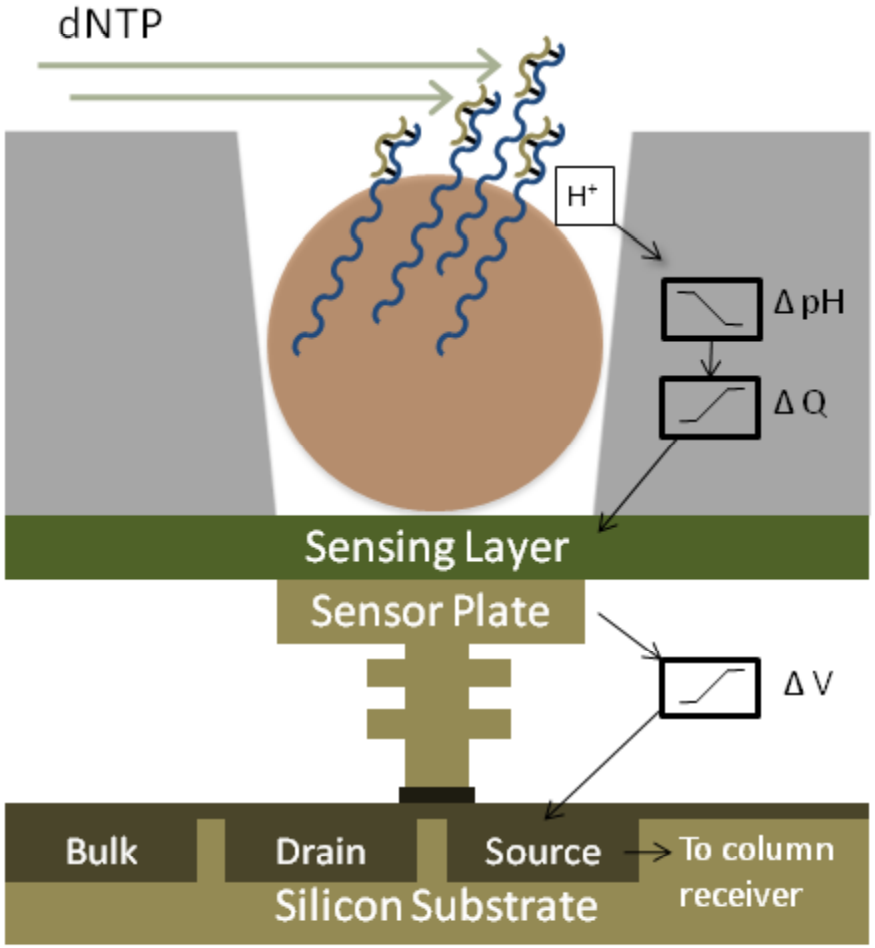
Ion Torrent

Single Day Workflow

- ~2 hour sequencing runs – enabled by PostLight™ Sequencing
- Innovative automated template preparation for PGM sequencer matches the speed of semiconductor sequencing
- Complete end-to-end workflow within 1 day or multiple samples per day

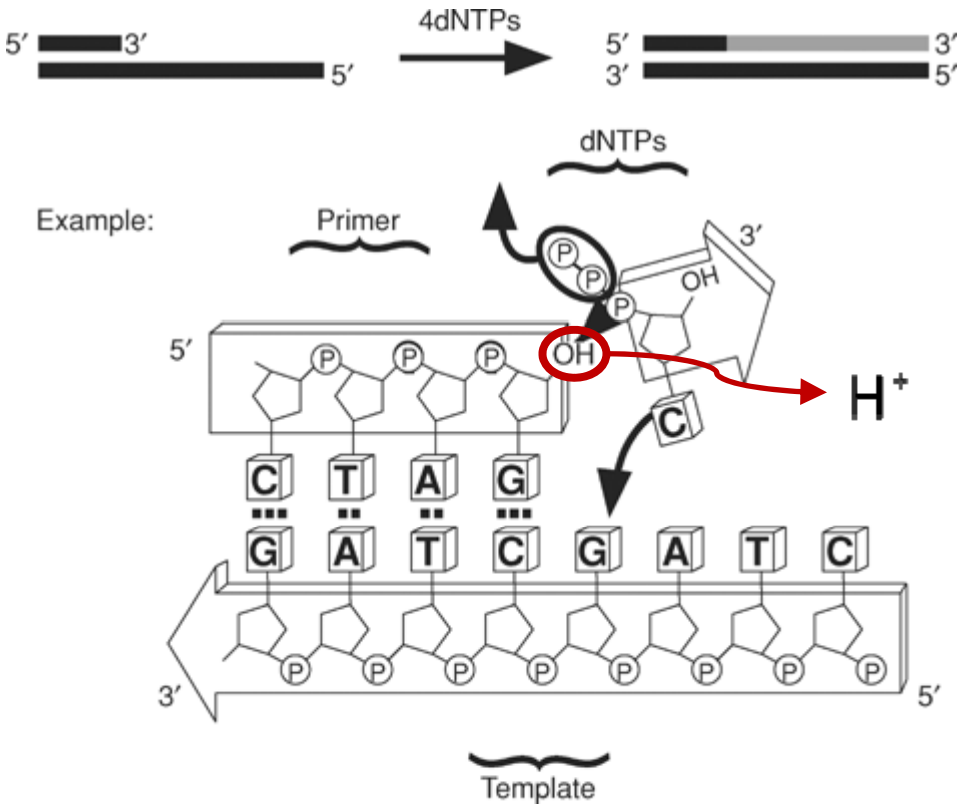


Schematic cross-section of a single well of an Ion Torrent sequencing chip



Semi-conductor technology

Chemistry



Eliminate source of sequencing errors:

- Modified bases
- Fluorescent bases
- Laser detection
- Enzymatic amplification cascades

Eliminate source of read length limitations:

- Unnatural bases
- Faulty synthesis
- Slow cycle time

Delivers highly uniform genome coverage

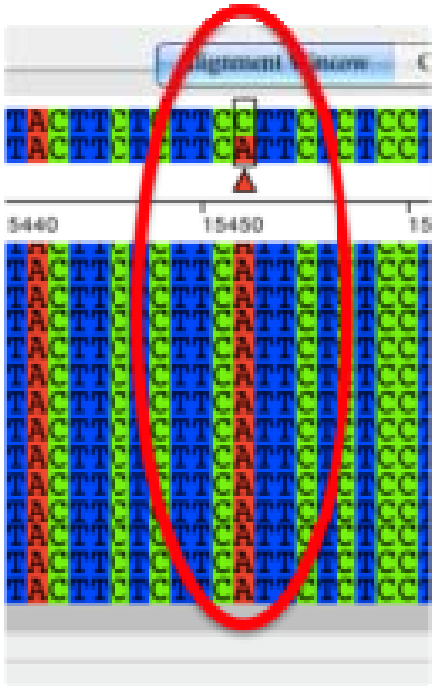
- In principle similar to pyrosequencing
- But simpler

Human Mitochondrial Sequencing

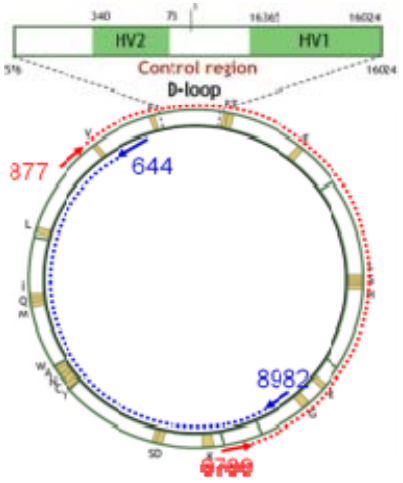
- Deep sequence for heteroplasmy detection
(> 1000x coverage on Ion 314)

- Ability to do 16 samples per run with barcoding

- Accurate variant calling, especially in hypervariable regions of mitochondria



Mutation detected on position 15450
Prof/. Stefan Schuster Penn. State University

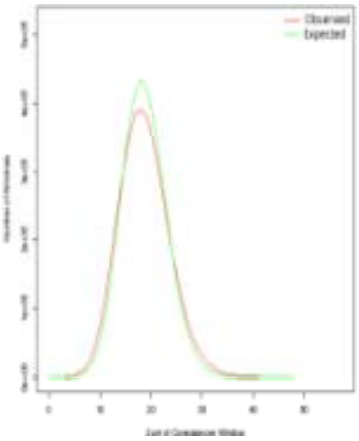


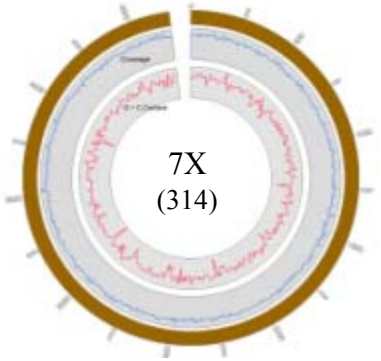
Amplify mtDNA via two overlapping long range PCR
Fragment via mechanical or enzymatic shearing

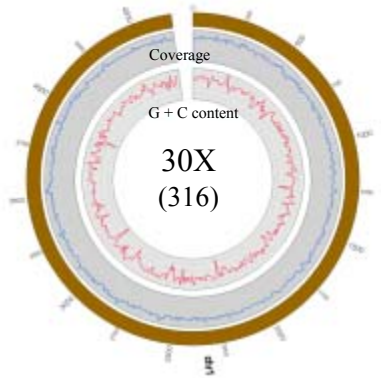
Library | **Template** | **Sequence** | **Analysis**

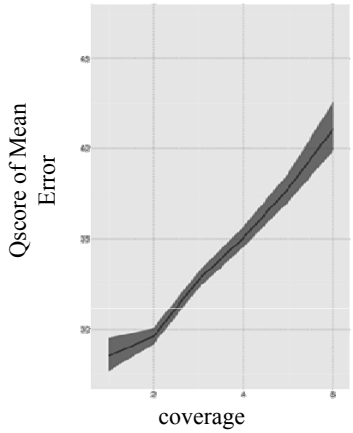
Microbial Sequencing

- Highly uniform coverage (equivalent to predicted) allows more efficient sequencing
- Up to 99.999% consensus accuracy
- 100 bp runs today (200 bp late 2011)







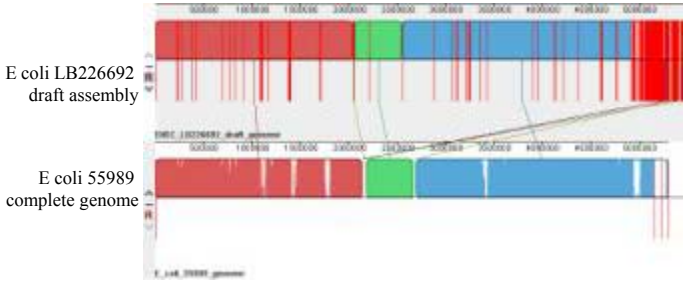


Fragment via mechanical or enzymatic shearing

European *E. coli* Outbreak Strain Identified using Ion PGM™ in 3 days

Monday May 30*	Library preparation	O104:H4 and HUSC41 samples (reference) strain libraries prepared
Tuesday May 31	Sequencing runs	0104:H4 amplified and sequenced 2 x 2 runs (Ion 314)
Wednesday June 01	Sequencing runs	0104:H4 sequenced 3 x 2 runs (Ion 314)
Thursday June 02	Assembly	Draft Genome identified, Assembled, Submitted and Released from NCBI
Friday June 03	Assay Design	TaqMan Assays Designed

*May 22 CEDC reports significant increase in patients with hemolytic uremic syndrome



A%	C%	G%	T%	Sum Contig Length (bp)	Num Contigs	Mean Contig Length (bp)	Median Contig Length (bp)	N50 Contig Length (bp)	Max Contig Length (bp)
24	25	25	24	5,450,264	364	14,973	762	181,540	475,662

Life Technologies Assembly

"The biggest advantage [of the PGM] from my point of view as a public health official is that it's speedy, and speed is what is needed at the moment,"

Prof. Dr. Med Dag Harmen, University Hospital Muenster

"[The PGM] takes the shortest time to generate genomic data."

Junjie Qin, BGI

ACKNOWLEDGMENTS

- Angela van Daal
- Life Technologies
- Promega Corporation
- Abbott/Ibis
- Illumina