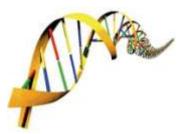


International Symposium on Forensic DNA in Law

Seoul, Republic of Korea November 7-8, 2012



The Future of Forensic DNA

John M. Butler, PhD

National Institute of Standards and Technology Gaithersburg, Maryland, **United States of America**





Presentation Outline

Introduction to NIST

- Our role with forensic DNA in the United States
- Some current projects

Near-term future

- New autosomal STR loci for expanded core loci
- Expanded use of databases (e.g., familial searching)
- Rapid DNA testing

More distant future

- Loci besides STRs for identity testing?
- Phenotyping capabilities?
- Next-generation DNA sequencing?

NIST History and Mission

- National Institute of Standards and Technology (NIST) was created in 1901 as the National Bureau of Standards (NBS). The name was changed to NIST in 1988.
- NIST is part of the U.S. Department of Commerce with a mission to develop and promote measurement, standards, and technology to enhance productivity, facilitate trade, and improve the quality of life.
- NIST supplies over 1,300 Standard Reference Materials (SRMs) for industry, academia, and government use in calibration of measurements.
- NIST defines time for the U.S.

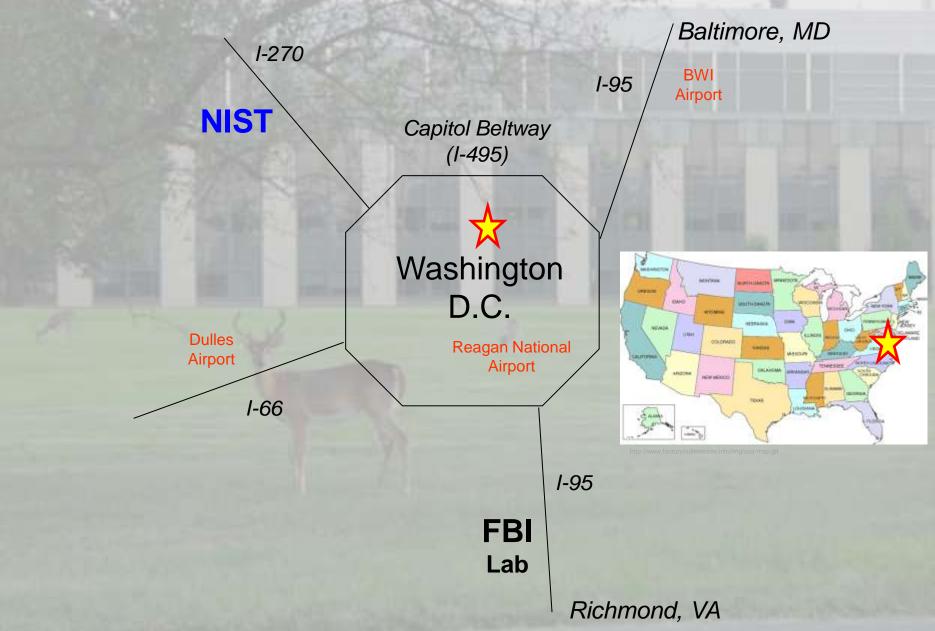


\$686 for 3 jars



DNA typing standard

Location of NIST



NIST Today

Major Assets

- ~ 2,900 employees
- ~ 2600 associates and facilities users
- ~ 400 NIST staff on about 1,000 national and international standards committees
- 4 Nobel Prizes in Physics in past 15 years (including 2012 to David Wineland for quantum physics)



Major Programs

- NIST Laboratories
- Baldridge National Quality Program
- Hollings Manufacturing Extension Partnership
- Technology Innovation Program

Joint NIST/University Institutes:

- JILA
- Joint Quantum Institute
- Institute for Bioscience & Biotechnology Research
- Hollings Marine Laboratory



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Group Leader NIST Applied Genetics Group





John Butler

Mike Coble



Margaret Kline



Marcia Holden



Pete Vallone





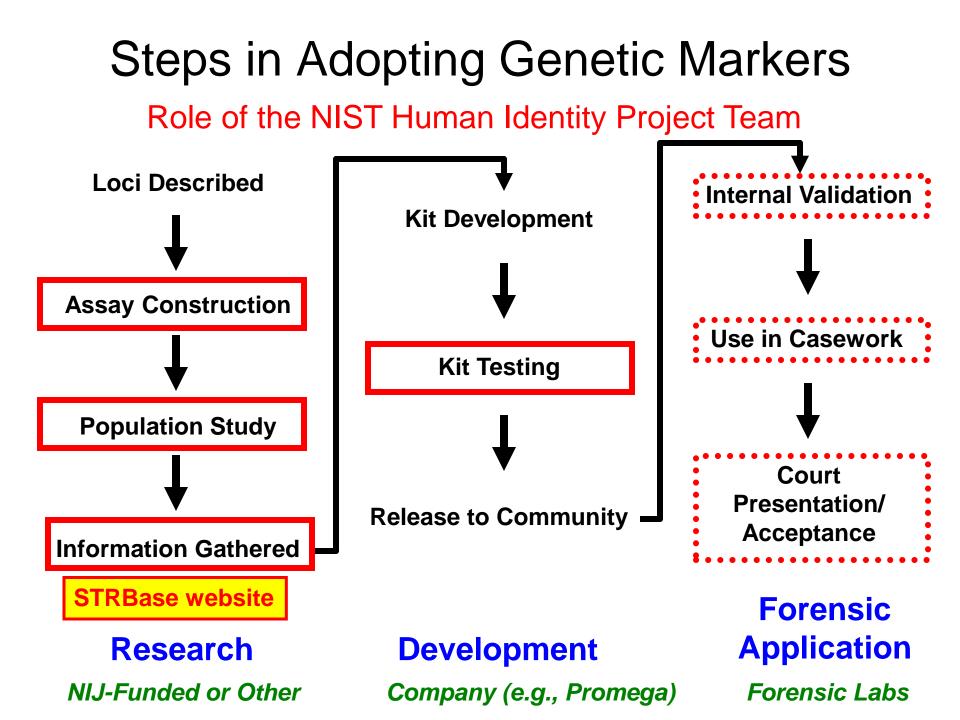






Patti RohmillerBeckyRossEricaKevinOffice ManagerHillHaynesButtsKiesler

Bringing calibration to clinical DNA diagnostics, speed to DNA testing, and technology to the scales of justice



Current Activities at NIST

Standard Reference Materials

- SRM 2372 (DNA quantitation standard)
- SRM 2391c (STR typing)

Technology Evaluation and Development

- Rapid multiplex PCR protocols (multiplex STR amplification in <35 min)
- Low-level DNA studies
- Mixture interpretation research and training materials
- Unusual STR allele characterization
- New STR loci and assays (STR 26plex, kit concordance, InDels & SNPs)

Training Materials

- Workshops on mixture interpretation and CE troubleshooting
- Third edition of *Forensic DNA Typing* textbook (2009, 2011, & 2013)

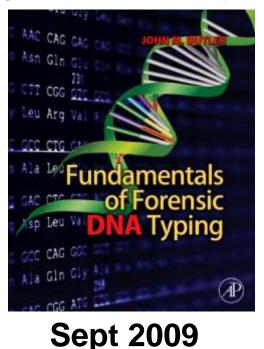
Forensic DNA Typing Textbook 3rd Edition is Three Volumes

Now part of my job at NIST (no royalties are received)



John Butler

For beginning students, general public, & lawyers



~500 pages

Advanced Topics in FORENSIC DNA TYPING: DNA TYPING: DNA TYPING: CCC CAG GOC La Gin Giv

August 2011 ~700 pages

Currently being written Advanced Topics in FORENSIC **DNA TYPING: INTERPRETATION** John M. Butler C CAG GO Ala Gln Gly

> 2013 ~500 pages

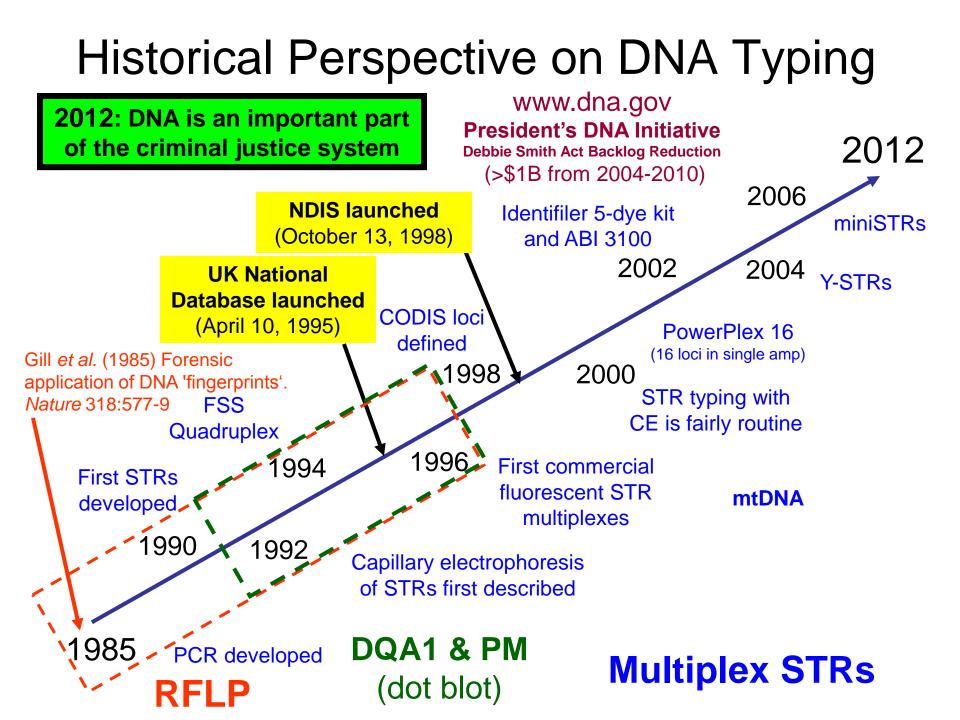
Value of a Historical Review

"If you want to understand today, you have to search yesterday." **Pearl Buck**

- Attributed to Pearl Buck

(http://www.quotegarden.com/history.html)





Stages of Forensic DNA Progression

Stages	Time Frame	Description
Exploration	1985-1995	Beginnings, different methods tried (RFLP and early PCR)
Stabilization	1995-2005	Standardization to STRs, selection of core loci, implementation of Quality Assurance Standards
Growth	2005-2012	Rapid growth of DNA databases, extended applications pursued
Sophistication	The Future	Expanding tools available, confronting privacy concerns

National Commission on the Future of DNA Evidence



U.S. Department of Justice Office of Justice Programs Natural Justice of Justice



The Future of Forensic DNA Testing

Predictions of the Research and Development Working Group

A Report Fro

•Report published in Nov 2000

•Asked to estimate where DNA testing would be 2, 5, and 10 years into the future

Conclusions

STR typing is here to stay for a few years because of DNA databases that have grown to contain millions of profiles

http://www.ojp.usdoj.gov/nij/pubs-sum/183697.htm

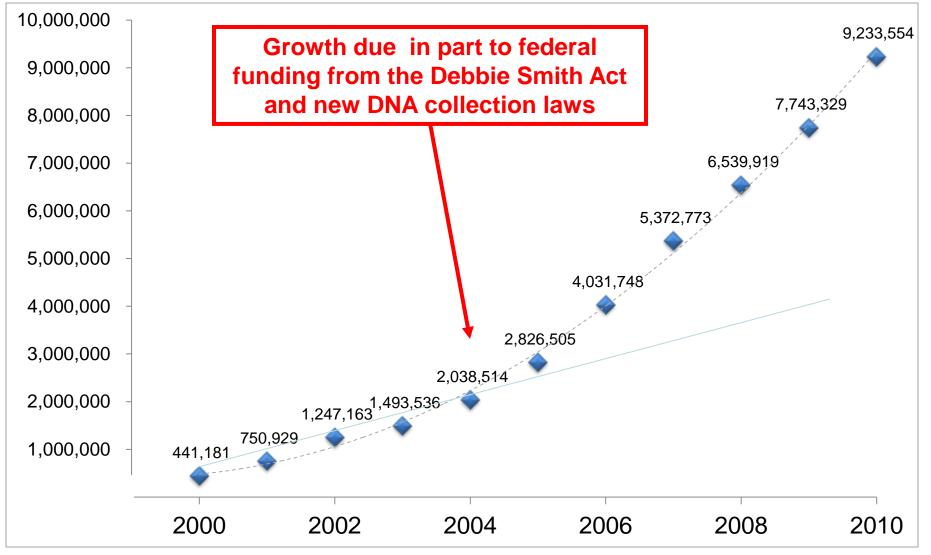
Growth in Numbers of DNA Profiles Present in Various NDIS Indices

(cumulative totals by year)

Year ending Dec 31	Forensic	Convicted Offender	Arrestee	Total Offender*
2000	21,625	441,181		441,181
2001	27,897	750,929		750,929
2002	46,177	1,247,163		1,247,163
2003	In the les	t two wooro	of data (2)	
2004			•	<u>009 & 2010)</u> :
2005		<mark>forensic</mark> sa		
2006	2,693,63	5 offender :	samples a	dded
2007	203,401	5,287,505	85,072	5,372,773
2008	248,943	6,398,874	140,719	6,539,919
2009	298,369	7,389,917	351,926	7,743,329
2010	351,951	8,559,841	668,849	9,233,554

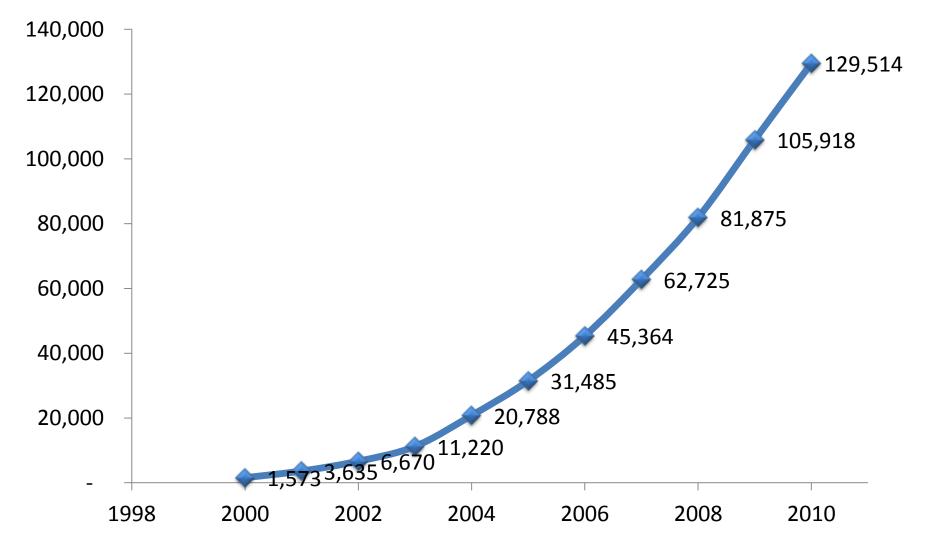
Source: FBI Laboratory's CODIS Unit

Number of Offender DNA Profiles in the U.S. National DNA Database



Source: FBI Laboratory's CODIS Unit

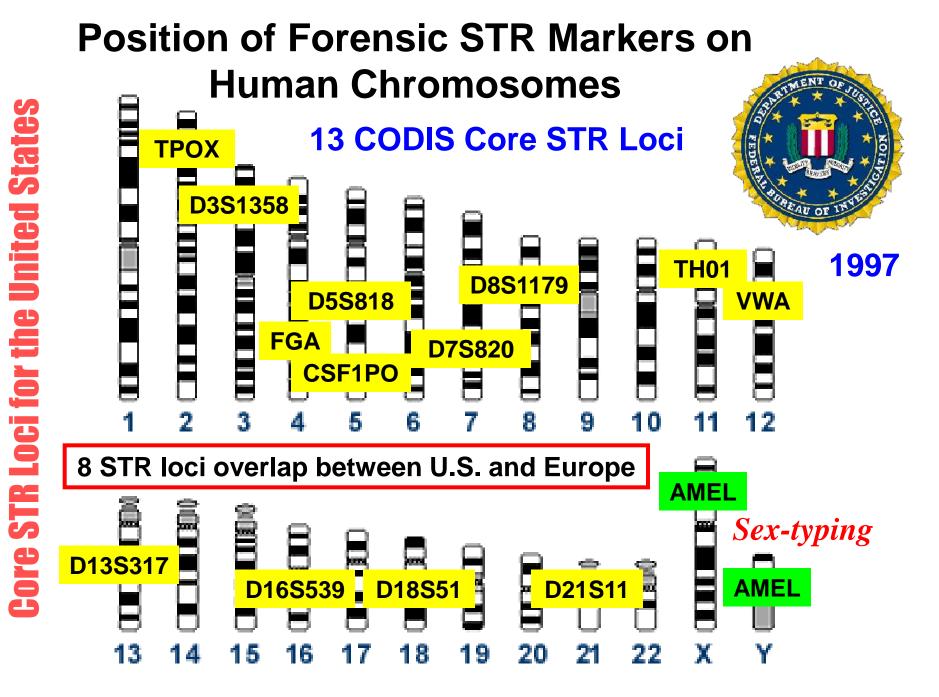
Number of Investigations Aided in the U.S. National DNA Database



Source: FBI Laboratory's CODIS Unit

Growth of DNA Databases

- Within the U.S., we have benefited from significant federal funding over the past decade
- Expanded laws now enable more offenders to be included (currently 26 states and federal government have laws to collect DNA from arrestees)
- Have effectively locked technology with core STR markers used to generate DNA profiles that now number greater than 10 million profiles



Expanding the U.S. CODIS Core Loci

D.R. Hares (2012) Expanding the CODIS Core Loci in the United States. *Forensic Sci. Int. Genet.* 6(1): e52-e54 Addendum to expanding the CODIS core loci in the United States, Forensic Sci. Int. Genet. (2012) 6(5): e135



Letter to the Editor

Expanding the CODIS core loci in the United States

CODIS Core Loci Working Group

Formed in May 2010 to make recommendations to FBI CODIS Unit

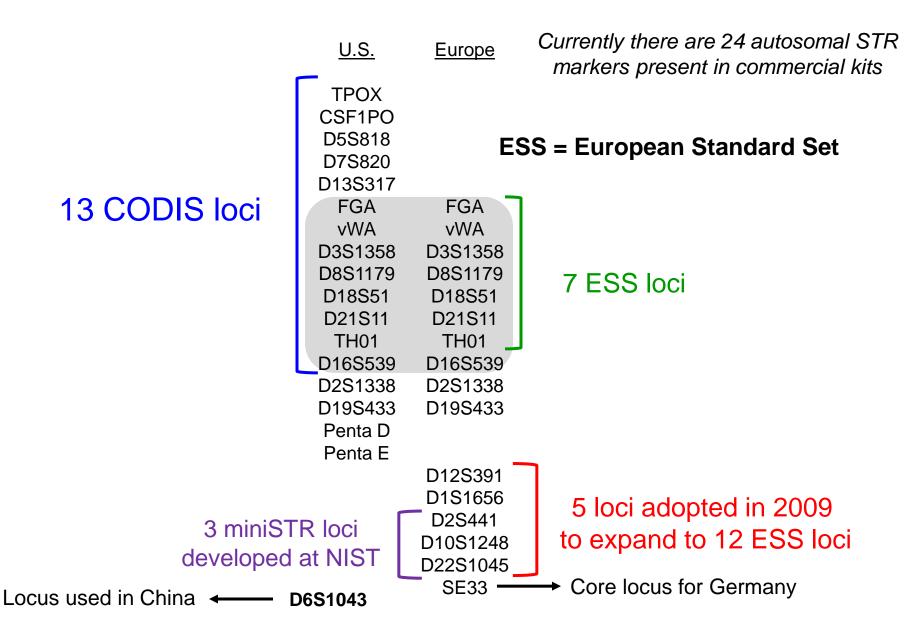
Douglas Hares (Chair) – FBI John Butler – NIST Cecelia Crouse – FL PBSO Brad Jenkins – VA DFS Ken Konzak – CA DOJ Taylor Scott – IL SP major reasons for expanding the CODIS core loci in the United States:

- (1) To reduce the likelihood of adventitious matches [7] as the number of profiles stored at NDIS continues to increase each year (expected to total over 10 million profiles by the time of this publication). There are no signs that this trend will slow down as States expand the coverage of their DNA database programs and increase laboratory efficiency and capacity.
- (2) To increase international compatibility to assist law enforcement data sharing efforts.
- (3) To increase discrimination power to aid missing persons cases.

Three major reasons for expanding the CODIS core loci in the United States D.R. Hares (2012) Forensic Sci. Int. Genet. 6(1):e52-e54

- To reduce the likelihood of adventitious matches as the number of profiles stored at NDIS continues to increase each year
- To increase international compatibility to assist law enforcement data sharing efforts
- To increase discrimination power to aid missing persons cases

International Comparability



Determination of Additional CODIS Core Loci

D.R. Hares (2012) Expanding the CODIS Core Loci in the United States. *Forensic Sci. Int. Genet.* 6: e52-e54 *Addendum to expanding the CODIS core loci in the United States*, Forensic Sci. Int. Genet. (2012) doi:10.1016/j.fsigen.2012.01.003

What	Why	Who/How	When
Form a Working Group (WG) to discuss initial selection	Establishes target goals	CODIS Core Loci Working Group with FBI Chair and 5 members; Web meetings	May 2010 - present
Announce proposed additional CODIS core loci	Sets desired target goals and informs manufacturers	WG Chair; Publish proposed listing of CODIS core loci	April 2011 online (published Jan 2012)
Ongoing Progress Reports	Provides updates for DNA community	WG Chair; Present updates on status of CODIS Core Loci project at meetings	2010-2012
Implementation Considerations & Strategy	Identify issues for implementation and timeline	WG	June 2011 - present
Manufacturers develop prototype kits	Creates tools to meet target goals	Manufacturers; Provide status reports to WG for timeline	2011-2012
Test and validate prototype kits	Examines if target goals can be met	Validation Laboratories; Follow QAS compliant validation plan	Beginning in 2012
Review and evaluate data from validation	Evaluates if desired performance is obtained	NIST, SWGDAM and FBI; Provide feedback, if any, to Manufacturers	In conjunction with and at the conclusion of validation
Selection of new CODIS core loci	Allows protocols to be established	FBI; seek input from DNA community and stakeholders; Notify Congress	After evaluation of validation data and kit production factors
Implementation of new CODIS core loci at the National DNA Index System	Enables target goals to be met	All NDIS-participating labs	~ 24 months after selection of new CODIS core loci

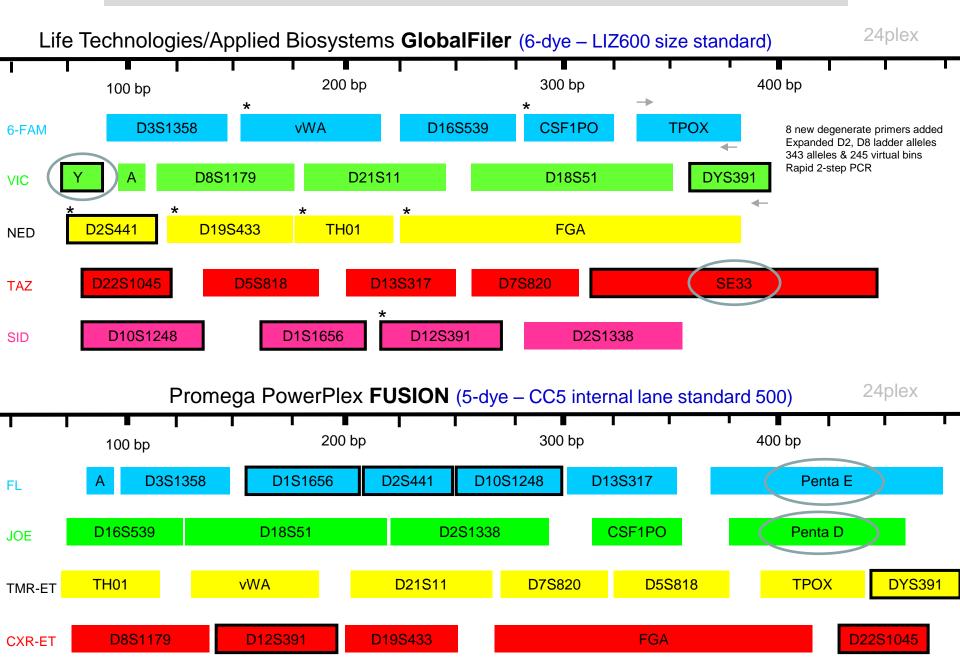
http://www.fbi.gov/about-us/lab/codis/planned-process-and-timeline-for-implementationof-additional-codis-core-loci

STR Loci Covered in Currently Available Commercial Kits

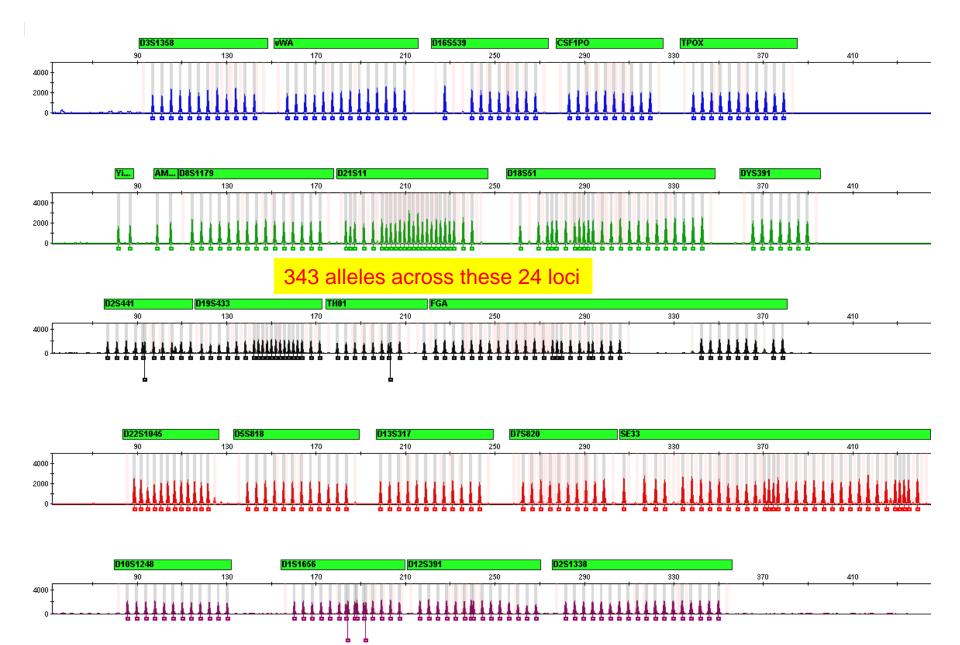
	present)	present) e)	esent)			16	17			usio 1ple								GlobalFiler 24plex								
Chr	Locus	CODIS 13 (US 1997-present)	CODIS 20 (US future)	ESS 12 (EU 2009-present)	PowerPlex 16	PowerPlex 18D	PowerPlex ESI/ESX 16	PowerPlex ESI/ESX 17	PowerPlex 21	PowerPlex CS7	PowerPlex Fusion	Profiler Plus	SGM Plus			MiniFiler	Identifiler	NGM	NGM SElect	GlobalFiler	ESSplex	ESSplex SE	Hexaplex ESS	Nonaplex ESS	Decaplex SE	IDplex
		re	equire	əd		P	rome	ga S	TR k	its			 Life	Tech	nolo	gies (A	<i>BI)</i> S	TR k	its			Qiagen STR kits				
1q	D1S1656																									
1q	F13B																					 				
2р	TPOX																									
2р	D2S441																									
2q	D2S1338																									
Зр	D3S1358																									
4q	FGA																									
5q	CSF1PO																									
5q	D5S818																									
6р	F13A01																									
6q	D6S1043																									
6q	SE33																									
7q	D7S820																									
8p	LPL																									
8q	D8S1179																									
9p	Penta C																									
10q	D10S1248																									
11p	TH01																									
12p	D12S391																									
12p	WA																									
13q	D13S317																									
15q	FESFPS																					1				
15q	Penta E		1	1																		1	1			
16q	D16S539																									
18q	D18S51																									
19q	D19S433																									
21q	D21S11																									
21q	Penta D																									
22q	D22S1045																-									
Xp, Yp	Amelogenin																									
Yq Yq	DYS391																									
14	010001	I					I										L	I				L	<u> </u>			

Butler, J.M., & Hill, C.R. (2013) *Topics on Forensic DNA Analysis: Current Practices and Emerging Technologies* (CRC Press). Chapter 9. Biology and Genetics of New Autosomal STR Loci Useful for Forensic DNA Analysis (in press)

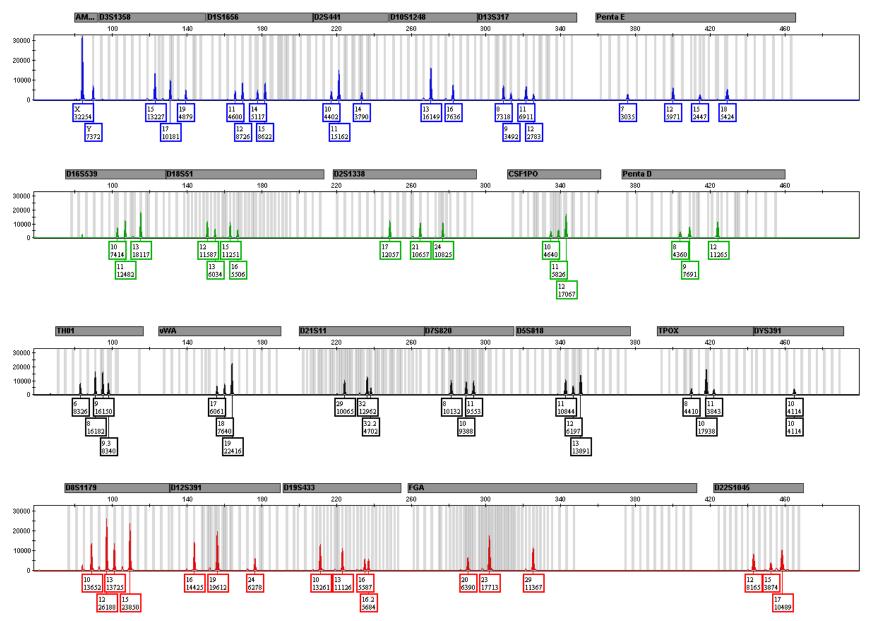
STR Kit Layouts by Dye Label and PCR Product Size



GlobalFiler Allelic Ladder

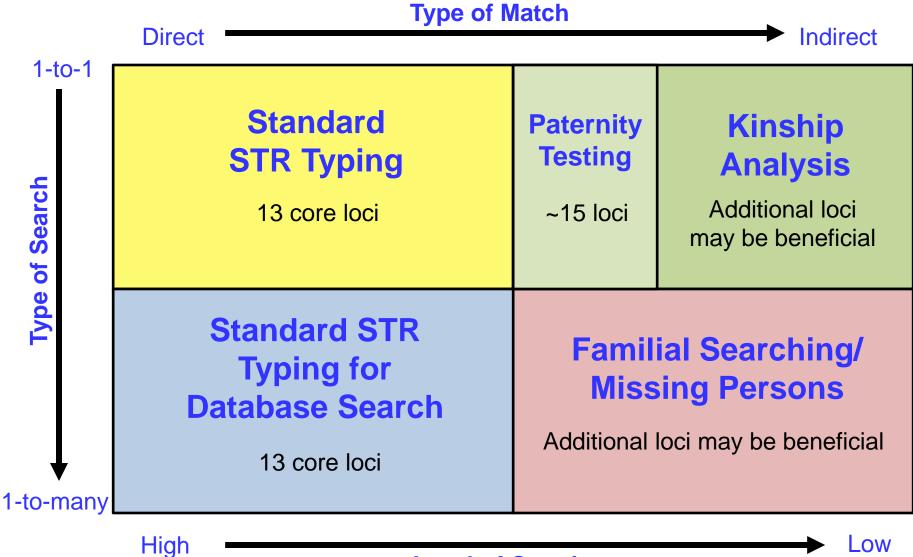


DNA Mixture Detected with PowerPlex Fusion (24plex STR kit)



Size standard not shown

Expanding the Forensic Core Competency



Level of Certainty



Slide originally from Kristen O'Connor (NIST) presentation at 21st International Symposium on Human Identification

Familial Searching in the U.S.

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

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

July 25th, 2010 8:23 am CT

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

DNA DATABASE Milwaukee police on hunt for serial killer linked to 7 deaths

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

Familial DNA hunt sought in East Coast rape case

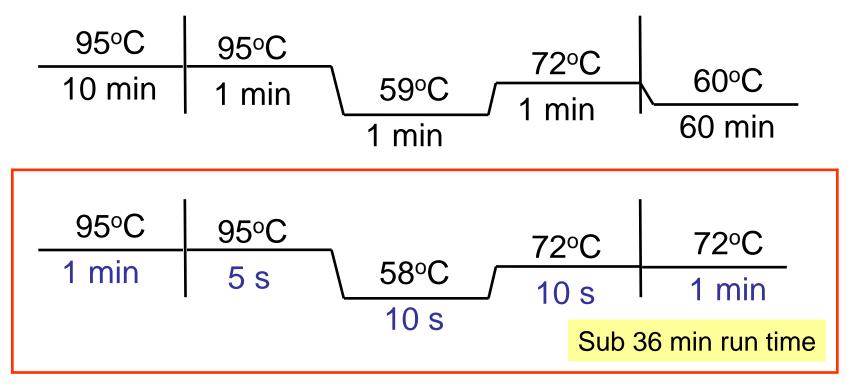
http://www2.insidenova.com/news/2010/aug/04/familial_dna_hunt_sought_in_east_coast_rape_case-ar-428231/

March 21, 2011 Virginia announced familial searching capability Wednesday December 1, 2010

Virginia could become 3rd state to use familial DNA searches

Some concerned practice could stigmatize those related to criminals

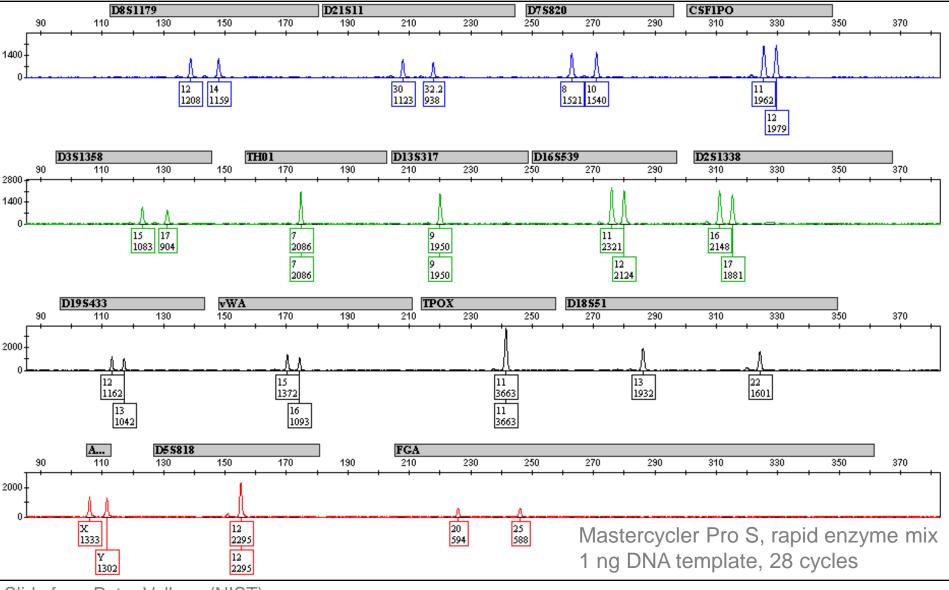
Rapid PCR Thermal Cycling Profile Identifiler STR kit 28 cycles of PCR



Maximum heating/cooling rate of ~2 to 6°C/s (cycler dependent)

Slide from Peter Vallone (NIST)

Full Identifiler STR Profile with 19 min PCR



Slide from Peter Vallone (NIST)

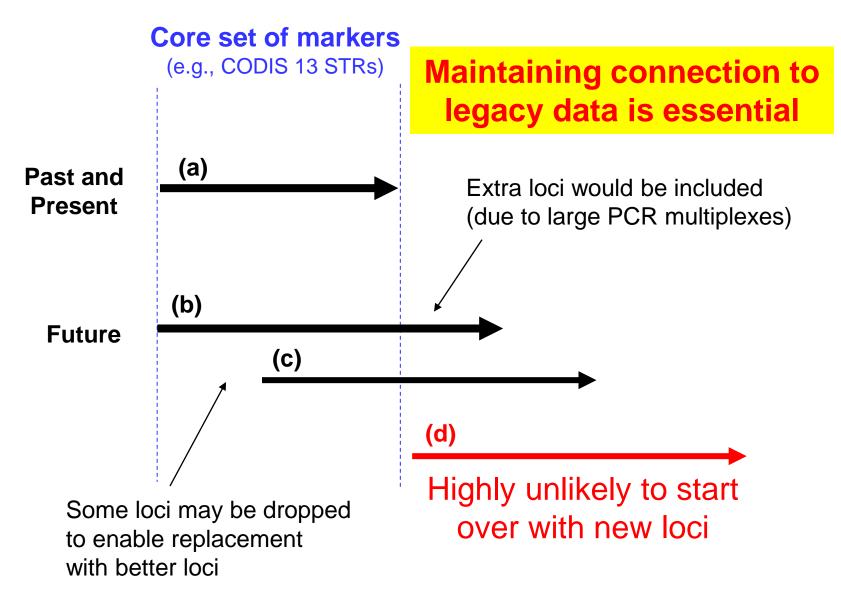
Potential Applications with Rapid PCR Capabilities

- Improve overall laboratory throughput
 - Multiplex PCR amplification is already in many situations the longest part of the DNA analysis process (depending on DNA extraction and DNA quantitation methods)
 - With increased use of robotic sample preparation and expert system data analysis, bottleneck for sample processing will shift to time for PCR amplification...
- Enable new potential DNA biometric applications (because the overall DNA analysis process is faster)
 - Permit analysis of individuals at a point of interest such as an embassy, an airport, or a country border

A "Crystal Ball" to the Future?



Possible scenarios for extending sets of genetic markers to be used in national DNA databases



STRs vs SNPs Article

Butler et al. (2007) STRs vs SNPs: thoughts on the future of forensic DNA testing. Forensic Science, Medicine and Pathology 3:200-205.

Forensic Sci Med Pathol (2007) 3:200–205 DOI 10.1007/s12024-007-0018-1

ORIGINAL PAPER

STRs vs. SNPs: thoughts on the future of forensic DNA testing

John M. Butler · Michael D. Coble · Peter M. Vallone

- SNPs are unlikely to replace STRs for routine forensic DNA testing due to challenges with high-level multiplexing and mixture detection/interpretation
- Most likely use of SNPs will be as ancestry-informative markers (AIMs) for sample ethnicity estimation



Compromised Sample Improvements

- Better DNA extraction/recovery
- Continued use of miniSTRs
 - to improve success rates for recovery of information from compromised DNA evidence
- Replicate results for reproducibility
 - to improve reliability with low-template DNA testing

Going Beyond the Core Competencies of Forensic DNA Testing

Core Competency

Standard STR Typing (DNA Profile)

Sufficient DNA quantity (ng)

Lower amounts of DNA being tested Direct Matching (or Parentage)

Challenging kinship search questions

Familial Searching Attempts (fishing for brothers or other relatives)

Solution: Additional Markers (Y-chromosome, more STRs) and Multiple Reference Samples

Touch DNA Attempts (poor quality, mixtures, low-level stochastic effects)

Solution: Replicate Testing

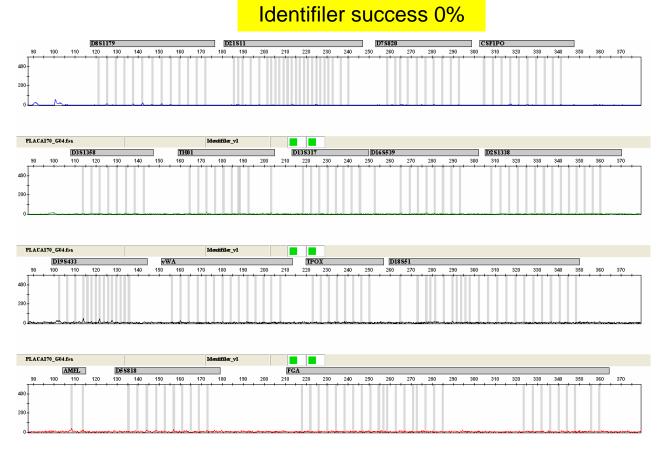
Be very cautious when outside the box...



Highly degraded DNA

SNP genotyping in an extreme degradation case Corpse half buried in a forest for ten years

- Uncovered by a forest fire
- Calcinated remains



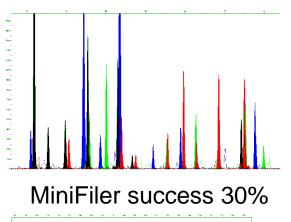


Highly degraded DNA

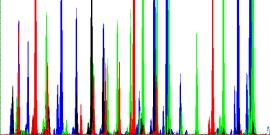
SNP genotyping in extreme degradation case Corpse half buried in a forest for ten years

- Uncovered by a forest fire
- Calcinated remains

HID 52plex Auto 1: success 100%







P: - 99.993

+SNPs

STRs

Slide from Manuel Fondevila (NIST, USC)

Geographical Origin Prediction

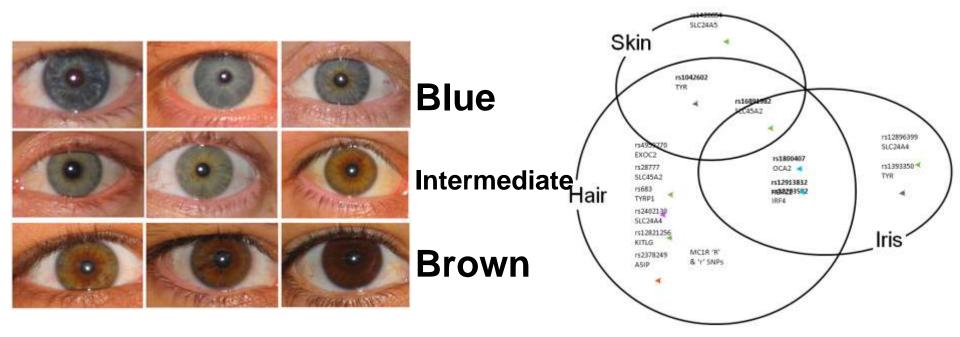


- Lao O, van Duijn K, et al. (2006) Proportioning whole-genome single-nucleotide-polymorphism diversity for the identification of geographic population structure and genetic ancestry. Am J Hum Genet 78: 680-90.
- Phillips, C., Salas, A., et al. (2007) Inferring ancestral origin using a single multiplex assay of ancestry-informative marker SNPs. FSI: Genetics 1: 273-280.
- Halder, I., Shriver, M., et al. (2008) A Panel of Ancestry Informative Markers for Estimating Individual Biogeographical Ancestry and Admixture From Four Continents: Utility and Applications. Hum Mut 29: 648-658.
- Pereira R., Phillips C., et al. (2012) Straightforward inference of ancestry and admixture proportions through ancestry-informative insertion deletion multiplexing. PLoS One;7(1):e29684.

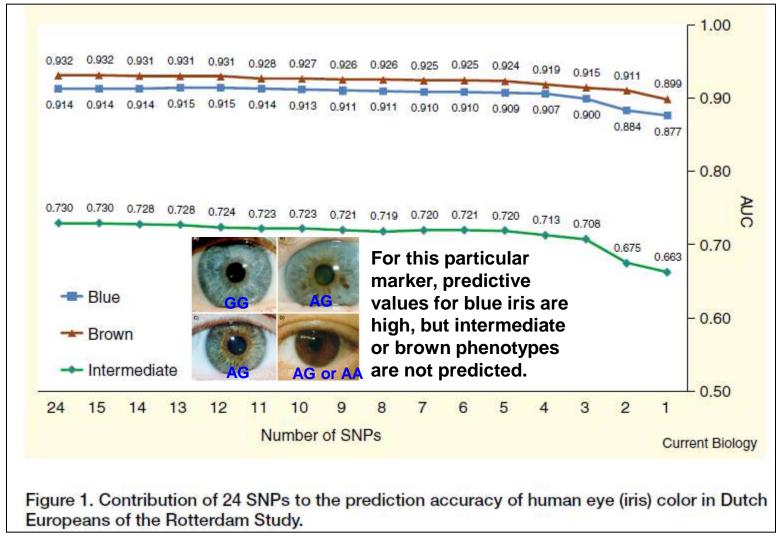
Phenotypic Trait Prediction

Traits of interest

- Traits whose variation may be classified on discreet categories.
- Regulated by a relatively low number of genes.
- Fine example: Iris and hair pigmentation.



Phenotypic trait prediction



Liu F., et al. (2009). Eye color and the prediction of complex phenotypes from genotypes, Curr. Biol. 19:R192–R193

Phenotypic trait prediction

- Currently several research groups are working on the prediction of phenotypical traits by SNP typing.
- Best predictions have been achieved on iris pigmentation.
- However the achieved predictive values are still different for each variant. Research is not yet completed.
- Branicki W, Kayser M et al. (2011). Model-based prediction of human hair color using DNA variants. *Human Genetics;* DOI 10.1007/s00439-010-0939-8.
- Walsh S., et al. (2010) IrisPlex: A sensitive DNA tool for accurate prediction of blue and brown eye colour in the absence of ancestry information. Forensic Sci. Int. Genet. (Epub)
- Kayser M., Schneider P.M. (2009) DNA-based prediction of human externally visible characteristics in forensics: motivations, scientific challenges, and ethical considerations. Forensic Sci. Int. Genet. 3(3):154-61.
- Ruiz Y., C. Phillips et al.(2012) Further development of forensic eye color predictive tests. Forensic Sci. Int. Genet. (accepted for publication).

NGS Platforms

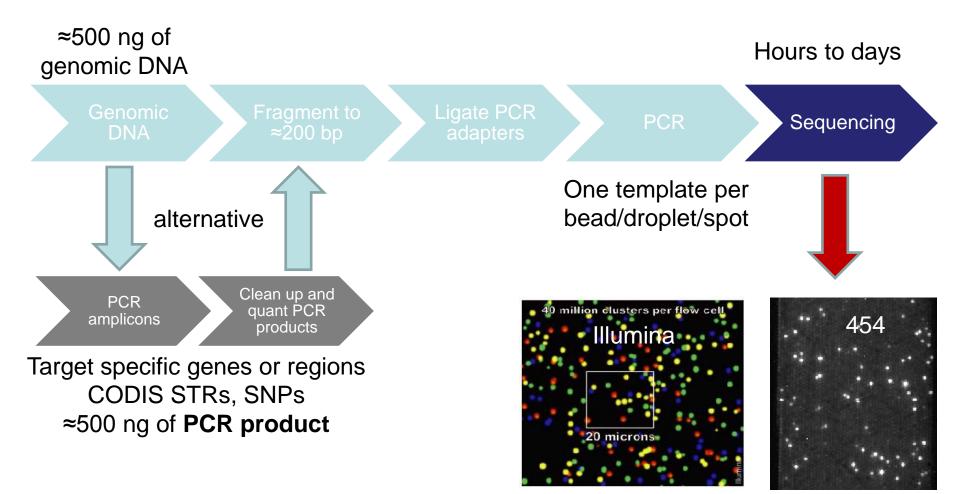
- Roche
 - 454 FLX
 - 454 GS Junior
- PacificBio
 - Pacbio RS
- Illumina
 - GAIIx
 - HiSeq
 - HiScanSQ
 - MiSeq
- Life Tech
 - 5500 series
 - Ion torrent Proton
 - Ion torrent **PGM** (personal genome machine)





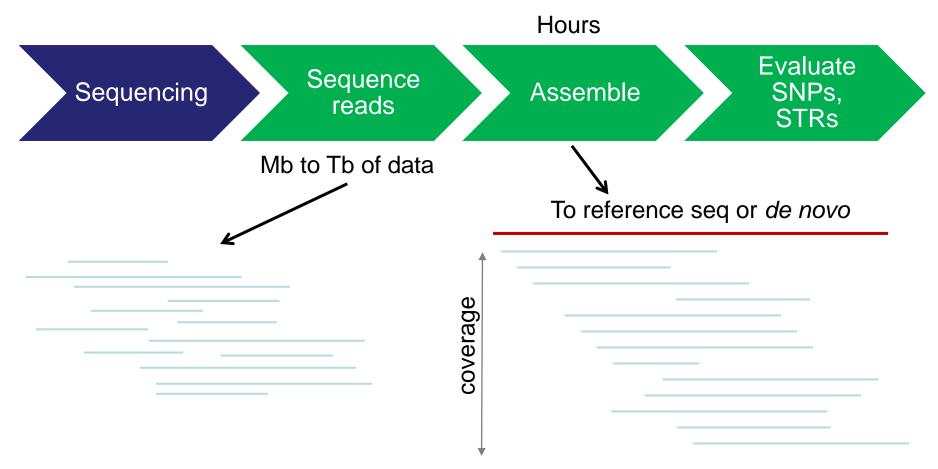


Generalized NGS Workflow



Slide from Peter Vallone (NIST)

Generalized NGS Workflow

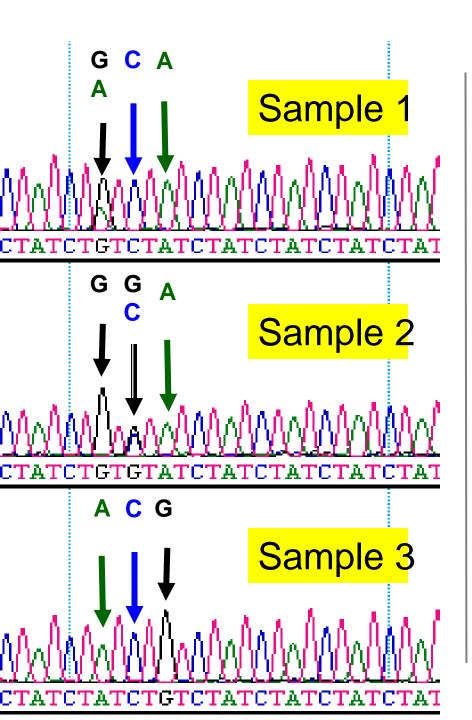


Slide from Peter Vallone (NIST)

Next Generation Sequencing Forensic Applications

- Going in depth into STR loci and beyond
 - STRs are useful for legacy (databases)
 - SNPs within STRs identify 'sub-alleles'
 - Millions of bases of sequence variants (SNPs)
- Opens up new human identity applications: biogeographical ancestry, externally visible traits, complex kinship, degraded samples, mixtures, other applications

Applications are currently being addressed by the forensic genetics community (Kayser and deKnijff 2011)



SNPs within the D8S1179 repeat All 3 samples '13,13' [TCTA]₁₃

Allele A - $[TCTA]_{13}$ Allele B - TCTA TCT<u>G</u> $[TCTA]_{11}$

Allele B - TCTA TCTG [TCTA]₁₁

Allele C - TCTA TCTG TGTA [TCTA]₁₀

There are **4** different '13' alleles in these 3 samples.

Allele D - $[TCTA]_2 TCTG [TCTA]_{10}$ Allele D - $[TCTA]_2 TCTG [TCTA]_{10}$

Data provided by Margaret Kline and Becky Hill

Specific issues with STRs

- Typically comprised of tetra nucleotide repeats
- Range 70 450+ bp regions
- Longer STRs can be difficult to assemble based on read length
- Illumina GAIIx (read length 150 bp)
 - Generated 1000-2500 bp amplicons (13 core loci)
 - Problems detecting D21S11 32.2 and 34.2 alleles
 - Issues detecting D18S51
 - Custom informatics tools for assembling STRs

Bornman et al., 2012 Biotechniques Rapid Dispatch: 1-6

Next Generation Sequencing

- Challenges
 - Repeating sequences (STRs) and read lengths
 - Sample amount requirements (10 ng to 5 µg)
 - Cost and time per unit of information
 - Data analysis (storage, assembly, interpretation)
 - Policy, privacy, disease related markers
 - Validation
 - Standards/reference materials
 - Nomenclature
 - Accuracy of sequence information
 - Errors, platform and bioinformatics-based bias

Next Generation Sequencing Workshop

- Interagency Workshop on the use of Next-Generation DNA Sequencing for Human Identification and Characterization (Jan 31 2012)
- Discussion of forensic applications of NGS (NIST, DoD, FBI, DHS) – materials can be found at:
 - <u>http://www.nist.gov/mml/bmd/genetics/ngs_hid_workshop.cfm</u>
- We are in the process of looking at platforms to characterize forensic markers (mitochondrial, STRs, SNPs)
- Evaluate accuracy, reproducibility, identify initial requirements for a NGS forensic reference material

Some Thoughts on the Future...

PCR amplification

- Faster enzymes to enable rapid PCR
- More robust enzymes and master mixes to overcome inhibition

Instrumentation

- More dye colors to aid higher levels of multiplexing
- Rapid, integrated devices
- Alternatives to capillary electrophoresis: PLEX-ID and NGS

Quantitative information

- qPCR and digital PCR

Marker systems

- Expanding sets of STR loci for growing DNA databases
- Other marker systems: SNPs, InDels, X-STRs, RM Y-STRs
- Body fluid identification with mRNA, miRNA, and DNA methylation
- Phenotyping for external visible characteristics
- Challenges with potential whole genome information

Data interpretation

- Probabilistic genotyping for low-level DNA and mixture interpretation
- Probability of dropout

We Need Continued Efforts to Improve DNA Interpretation (especially low-level DNA and mixtures)

Forensic Science International: Genetics 6 (2012) 677-678

Contents lists available at SciVerse ScienceDirect



Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Editorial

Focus issue—Analysis and biostatistical interpretation of complex and low template DNA samples

December 2012 – Forensic Science International: Genetics, volume 6, issue 6

Forensic Science International: Genetics 6 (2012) 679-688



DNA commission of the International Society of Forensic Genetics: Recommendations on the evaluation of STR typing results that may include drop-out and/or drop-in using probabilistic methods

P. Gill ^{a,b,*}, L. Gusmão^c, H. Haned^d, W.R. Mayr^e, N. Morling^T, W. Parson^g, L. Prieto^h, M. Prinz¹, H. Schneider^J, P.M. Schneider^k, B.S. Weir¹

www.DNA.gov Website

ne Grant Funding Training Statistics Re	search Publications State Profiles	Search
roviding funding, training and assistanc solve crimes, protect the innocent and	e to ensure that forensic DNA reaches its full potential identify missing persons.	Training Course Catalog
Highlights	Highlights	Register for online courses
About Forensic DNA	VIDEO	Login and view your courses
DNA Databases	<u>NamUs Behind the Scenes: How It Works, Why It</u> <u>Matter (6 minutes)</u>	Reset your password
Reducing the Backlog	TRAINING	Request your username
Solving Crimes	Advanced DNA Technologies and Forensic Automation	Browse by Audience
Identifying Persons and Victims	Analyst Training Program	Officers/ Investigators
Postconviction Testing	PUBLICATIONS <u>Research articles from NIJ-funded projects updated</u>	Forensic Scientists
Tools for Forensic Scientists	through 2008	Officers of the Court
Services for Laboratories	AVAILABLE FUNDING Forensic DNA Unit Efficiency Improvement	Crime Lab Managers
	Social Science Research on Wrongful Conviction	Researchers

Research on	Statistics on	Grant funding for
Human DNA quantitation	Profiles in the database	Forensic backlog reduction
Y Chromosome	DNA crime labs	Convicted offender/arrestee
Compromised DNA ovidence	Backleg of complex	backlog reduction

Summary of NIJ-Funded Research



The Future of Forensic DNA

is Similar to the Olympic Motto of "Swifter, Higher, Stronger"

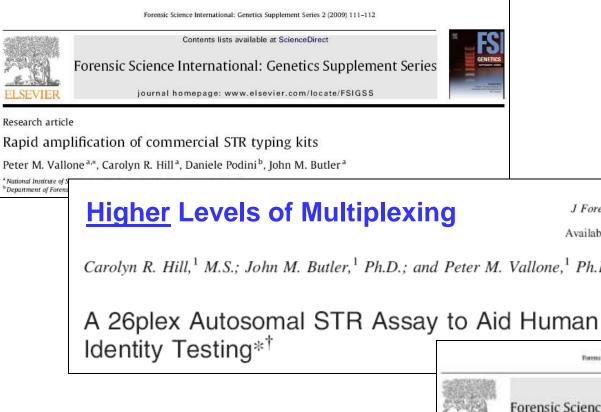


Resources

Training

Recent NIST Publications Demonstrating "Swifter, Higher, Stronger" DNA Analysis

Swifter PCR Amplification



Stronger Powers of Discrimination

J Forensic Sci, September 2009, Vol. 54, No. 5 doi: 10.1111/j.1556-4029.2009.01110.x Available online at: www.blackwell-synergy.com

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Forensic Science International: Genetics Supplement Series

journal homepage: www.elsevier.com/locate/FSIGSS

Research article

The single most polymorphic STR Locus; SE33 performance in U.S. populations

John M. Butler^{a,*}, Carolyn R. Hill^a, Margaret C. Kline^a, David L. Duewer^a, Cynthia J. Sprecher^b, Robert S. McLaren^b, Dawn R. Rabbach^b, Benjamin E. Krenke^b, Douglas R. Storts^b

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Thank you for your attention

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

Our team publications and presentations are available at: http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm