THE ROYAL SOCIETY

The Paradigm Shift for UK Forensic Science
Discussion Meeting

2 February 2015

The Future of **Forensic DNA** Analysis

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Gaithersburg, Maryland
United States of America

NIST

Presentation & Written Article Outline

- **Introduction to NIST and recent U.S. activities**
  - National Commission on Forensic Science (NCFS)
  - Organization of Scientific Area Committees (OSAC)
- **DNA capabilities**
- **The Past Reviewed**
  - Major themes and time periods
  - Research leadership
- **The Present Considered**
  - Genetic marker systems
  - DNA database growth and use in the U.S.
  - Critical challenges faced today
- **The Future Predicted**
  - *Faster* results
  - *Higher* sensitivity and information content
  - *Stronger* conclusions with challenging samples

Background Information on NIST

- Started in 1901 with roots back to the Constitution
- Name changed to **National Institute of Standards and Technology (NIST)** from National Bureau of Standards in 1988
- Primary campus in Gaithersburg, Maryland (just outside of Washington, D.C.)
- Part of the U.S. Department of Commerce
- >3,000 employees and >2,000 associates
- Supply >1300 reference materials
- Defines official time for the U.S.

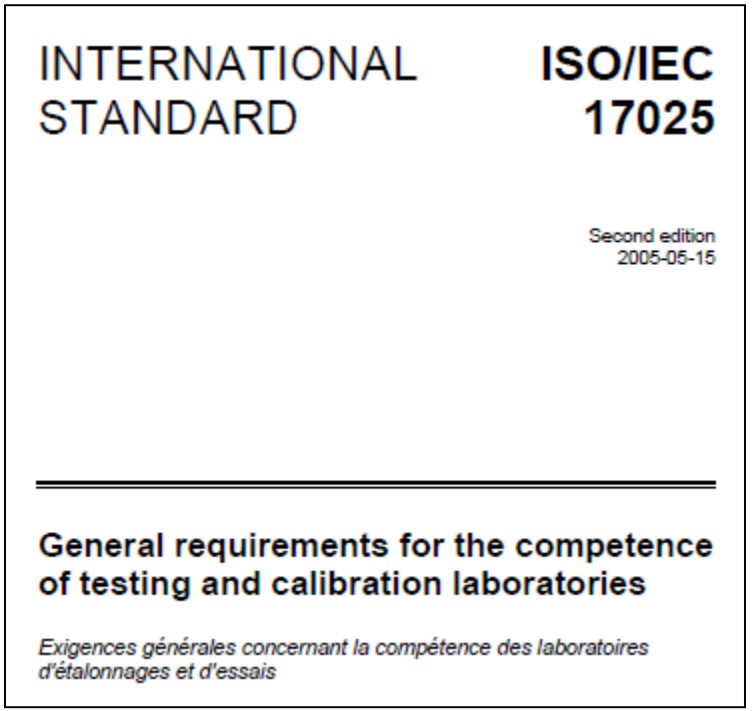
Types of Standards

physical (measurement) standards

Certified reference material to aid with calibration of measurements

http://www.nist.gov/srm/

documentary (technical) standards


INTERNATIONAL STANDARD

ISO/IEC 17025

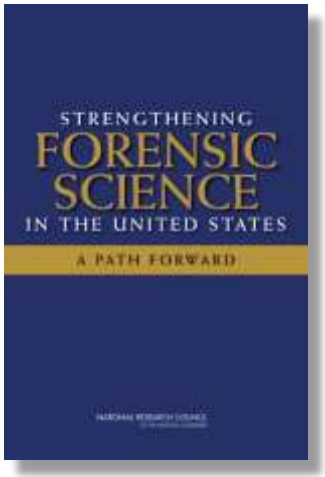
Second edition
2005-05-15

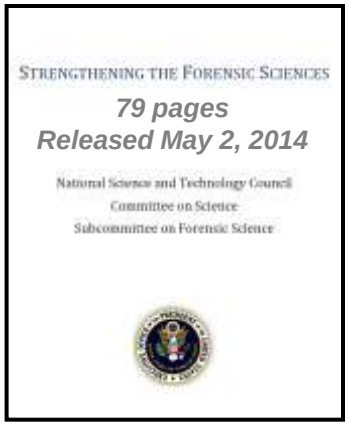
General requirements for the competence of testing and calibration laboratories

Exigences générales concernant la compétence des laboratoires d'étalonnages et d'essais

Specific requirements for the operation of a laboratory related to management system and competence

NCFS and OSAC:
U.S. Efforts to Strengthen Forensic Science





http://www.whitehouse.gov/sites/default/files/microsites/ostp/NSTC/strengthening_the_forensic_sciences_may_-_2014.pdf

- National Academy of Sciences **(NAS) report** issued in Feb 2009
- White House **Subcommittee on Forensic Science** (SoFS) operated from July 2009 to Dec 2012

DOJ/NIST Partnership (announced Feb 2013)

1. **NCFS** (National Commission on Forensic Science)
   - **First meeting held February 3-4, 2014 in Washington DC**
2. **OSAC** (Organization of Scientific Area Committees)
   - **Being organized; first public meetings to be held Feb 2015**

National Commission on Forensic Science (NCFS)

31 voting and 8 *ex-officio* members

Last meeting (5th): January 29-30, 2015

Next meeting (6th): April 30-May 1, 2015

Policy-focused

NCFS Leadership

Sally Q. Yates
Acting
Deputy Attorney General
DOJ Co-Chair

Willie E. May
Acting
Director of NIST
NIST Co-Chair

Nelson A. Santos
Vice-Chair (DOJ)

John M. Butler
Vice-Chair (NIST)

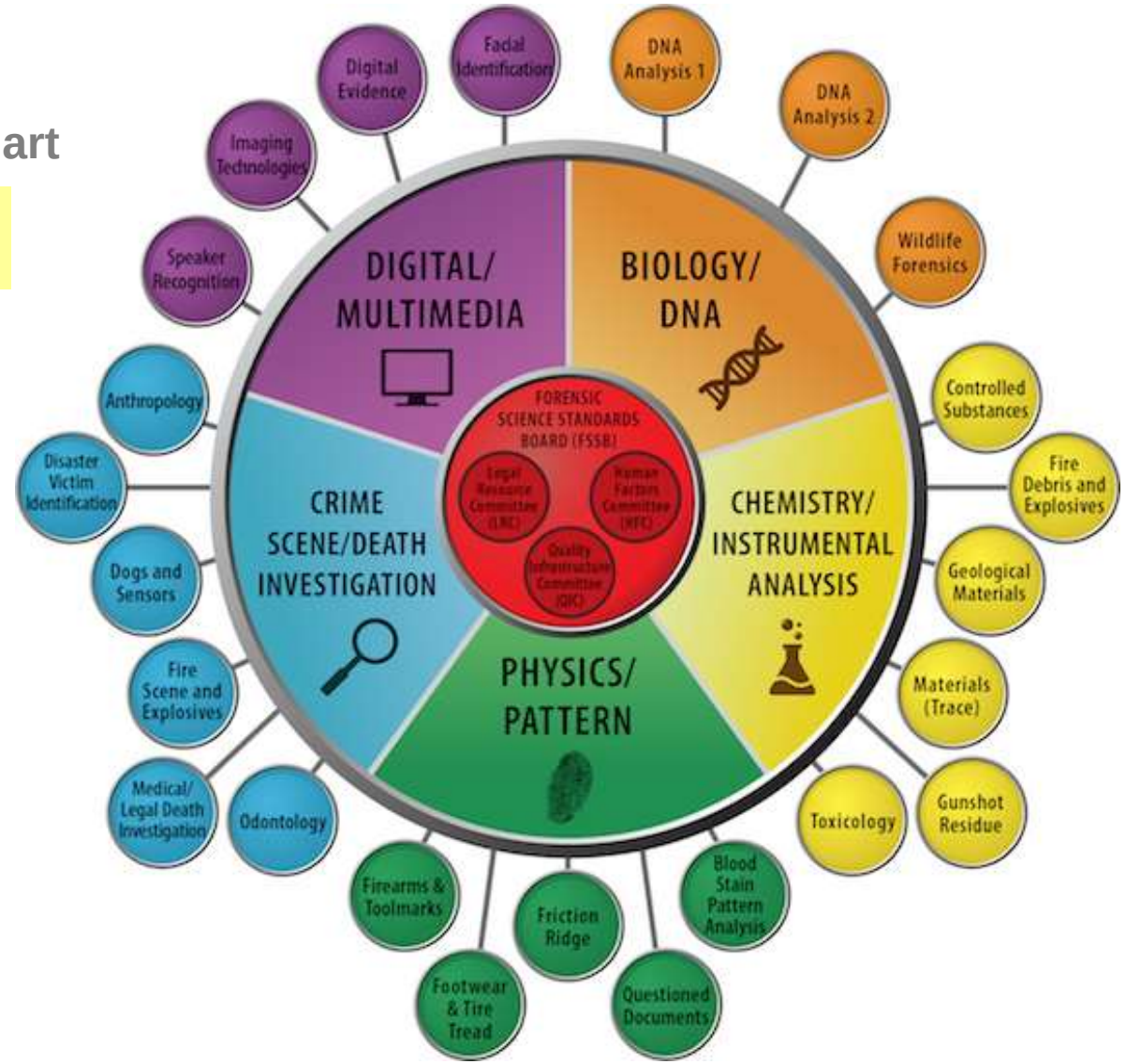
Organization of Scientific Area Committees

OSAC

Functional Organization Chart

Practice-focused

542 members and >1000 affiliates
as subject matter experts participating in 24 subcommittees, 5 scientific areas, 3 resource committees (legal, quality, human factors), and 1 governing board (Forensic Science Standards Board)



http://www.nist.gov/forensics/osac/index.cfm

Initial membership finalized Dec 22, 2014

Overview of Standards involved in the Forensic Science Enterprise

ILAC-G19:08/2014
Modules in a Forensic Science Process

ISO/IEC **17024**
Certification Provider

ISO/IEC **17043**
Proficiency Test Provider

ISO/IEC **17011**
Accrediting Body

Forensic Testing Laboratory

Technical standard: ISO/IEC **17025**

Supplemental material: DNA (QAS) | Latents | Firearms | …

Police Agency

ISO/IEC **17020**

Crime scene unit

M.E./Coroner

ISO **15189**

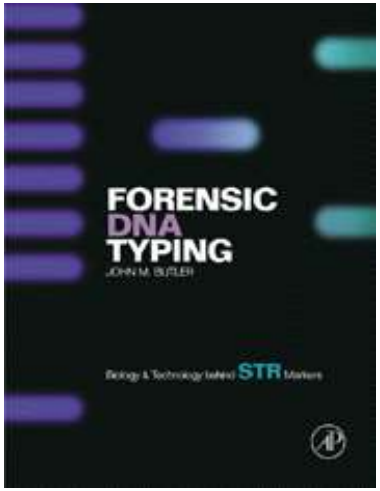
Autopsy | Virtopsy

OSAC work will help provide supplemental materials and new technical standards

Accreditation to appropriate quality standards should provide confidence for all stakeholders in what is being done

Forensic DNA Typing Textbooks Have Set the Standard for the Field

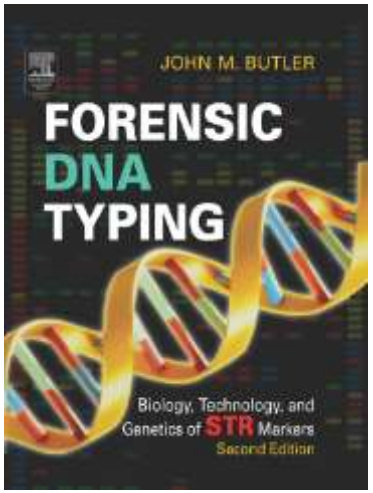
1st Edition



Jan 2001

335 pages

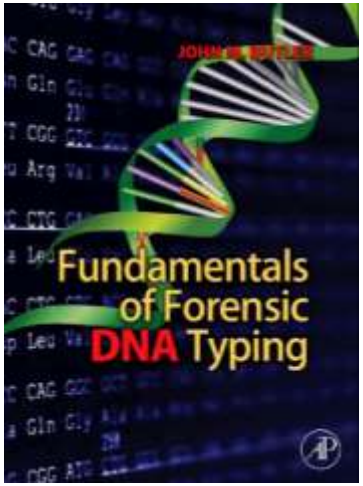
2nd Edition



Feb 2005

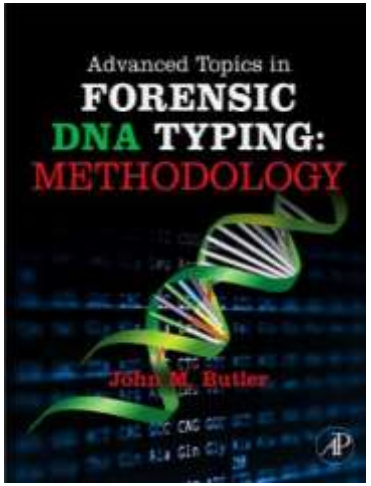
688 pages

3rd Edition (3 volumes)



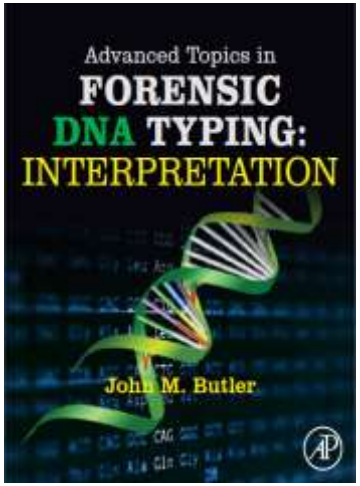
Sept 2009

520 pages



Aug 2011

704 pages

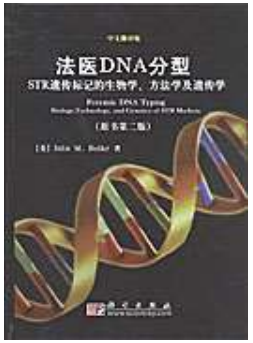


Oct 2014

604 pages

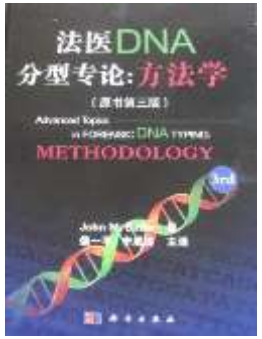
Language Editions

Chinese (2007)



Japanese (2009)

Chinese (2013)



Steps in Forensic DNA Analysis

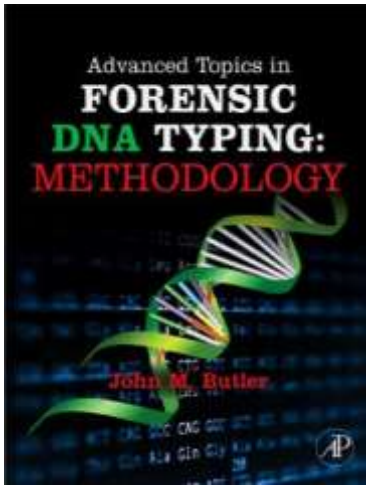
Understanding Results Obtained & Sharing Them

Gathering the Data

Interpretation

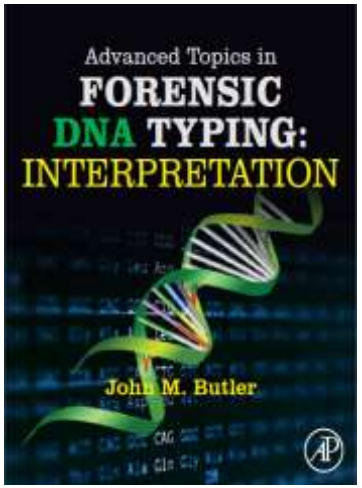
>1300 pages of information with >5000 references cited in these two books

Advanced Topics: Methodology



August 2011

Advanced Topics: Interpretation



October 2014

DNA Capabilities to Aid Forensic Investigations

1. The **ability to identify the perpetrator**
2. Weight-of-evidence based on established genetic principles and statistics (Hardy-Weinberg 1908)
3. Established characteristics of genetic inheritance enables close **biological relatives** to be used for reference points using kinship associations
4. Superb **sensitivity** with PCR amplification (opens the possibility for contamination)
5. Well-established **quality assurance measures**
6. New **technology development** aided by genomics

Successful interpretation of DNA (Q-to-K comparison) depends on quality of the crime scene evidence (Q) and availability of suitable reference samples (K)

The

Past

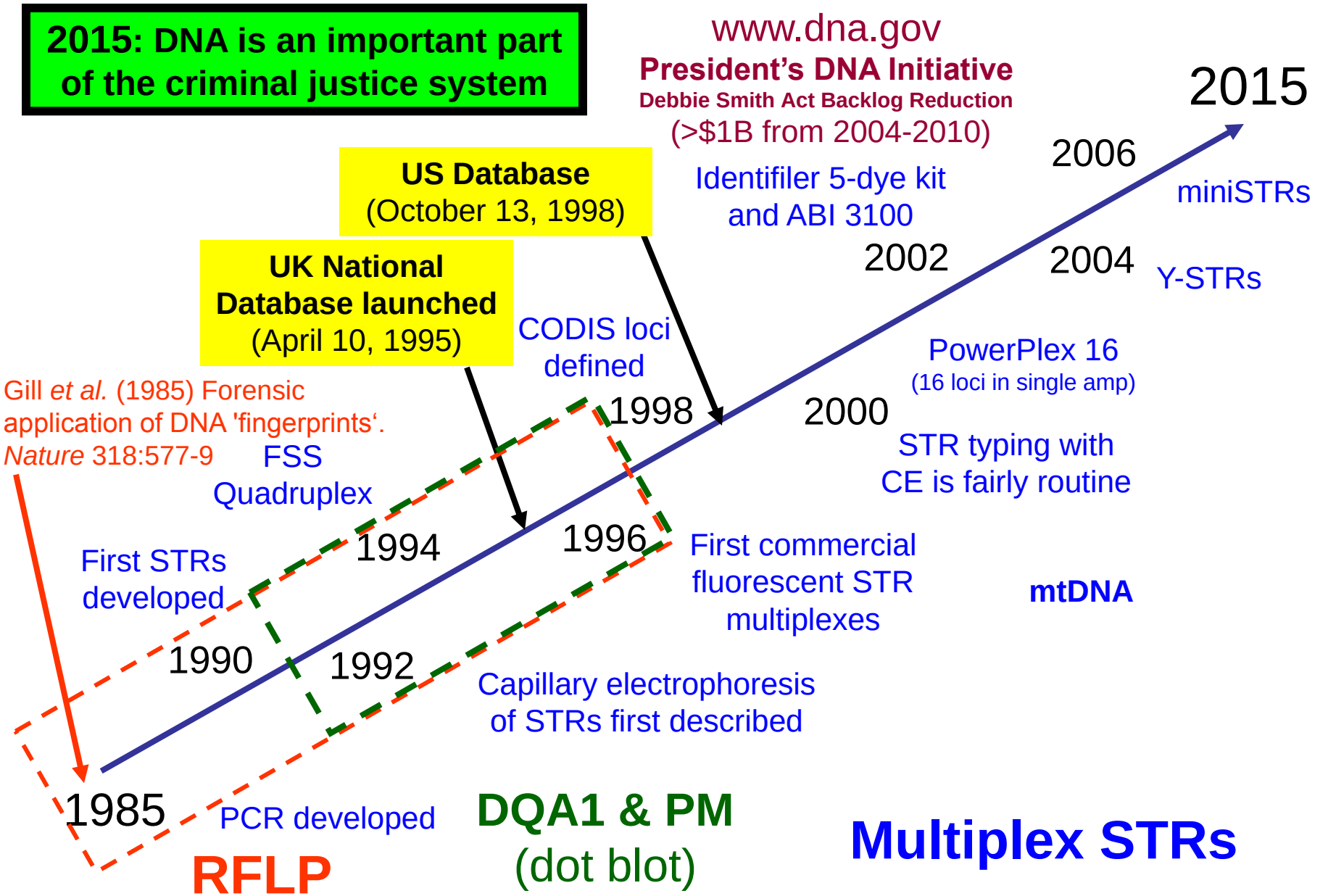
Reviewed

Stages of Forensic DNA Progression

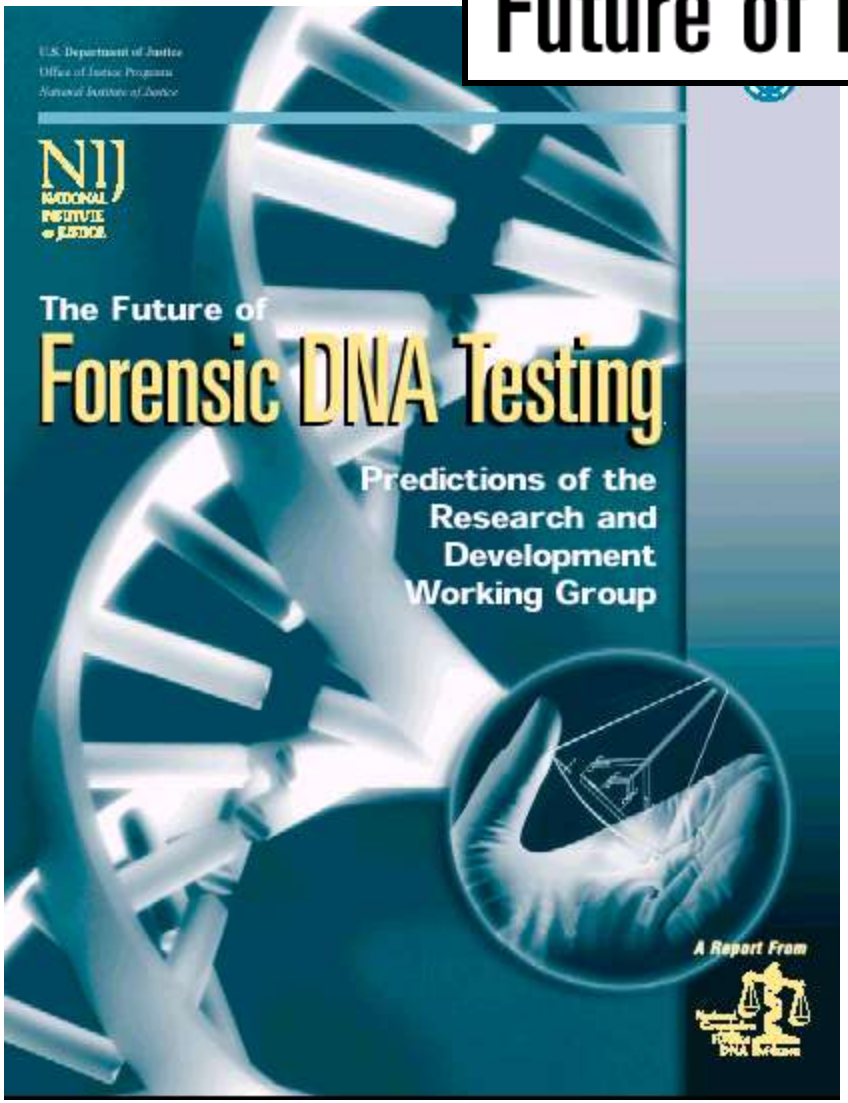
Stages	Time Frame	Description
Exploration	1985 - 1995	Beginnings, different methods tried (RFLP and early PCR)
Stabilization	1995 - 2005	Standardization to STRs, selection of core loci, implementation of Quality Assurance Standards
Growth	2005 - 2015	Rapid growth of DNA databases, extended applications pursued
Sophistication	*2015 to 2025 and beyond*	*Expanding tools available,* ***confronting privacy concerns***

Table 1 from J.M. Butler (2015) The future of forensic DNA analysis. *Phil. Trans. R. Soc. B (in press)*

History of Forensic DNA Testing



National Commission on the Future of DNA Evidence



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

- 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

Research Leadership in Forensic DNA

- During its existence, the **UK Forensic Science Service** played an important role in the development and application of forensic DNA techniques

- Other important centers of research include:
  - University of Innsbruck (Austria)
  - University of Copenhagen (Denmark)
  - University of Santiago de Compostella (Spain)
  - NIST Applied Genetics Group (USA)
  - University of North Texas Health Science Center (USA)
  - Netherlands Forensic Institute (Holland)
  - Institute of Environmental Science and Research (New Zealand)

The

Present

Considered

Current U.S. National DNA Database

As of December 2014, over **13 million samples**

- 11,548,720 offender DNA profiles
- 1,303,454 arrestee DNA profiles
- 601,664 forensic profiles

As of October 2014, China had >420 labs and >25 million STR profiles in their DNA database

- Has produced **270,326 hits** to help solve cases

- http://www.fbi.gov/about-us/lab/biometric-analysis/codis/ndis-statistics

Growth of DNA Databases

- Expanded laws now enable more offenders to be included (32 states and federal government have laws to collect from arrestees)
  - Has contributed to sample backlogs

U.S. Supreme Court decision (June 2013) in *Maryland v King*

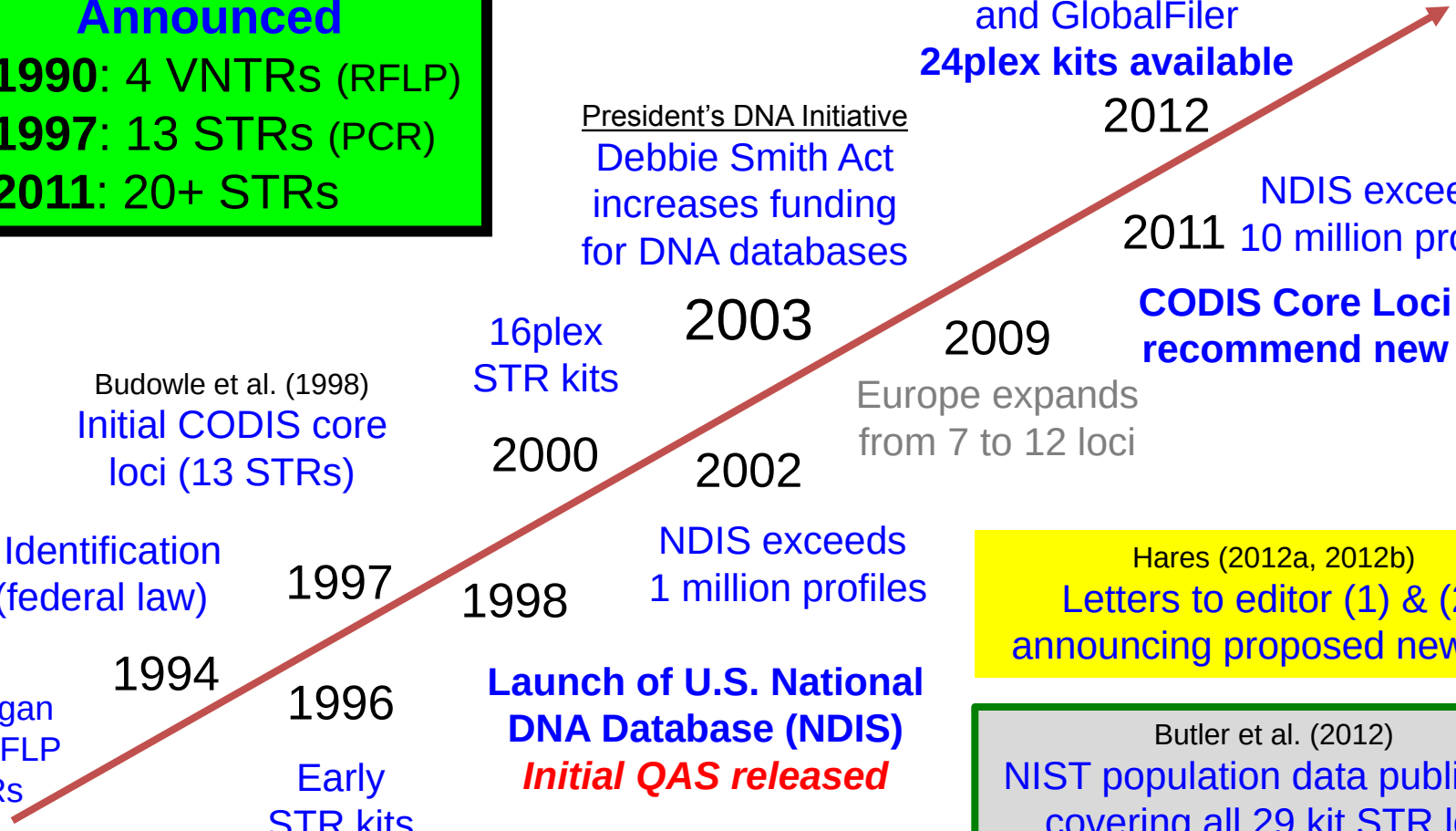
- Have benefited from significant federal funding since 2004 (>$1 billion for backlog reduction)

- **Have effectively locked technology with core STR markers used** to generate DNA profiles that now number in the millions

U.S. Core Loci Expansion Efforts

More loci added as databases grew…

U.S. Core Loci Goals Announced
1990: 4 VNTRs (RFLP)
1997: 13 STRs (PCR)
2011: 20+ STRs



1990 — U.S. began with 4 RFLP VNTRs

Baechtel et al. (1991)
Combined DNA Index System (CODIS) proposed

1994 — DNA Identification Act (federal law)

1996 — Early STR kits

1997 — Budowle et al. (1998) Initial CODIS core loci (13 STRs)

1998 — **Launch of U.S. National DNA Database (NDIS)** ***Initial QAS released***

2000 — 16plex STR kits

2002 — NDIS exceeds 1 million profiles

2003 — President's DNA Initiative; Debbie Smith Act increases funding for DNA databases

2009 — Europe expands from 7 to 12 loci

2011 — NDIS exceeds 10 million profiles; **CODIS Core Loci WG recommend new loci**

2012 — PowerPlex Fusion and GlobalFiler **24plex kits available**

2014 — Implementation to be required 2 years after announcement

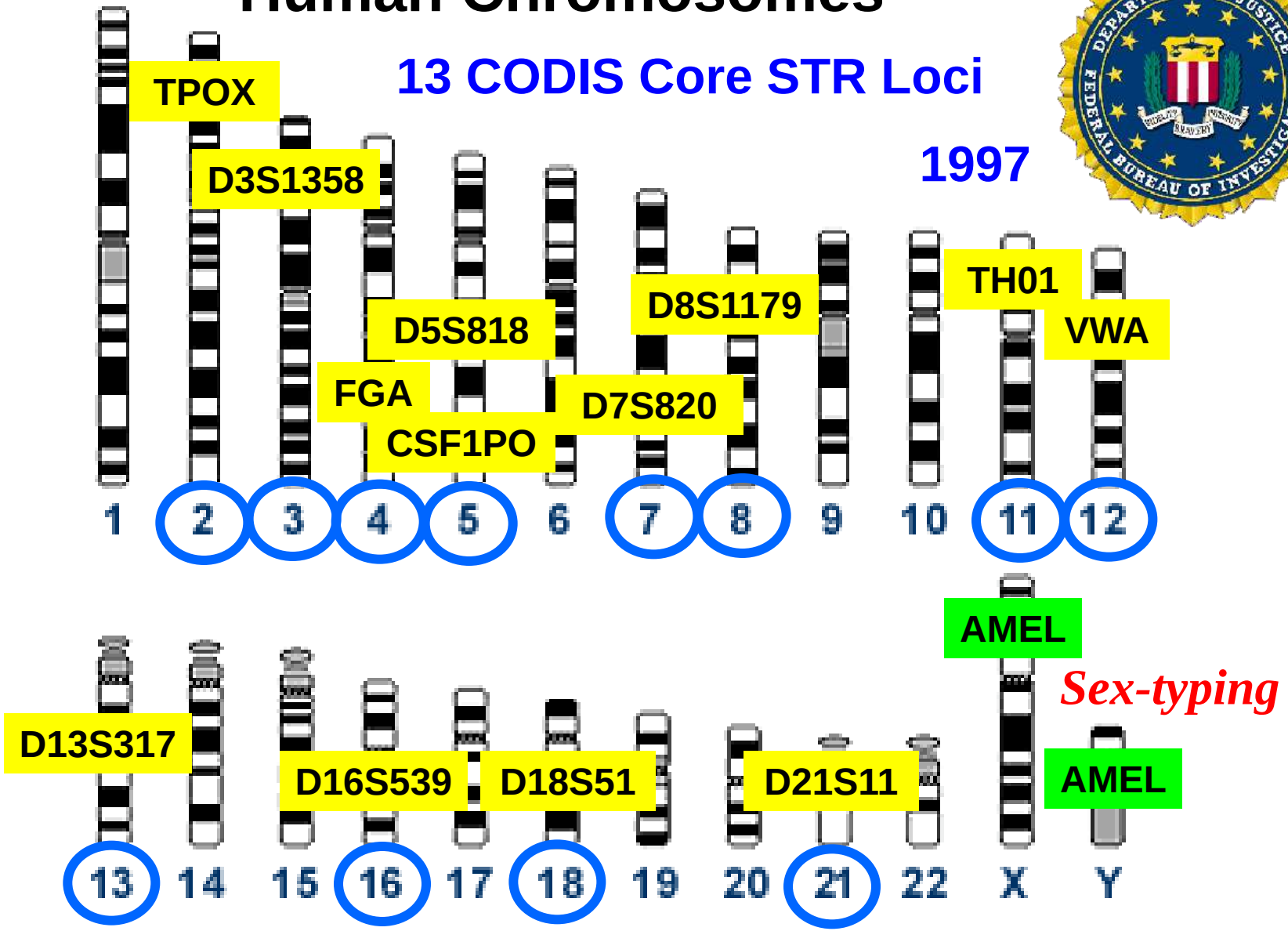
Hares (2012a, 2012b)
Letters to editor (1) & (2) announcing proposed new loci

Butler et al. (2012)
NIST population data published covering all 29 kit STR loci

QAS: Quality Assurance Standards

Position of Forensic STR Markers on Human Chromosomes

Core STR Loci for the United States

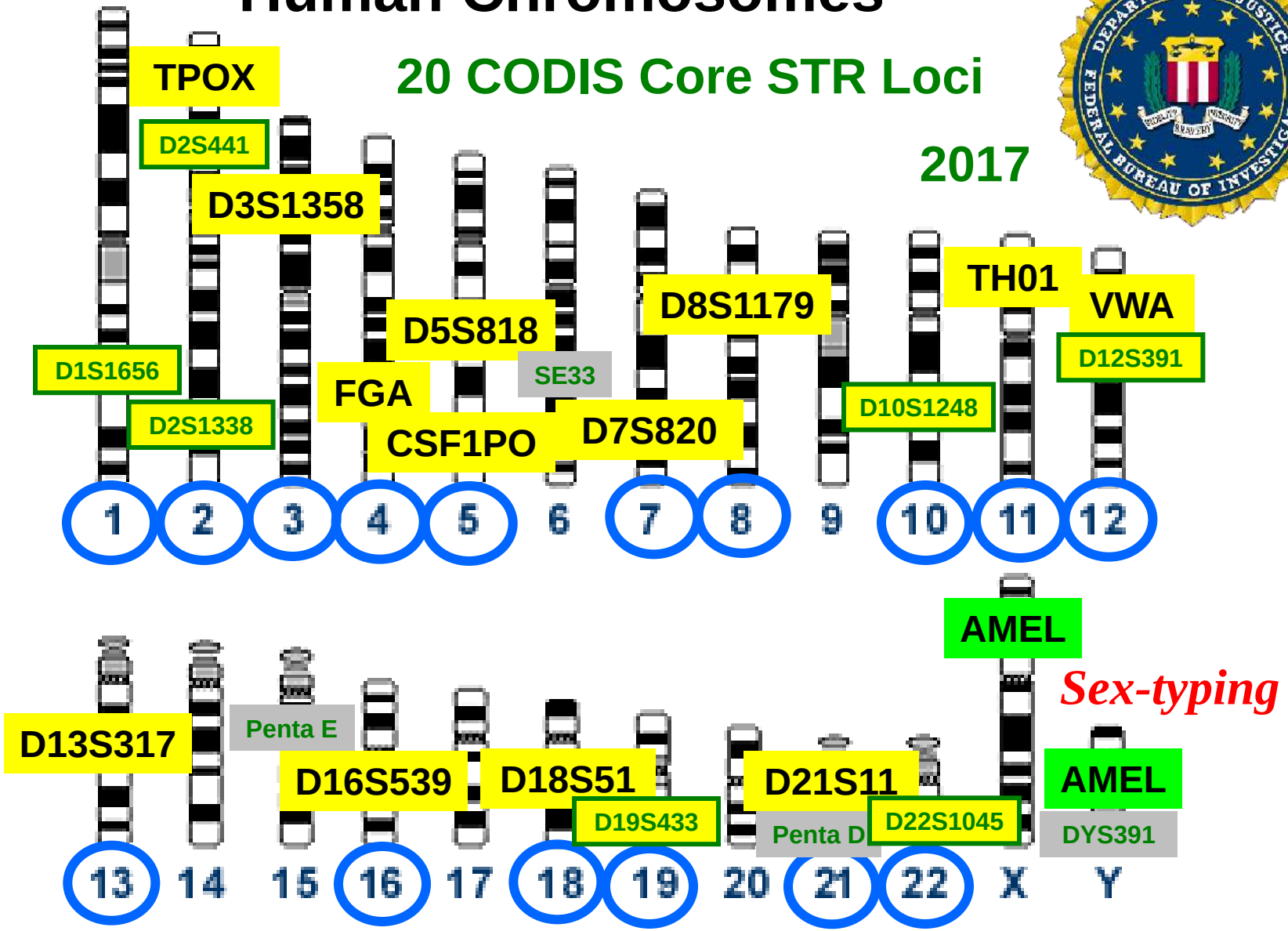


Position of Forensic STR Markers on Human Chromosomes

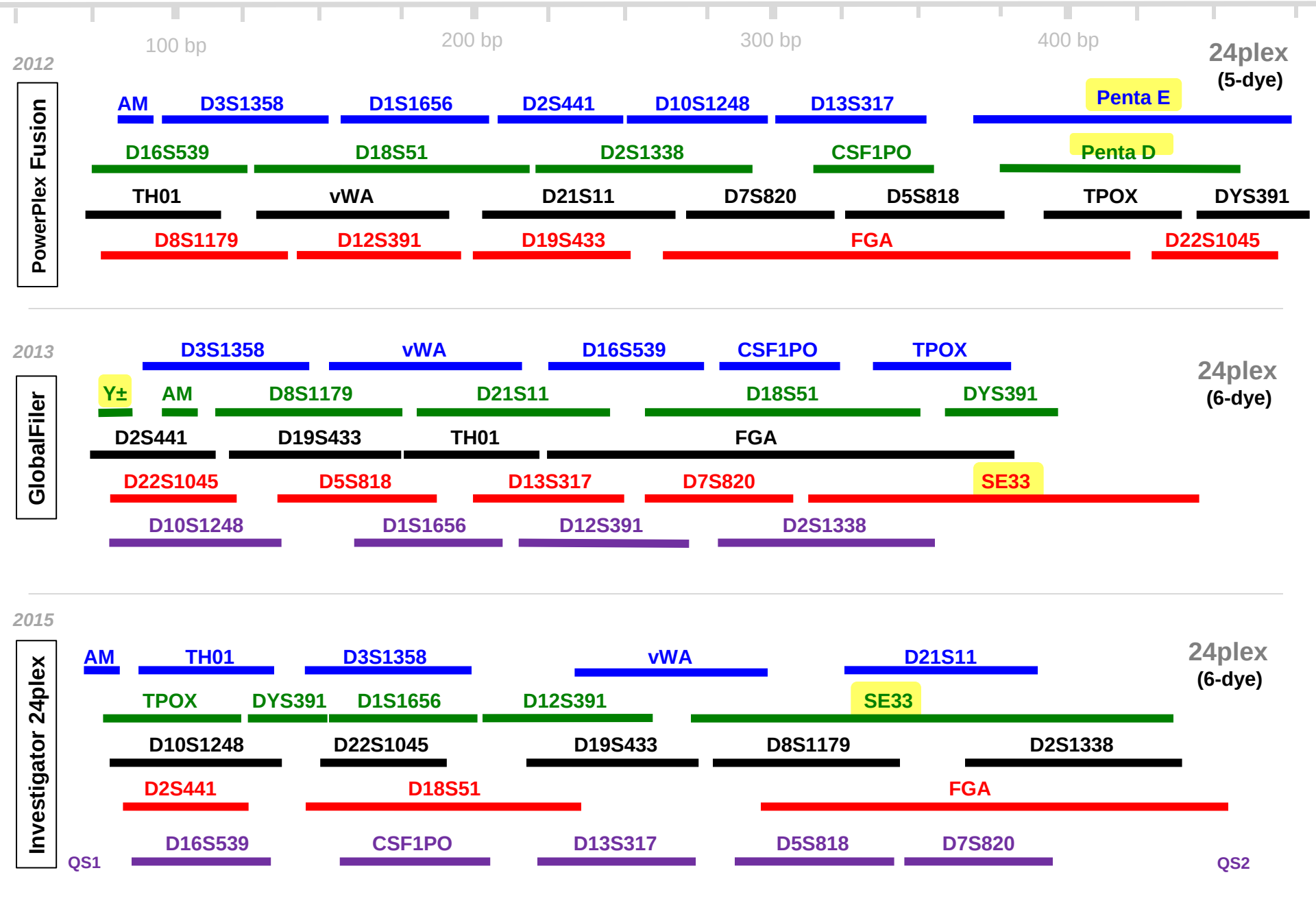
Expanded Core STR Loci for the United States

20 CODIS Core STR Loci

2017



Relative Sizes of STR Loci in 24plex Kits



The

Future

Predicted

New Trends in Forensic DNA

- ***Faster** results:* Rapid DNA capabilities and new sample-to-answer integrated instruments
- ***Higher** sensitivity:* New assays lowering the limits of detection, which makes interpretation more challenging
- ***Higher** information content:* Next-generation sequencing (NGS) for more markers & STR allele information
- ***Stronger** conclusions:* Mixture interpretation with probabilistic genotyping models

Rapid DNA Efforts


Pete Vallone **Erica Butts**

Accelerated Nuclear DNA Equipment (ANDE) developed by **NetBIo**

http://ishinews.com/wp-content/uploads/2012/10/Rapid-DNA-Miles-1.58MB.pdf

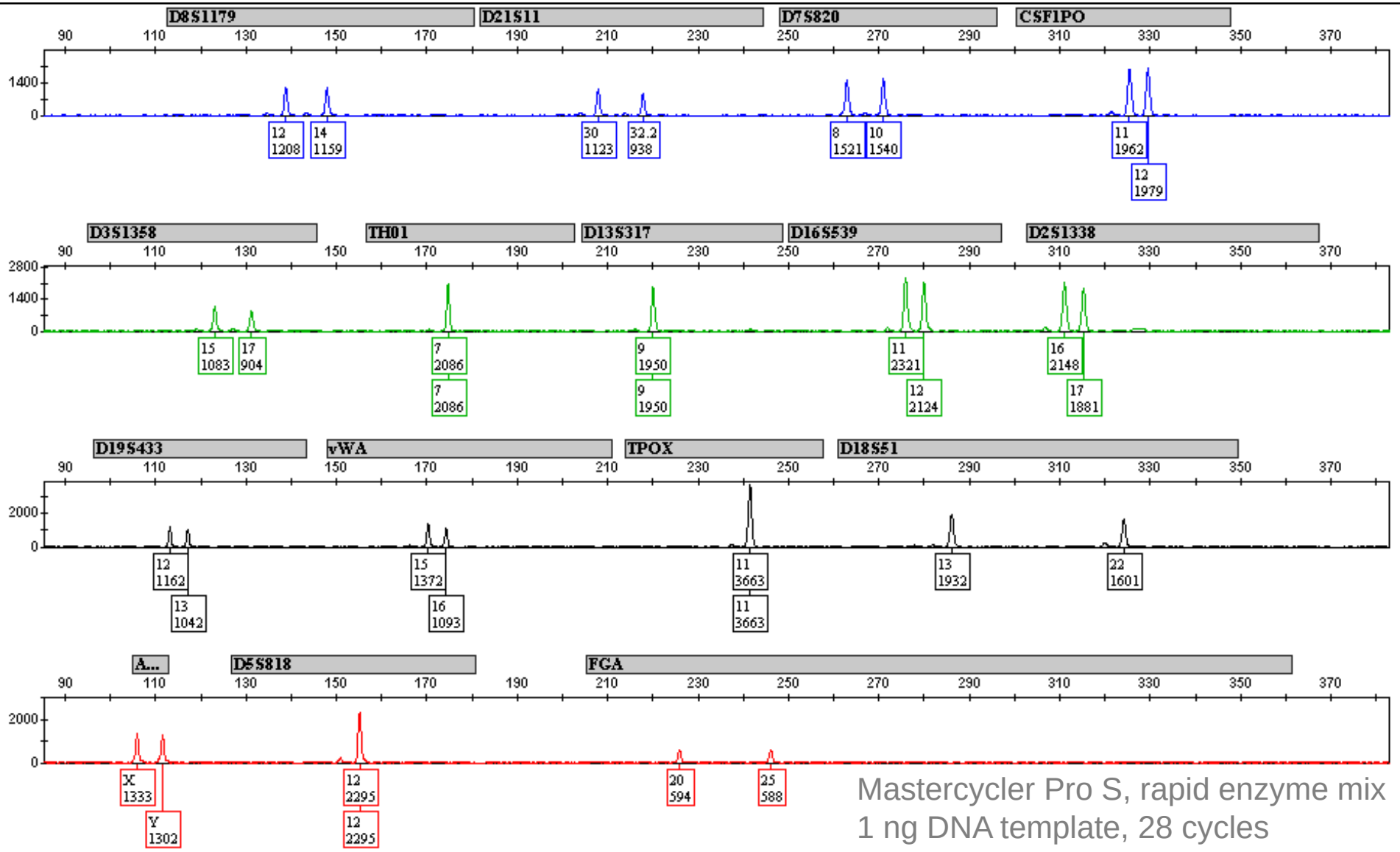
RapidHIT 200 developed by **IntegenX**

http://integenx.com/wp-content/uploads/2010/06/RapidHIT-200.png

- Evaluating ANDE (NetBio) and IntegenX rapid DNA instruments
  - both instruments are capable of swab in → STR profile out in less than 90 minutes without user intervention
- Exploring rapid DNA techniques including direct PCR and rapid PCR
  - STR profiles generated in <2 hours with standard lab equipment and rapid protocols
  - See ISHI 2012 poster available on STRBase “Rapid DNA Testing Approaches for Reference Samples”

Fastest results swab-to-profile (Identifiler): 57 minutes

Full Identifiler STR Profile with **19 min PCR**



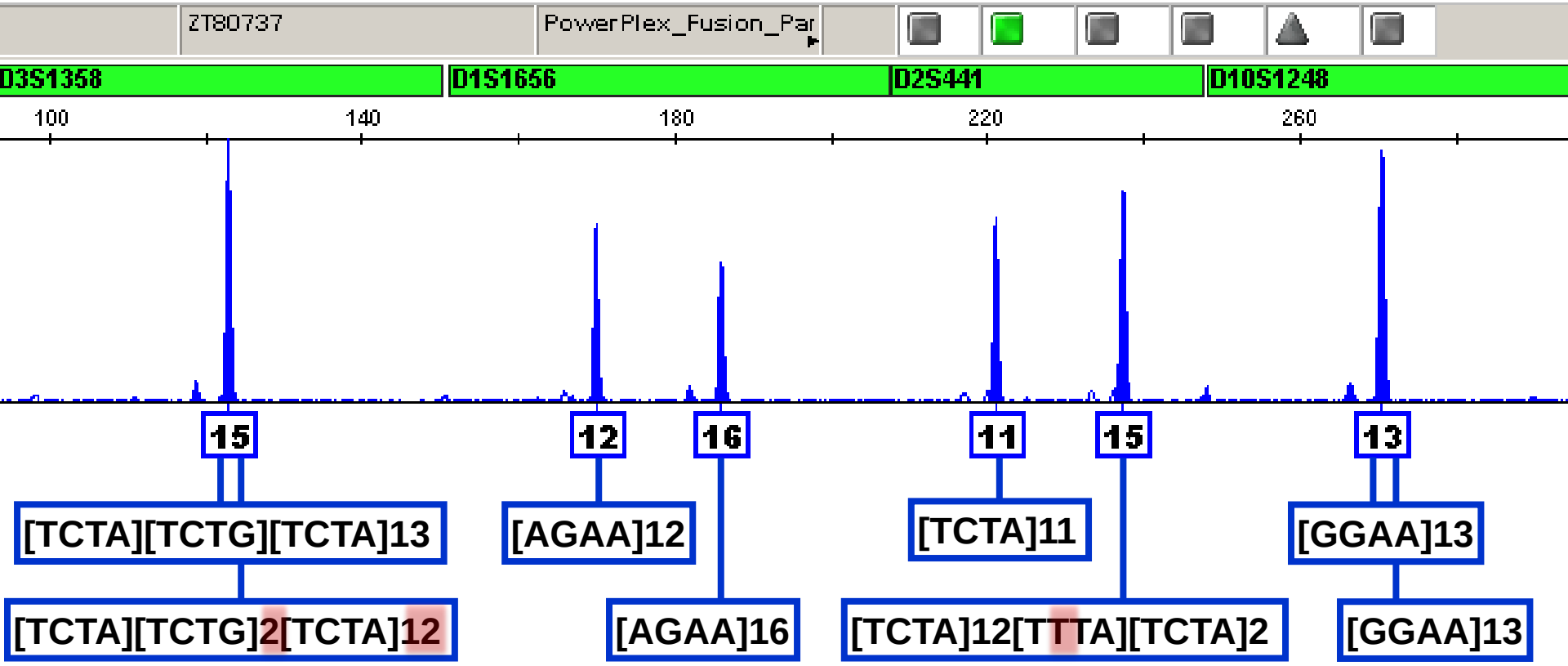
Mastercycler Pro S, rapid enzyme mix
1 ng DNA template, 28 cycles

Slide from Peter Vallone (NIST)

Next Generation Sequencing (NGS)

- **Higher information content** with sequence data
  - Expanded number of STR loci and other genetic markers such as SNPs and InDels
    - New markers may enable additional applications (e.g., biogeographical ancestry and phenotypic prediction)
  - **Deeper depth of information on STR alleles**
    - For example, eight different sequence versions of D12S391 alleles among 197 samples examined (Gelardi et al. 2014)

- **Significant challenges with BIG data**
  - STR allele nomenclature issues
  - Data storage (do you retain terabytes of data?)
  - Data analysis time will increase…
  - Privacy concerns with additional genomic information



Sequence-Based Heterozygote: A locus that appears homozygous in length-based measurements (such as CE), but is heterozygous by sequence

- Need better DNA extraction/recovery
- Need further efforts with evidence interpretation and understanding of data being generated with high sensitivity techniques (move towards probabilistic methods)

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

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

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

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 [f], W. Parson [g], L. Prieto [h], M. Prinz [i], H. Schneider [j], P.M. Schneider [k], B.S. Weir [l]

Summary of DNA Mixture Interlaboratory Studies Conducted by NIST

Study	Year	# Labs	# Samples	Mixture Types
MSS 1	1997	22	11 stains	ss, 2p, 3p
MSS 2	1999	45	11 stains	ss, 2p, 3p
MSS 3	2000-01	74	7 extracts	ss, 2p, 3p
MIX05	2005	69	4 cases (.fsa)	only 2p
MIX13	2013	108	5 cases (.fsa)	2p, 3p, 4p

ss = single-source
2p = 2-person
3p = 3-person
4p = 4-person

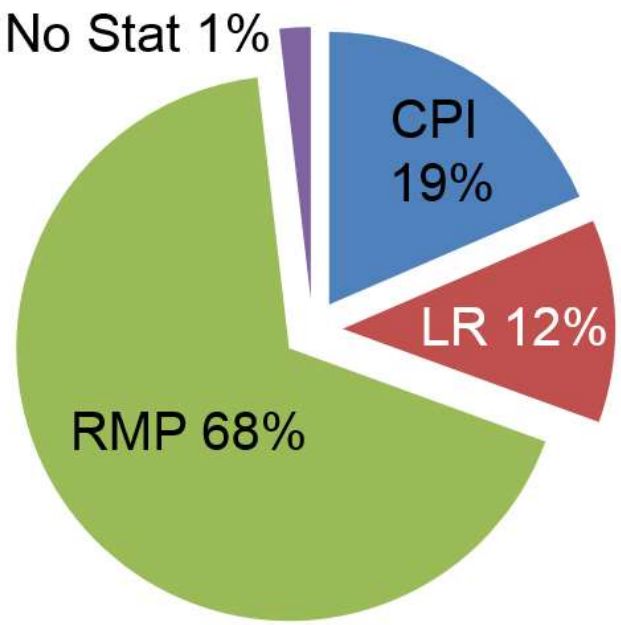
- Other recent studies
  - UK Regulator
  - USACIL

Studies have revealed significant variations in approaches among and within forensic laboratories

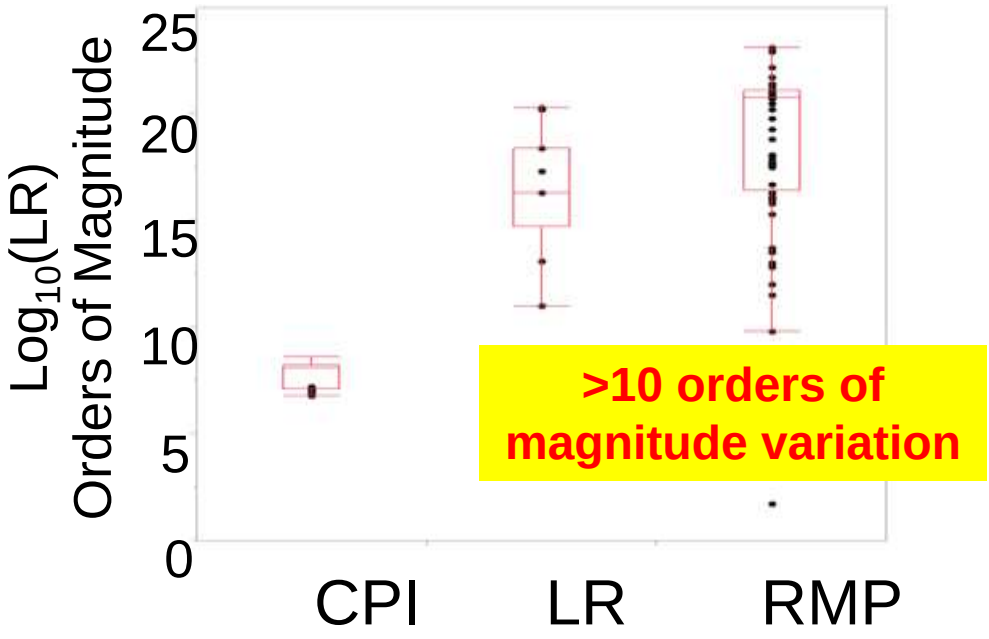
Slide from Mike Coble (NIST)

MIX13 Study Case 1 Results

- **Scenario:** Mock sexual assault, **2 person 50:50 mixture**, all alleles above a stochastic threshold of 150 RFU
- **Purpose:** How many labs would consider the victim's profile and determine genotypes through deconvolution and report a modified random match probability statistic?



Different Approaches Being Taken

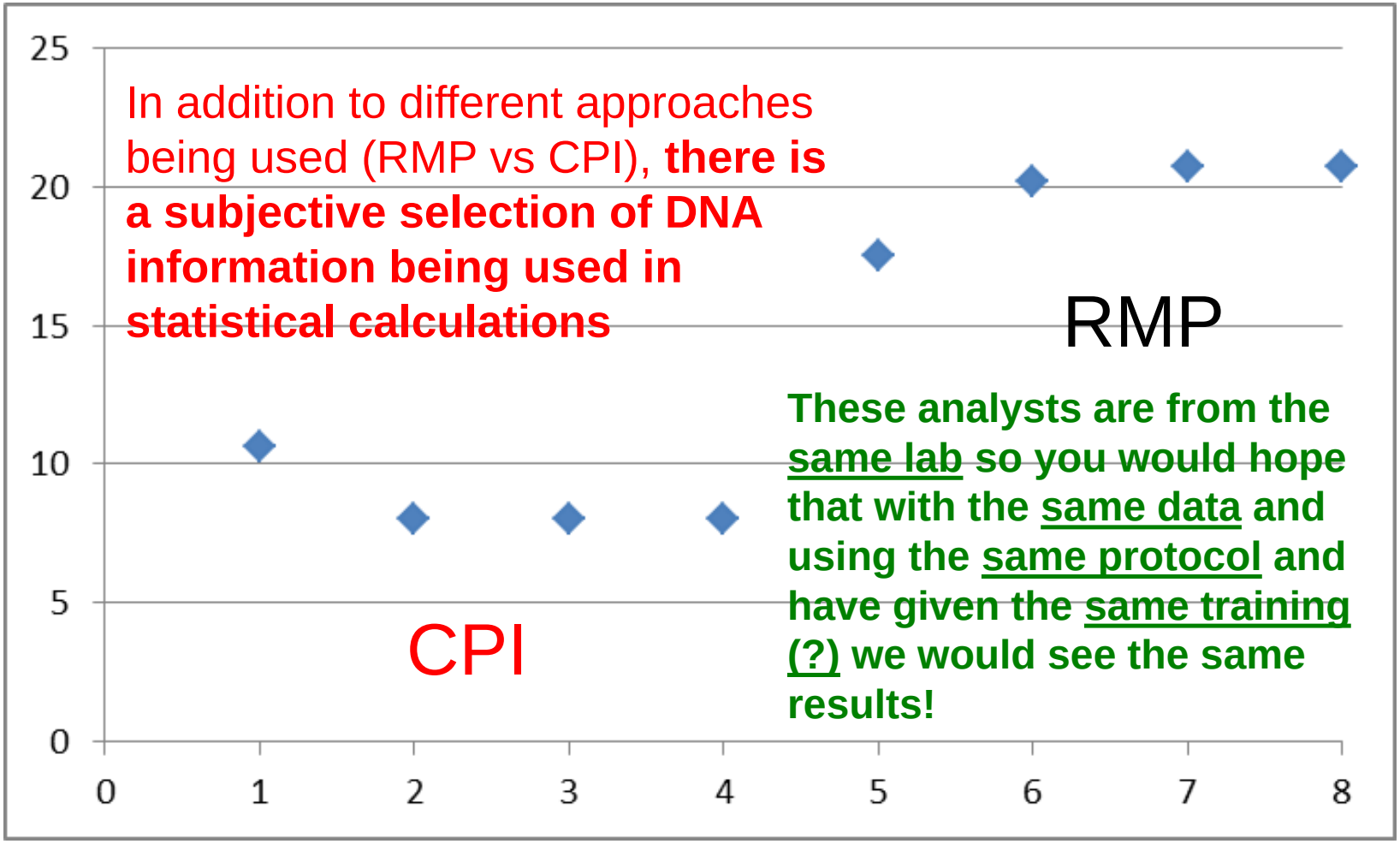


N = 108 laboratories

Data from Mike Coble (NIST)

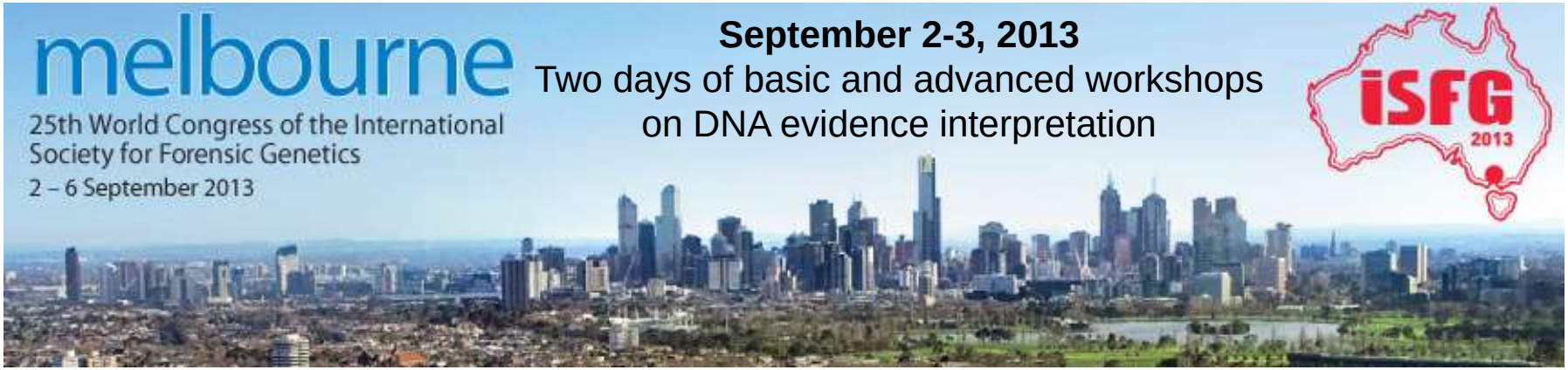
MIX13 Study Case 1 Results

Intra-Laboratory Results (n = 8)



Data from Mike Coble (NIST)

DNA Interpretation Training Workshops



Handouts and reference list available at
http://www.cstl.nist.gov/strbase/training/ISFG2013workshops.htm

Going Beyond the Core Competencies of Forensic DNA Testing…

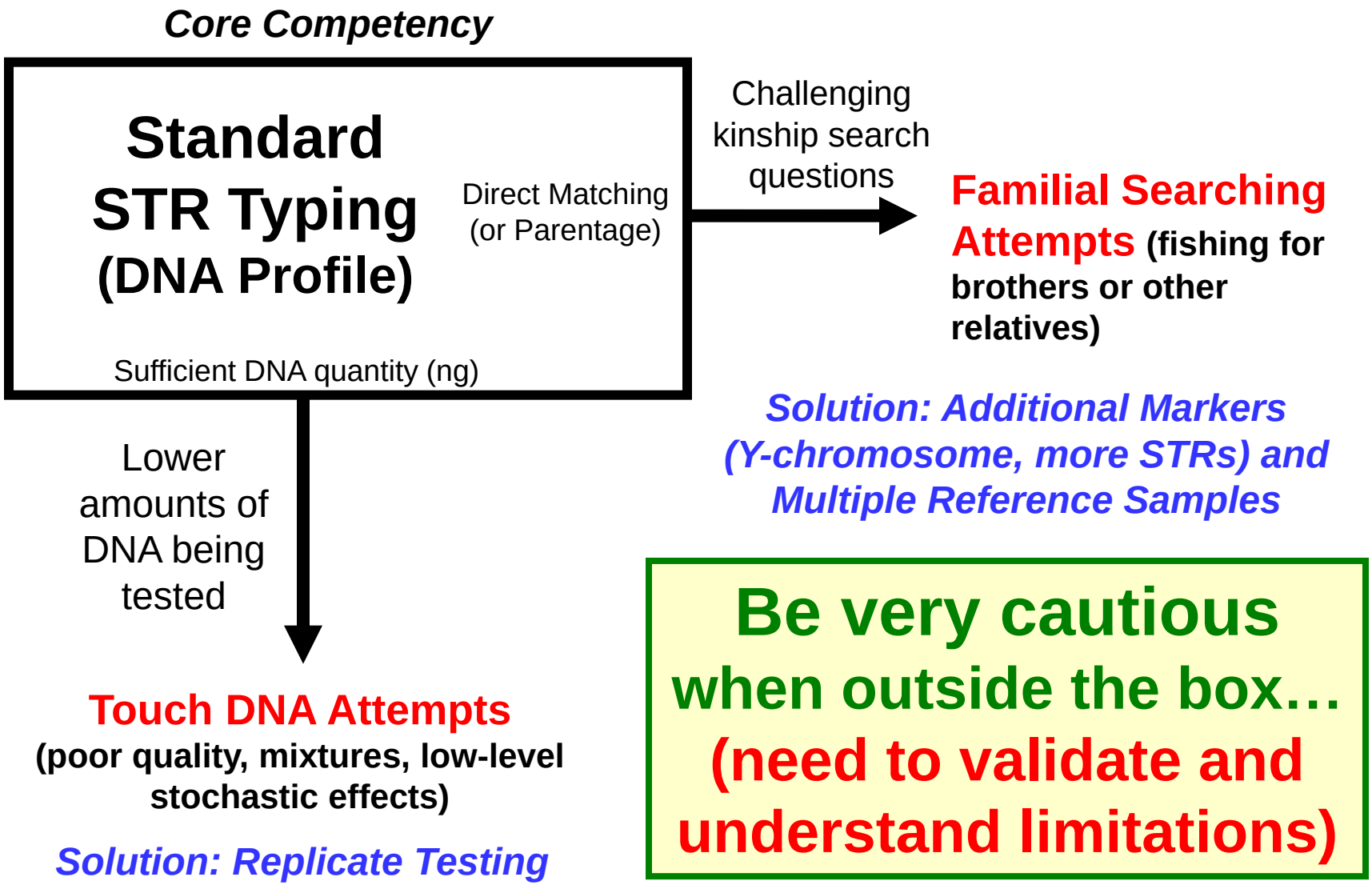


Figure 18.3 in forthcoming Butler (2009) *Fundamentals of Forensic DNA Typing*

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

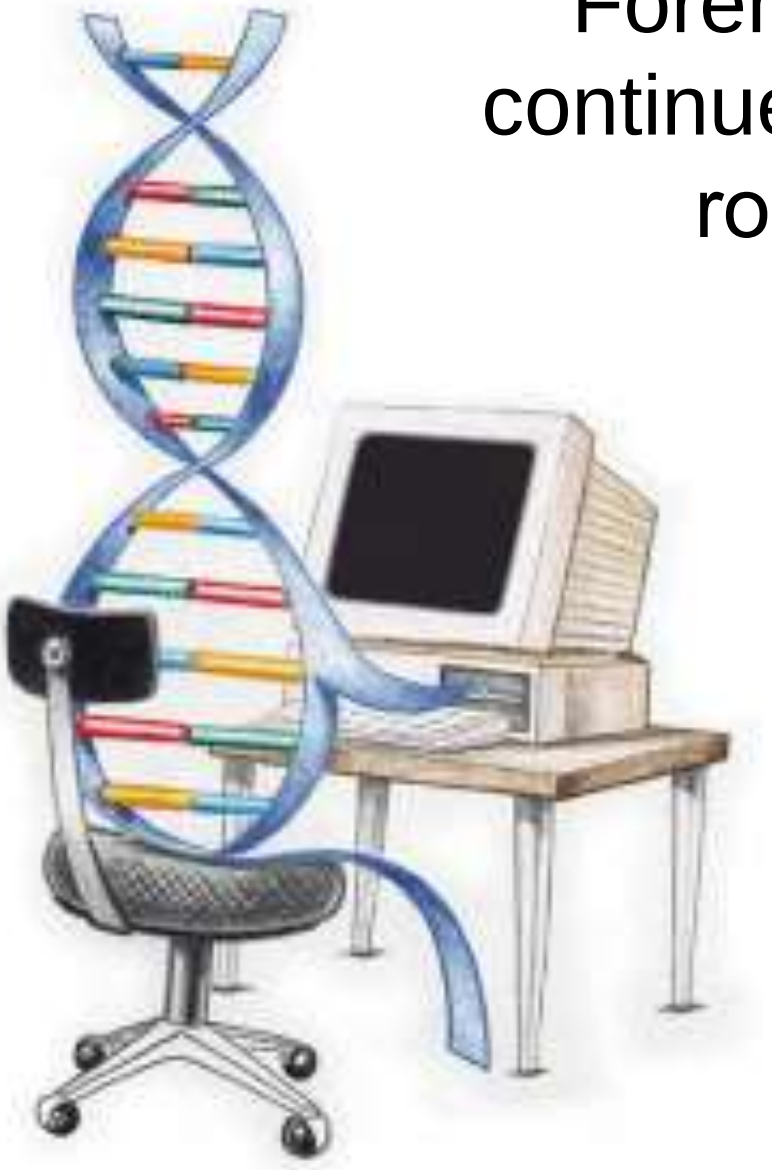
The Future of Forensic DNA

is Similar to the Olympic Motto of

"Swifter, Higher, Stronger"

Resources **Training** **Action**

Forensic DNA testing will continue to play an increasing role in the future…



http://www.manastungare.com/publications/genetic/dna.gif

- Improved sensitivities lead to contamination concerns and DNA case relevance
- Costs will play a role
  - Competition
  - Centralization
  - Communication
- New technology adoption?
  - Political factors
  - Legal factors
  - Legacy data
  - Privacy concerns

National Commission on Forensic Science (NCFS):
www.justice.gov/ncfs

Organization of Scientific Area Committees (OSAC):
www.nist.gov/forensics/osac/index.cfm

NIST FORENSIC SCIENCES

www.nist.gov/forensics

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Mathematical Analogy to Forensic DNA

- **Forensic DNA testing** can be equated to different levels of **mathematics**:
  1. **Single-source samples** (e.g., reference samples) are like **basic arithmetic**
  2. **Two-person mixtures** (e.g., sexual assault evidence) are like **algebra**
  3. **Complex mixtures** (e.g., touch DNA) are like **calculus**

- Validation studies can be considered classroom instruction to help understand the topic and prepare for the casework "exams"

- Proficiency tests are like homework – a graded experience where feedback is received to prepare students for the casework exams when the true answers are not known to the test takers

- If homework is not challenging enough or if your classroom instruction is not to the level needed to be prepared, how can you hope to pass the test? Algebraic principles are necessary for calculus (just like two-person mixture principles apply to complex mixtures), but to truly solve calculus problems and complex mixtures a different level of thinking is required and more study is necessary. ***Would you want to go into a calculus final with only instruction in algebra and experience doing only basic math homework problems?***