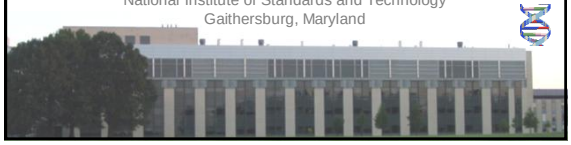
The Copenhagen Forensic Genetic Summer School
Advanced Topics in STR DNA Analysis
June 27-28, 2012

The Value of Additional STR Markers

Mike Coble and Becky Hill

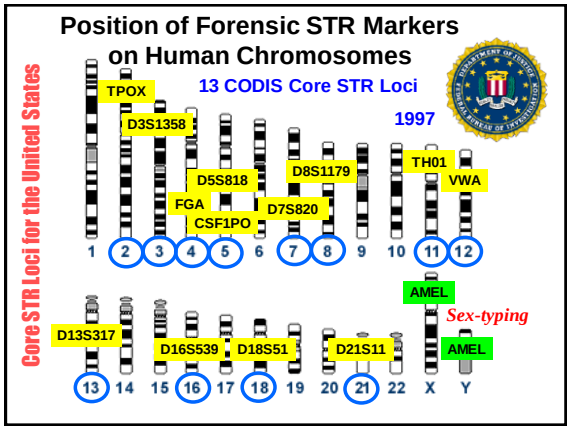
NIST Applied Genetics Group
National Institute of Standards and Technology
Gaithersburg, Maryland

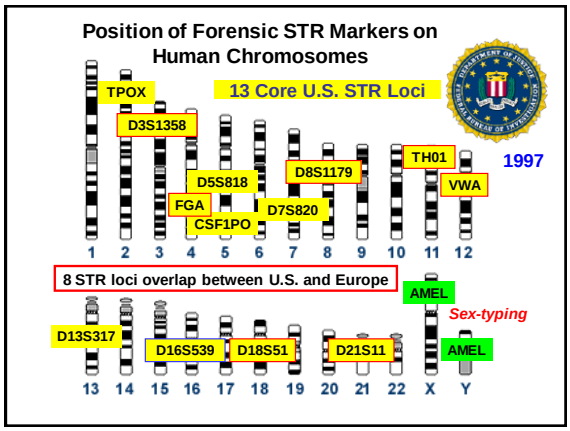


Expanding CODIS Core Loci

Additional STR Loci in the Future?

- Will be needed for more complex kinship analyses and extended applications
  - Example: Y-STRs needed for familial searching
- Immigration testing needs more than 13 STRs
- Larger DNA databases will require more loci





Expanding the 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

Contents lists available at ScienceDirect

Forensic Science International: Genetics

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

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.

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

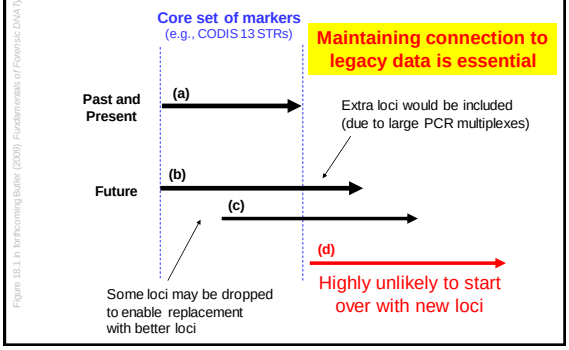


Figure 18.1 in forthcoming Butler (2009) *Fundamentals of Forensic DNA Typing*

Proposed Expanded CODIS Core Loci

D.R. Hares (2012) *Forensic Sci. Int. Genet.* 6(1):e52-e54

20 required loci
Amelogenin (for sex-typing)
18 autosomal STRs
1 Y-STR (DYS391)

	Locus
Section A (required)	Amelogenin
	D18S51
	FGA
	D21S11
	D8S1179
	vWA
	D13S317
	D16S539
	D7S820
	TH01
	D3S1358
	D5S818
	CSF1PO
	D2S1338
	D19S433
	D1S1656
	D12S391
	D2S441
	D10S1248
	~~Penta E~~
	DYS391

	Locus
Section B (in order of preference)	TPOX
	D22S1045
	SE33
	~~Penta D~~

Current CODIS 13 loci in red font

Criteria for Acceptance of Additional Loci

D.R. Hares (2012) *Forensic Sci. Int. Genet.* 6(1):e52-e54

Considered only short tandem repeat (STR) loci due to need for compatibility to existing database of >10 million STR profiles

- **STR Loci**
  - **No known association to medical conditions or defects**
  - **Low mutation rate**
  - **High level of independence**
  - **High level of discrimination**
  - **Use by international forensic DNA community**
  - **Number of loci vs. discrimination factor**
  - **Compliance with Quality Assurance Standards (QAS)**
- **Kit performance**
  - **Balance between loci**
  - **Reliable**
  - **Reproducible**
  - **Sensitive**
  - **Quality results**
  - **Adaptable for use by NDIS laboratories (# of amplifications, ability of kit manufacturers to produce)**
  - **QAS compliant (documentation and availability of validation requirements)**

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

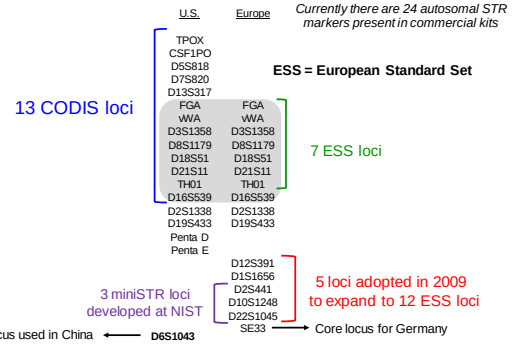
- **To reduce the likelihood of adventitious matches** as the number of profiles stored at NDIS continues to increase each year
- **To increase international compatibility** to assist law enforcement data sharing efforts
- **To increase discrimination power to aid missing persons cases**

Adventitious Matches

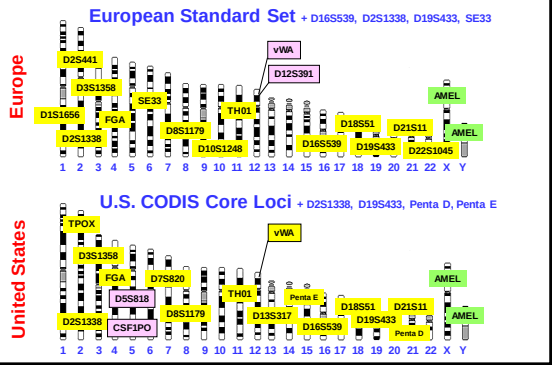
- The only published account of a false match from a DNA database came in 1999 when the UK database then consisting of 660,000 profiles with only 6 STR loci (SGM assay) lead to a "hit" between two individuals whose 6-locus random match probability was 1 in 37 million (R. Willing, *USA Today*, Feb 8, 2000; "Mismatch calls DNA test into question").
- Further testing with four additional STRs (SGM Plus loci) showed that the samples were from different individuals. **The UK expanded the number of core loci from 6 to 10 with the adoption of the SGM Plus kit to try and prevent another adventitious match**.
- The growth of DNA databases necessitates the inclusion of additional loci to avoid this problem.

For further information, see D.N.A. Box 8.3 in Butler, J.M. (2012) *Advanced Topics in Forensic DNA Typing: Methodology*, p. 251

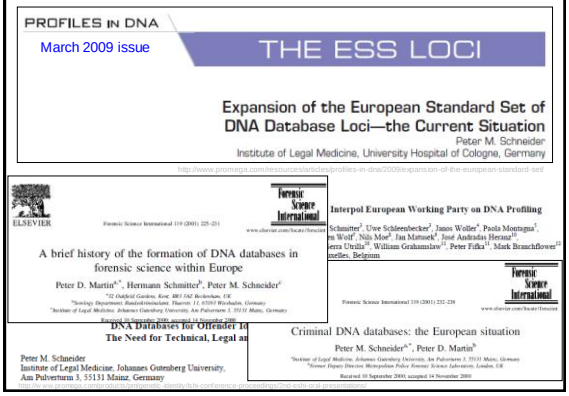
International Comparability



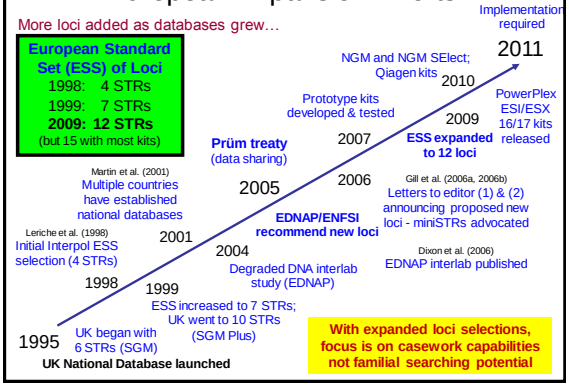
Common Forensic STR Loci



What can we learn from European Standard Set (ESS) expansion experience?



European Expansion Efforts



Lessons from European ESS Expansion

- **Data studies should drive decisions**
  - Interlaboratory study with degraded DNA (Dixon et al. 2006 article was key)
- Casework capabilities are a primary goal
  - miniSTRs and desire for kits with ability to overcome inhibitors
- **Initial locus selection announced through Letters to the Editor** of the leading forensic DNA journal (Gill et al. 2006a, 2006b)
- Companies responded with prototype kits for evaluation
- Expanded ESS loci were selected and voted upon after data review by ENFSI labs **(4 years after initial recommendations were made)**
- EU adopted recommendations of ENFSI
- Commercial kits became available to meet expanded ESS requirements
- Population data gathered and software developed
- European labs must be compliant by Nov 30, 2011 (**2 years after adoption**)
- Casework capabilities not familial searching potential were the intent of the core loci selection

EDNAP Study Showed Value of miniSTRs

Analysis of artificially degraded DNA using STRs and SNPs—results of a collaborative European (EDNAP) exercise

L.A. Dixon [a,*], A.E. Dobbins [a], H.K. Pulker [a], J.M. Butler [b], P.M. Vallone [b], M.D. Coble [b], W. Parson [c], B. Berger [c], P. Grubwieser [c], H.S. Mogensen [d], N. Morling [d], K. Nielsen [d], J.J. Sanchez [d], E. Petkovski [e], A. Carracedo [f], P. Sanchez-Diz [f], E. Ramos-Luis [f], M. Brión [f], J.A. Irwin [g], R.S. Just [g], O. Loreille [g], T.J. Parsons [g], D. Syndercombe-Court [h], H. Schmitter [i], B. Stradmann-Bellinghausen [i], K. Bender [j], P. Gill [a]

[a] The Forensic Science Service, Research and Development, Trident Court, Birmingham, UK
[b] National Institute of Standards and Technology, Gaithersburg, MD, USA
[c] Institute of Legal Medicine, Innsbruck Medical University, Austria

"Recently, there has been much debate about what kinds of genetic markers should be implemented as new core loci that constitute national DNA databases. The choices lie between conventional STRs, ranging in size from 100 to 450 bp; mini-STRs, with amplicon sizes less than 200 bp; and single nucleotide polymorphisms (SNPs)…Results were collated and analysed and, in general, **mini-STR systems were shown to be the most effective**…"

Data Driven Decisions

Short communication

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

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

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

Received 25 May 2005; accepted 26 May 2005
Available online 5 July 2005

"Following a recent meeting by the ENFSI and EDNAP groups on the 4–5 April, 2005, in Glasgow, UK, it was unanimously agreed that the process of standardization within Europe **should take account of recent work that unequivocally demonstrated that chance of obtaining a result from a degraded sample was increased when small amplicons (mini-STRs) were analysed**…"

Characterizing New STR Loci


John Butler


Becky Hill

Main Points:

- In April 2011, the FBI announced plans to expand the core loci for the U.S. beyond the current 13 CODIS STRs
- Our group is collecting U.S. population data on new loci and characterizing them to aid understanding of various marker combinations
- We are collecting all available information from the literature on the 24 commonly used autosomal STR loci

Presentations/Publications:

- AAFS 2011 presentation
- Hill et al (2011) *FSI Genetics* 5(4): 269-275
- Hares (2012) Expanding the U.S. core loci… *FSI Genetics* 6(1): e52-e54
- Butler & Hill (2012) *Forensic Sci Rev* 24(1): 15-26

Article in the January 2012 issue of *Forensic Science Review*

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

Biology and Genetics of New Autosomal STR Loci Useful for Forensic DNA Analysis

REFERENCE: Butler JM, Hill CR: Biology and genetics of new autosomal STR loci useful for forensic DNA analysis; *Forensic Sci Rev* 24:15; 2012.

ABSTRACT: Short tandem repeats (STRs) are regions of tandemly repeated DNA segments found throughout the human genome that vary in length (through insertion, deletion, or mutation) with a core repeated DNA sequence. Forensic laboratories commonly use tetranucleotide repeats, containing a four base pair (4-bp) repeat structure such as GATA. In 1997, the Federal Bureau of Investigation (FBI) Laboratory selected 13 STR loci that form the backbone of the U.S. national DNA database. Building on the European expansion in 2009, the FBI announced plans in April 2011 to expand the U.S. core loci to as many as 20 STRs to enable more global DNA data sharing. Commercial STR kits enable consistency in marker use and allele nomenclature between laboratories and help improve quality control. The STRBase website, maintained by the U.S. National Institute of Standards and Technology (NIST), contains helpful information on STR markers used in human identity testing.

Key Words: Autosomal genetic markers, CODIS STRs, core loci, DNA typing, European Standard Set, expanded U.S. core loci, short tandem repeat (STR), STR kits.

Discusses the 24 autosomal STR loci available in commercial kits

The 11 STR Loci Beyond the CODIS 13

	STR Locus	Location	Repeat Motif	Allele Range*	# Alleles*
	D2S1338	2q35	TGCC/TTCC	10 to 31	40
	D19S433	19q12	AAGG/TAGG	5.2 to 20	36
	Penta D	21q22.3	AAAGA	1.1 to 19	50
	Penta E	15q26.2	AAAGA	5 to 32	53
5 new European loci	D1S1656	1q42	TAGA	8 to 20.3	25
	D12S391	12p13.2	AGAT/AGAC	13 to 27.2	52
	D2S441	2p14	TCTA/TCAA	8 to 17	22
	D10S1248	10q26.3	GGAA	7 to 19	13
	D22S1045	22q12.3	ATT	7 to 20	14
	SE33	6q14	AAAG‡	3 to 49	**178**
	D6S1043	6q15	AGAT/AGAC	8 to 25	25

**Allele range and number of observed alleles from Appendix 1, J.M. Butler (2012) Advanced Topics in Forensic DNA Typing: Methodology; ‡SE33 alleles have complex repeat structure*

25 Alleles Reported in the Literature for **D1S1656**

15 NIST observed alleles circled in red

Allele (Repeat #)	Promega ESX 17	Promega ESI 17	ABI NGM	Repeat Structure [TAGA]$_n$[TGA]$_{0-1}$[TAGA]$_n$TAGG[TG]$_5$	Reference
8	133 bp	222 bp	171 bp	[TAGA]$_8$[TG]$_5$	Phillips *et al.* (2010)
9	137 bp	226 bp	175 bp	[TAGA]$_9$[TG]$_5$	Phillips *et al.* (2010)
10 (a)	141 bp	230 bp	179 bp	[TAGA]$_{10}$[TG]$_5$	Lareu *et al.* (1998)
10 (b)	141 bp	230 bp	179 bp	[TAGA]$_{10}$TAGG[TG]$_5$	Phillips *et al.* (2010)
11	145 bp	234 bp	183 bp	[TAGA]$_{11}$[TG]$_5$	Lareu *et al.* (1998)
12 (a)	149 bp	238 bp	187 bp	[TAGA]$_{12}$[TG]$_5$	Lareu *et al.* (1998)
12 (b)	149 bp	238 bp	187 bp	[TAGA]$_{11}$TAGG[TG]$_5$	Lareu *et al.* (1998)
13 (a)	153 bp	242 bp	191 bp	[TAGA]$_{12}$TAGG[TG]$_5$	Lareu *et al.* (1998)
13 (b)	153 bp	242 bp	191 bp	[TAGA]$_{13}$[TG]$_5$	Phillips *et al.* (2010)
13.3	156 bp	245 bp	194 bp	[TAGA]$_3$TGA[TAGA]$_{13}$TAGG[TG]$_5$	Phillips *et al.* (2010)
14 (a)	157 bp	246 bp	195 bp	[TAGA]$_{13}$TAGG[TG]$_5$	Lareu *et al.* (1998)
14 (b)	157 bp	246 bp	195 bp	[TAGA]$_{14}$[TG]$_5$	Phillips *et al.* (2010)
14.3	160 bp	249 bp	198 bp	[TAGA]$_4$TGA[TAGA]$_9$TAGG[TG]$_5$	Phillips *et al.* (2010)
15	161 bp	250 bp	199 bp	[TAGA]$_{14}$TAGG[TG]$_5$	Lareu *et al.* (1998)
15.3	164 bp	253 bp	202 bp	[TAGA]$_4$TGA[TAGA]$_{10}$TAGG[TG]$_5$	Lareu *et al.* (1998)
16	165 bp	254 bp	203 bp	[TAGA]$_{15}$TAGG[TG]$_5$	Lareu *et al.* (1998)
16.3	168 bp	257 bp	206 bp	[TAGA]$_4$TGA[TAGA]$_{11}$TAGG[TG]$_5$	Lareu *et al.* (1998)
17	169 bp	258 bp	207 bp	[TAGA]$_{16}$TAGG[TG]$_5$	Lareu *et al.* (1998)
17.1	170 bp	259 bp	208 bp	Not published	Schröer *et al.* (2000)
17.3	172 bp	261 bp	210 bp	[TAGA]$_4$TGA[TAGA]$_{12}$TAGG[TG]$_5$	Lareu *et al.* (1998)
18	173 bp	262 bp	211 bp	[TAGA]$_{17}$TAGG[TG]$_5$	Phillips *et al.* (2010)
18.3	176 bp	265 bp	214 bp	[TAGA]$_4$TGA[TAGA]$_{13}$TAGG[TG]$_5$	Lareu *et al.* (1998)
19	177 bp	266 bp	215 bp	Not published	Asamura *et al.* (2008)
19.3	180 bp	269 bp	218 bp	[TAGA]$_4$TGA[TAGA]$_{14}$TAGG[TG]$_5$	Lareu *et al.* (1998)
20.3	184 bp	273 bp	222 bp	Not published	Gamero *et al.* (2000)

from Appendix 1, J.M. Butler (2011) Advanced Topics in Forensic DNA Typing: Methodology

NIST **U.S. Population Allele Frequencies**

D1S1656 (15 different alleles)

15 different alleles

Allele	African American (N = 341)	Caucasian (N = 361)	Hispanic (N = 236)
10	0.01433	0.00277	0.00630
11	0.04871	0.07756	0.02731
12	0.06304	0.11773	0.08824
13	0.10029	0.06648	0.11555
14	**0.25788**	0.07895	0.11765
14.3	0.00716	0.00277	0.00420
15	0.15616	**0.14820**	0.13866
15.3	0.03009	0.05817	0.05042
16	0.11032	0.13573	**0.17437**
16.3	0.10029	0.06094	0.05462
17	0.02865	0.04709	0.04202
17.3	0.05014	0.13296	0.14496
18	0.00287	0.00554	0.00630
18.3	0.02436	0.05125	0.02521
19.3	0.00573	0.01385	0.00420

N = 938

(only unrelated samples used; fathers removed from this sample set)

< 5/2N

D1S1656 Characteristics

- **15 alleles** observed
- **92 genotypes** observed
- **>89% heterozygotes** (heterozygosity = 0.8934)
- **0.0220 Probability of Identity** (P_I)

$$P_I = \sum (genotype\ frequencies)^2$$

These values have been calculated for all 24 STR loci across the U.S. population samples examined

Loci sorted on Probability of Identity (P_I) values

23 STR Loci present in STR kits rank ordered by their variability

STR Locus	Alleles Observed	Genotypes Observed	Het. (obs)	P_I value N = 938
SE33	53	292	0.9360	0.0069
Penta E*	20	114	0.8799	0.0177
D2S1338	13	68	0.8785	0.0219
D1S1656	15	92	0.8934	0.0220
D18S51	21	91	0.8689	0.0256
D12S391	23	110	0.8795	0.0257
FGA	26	93	0.8742	0.0299
Penta D*	16	71	0.8754	0.0356
D21S11	25	81	0.8358	0.0410
D19S433	16	76	0.8124	0.0561
D8S1179	11	45	0.7878	0.0582
vWA	11	38	0.8060	0.0622
D7S820	11	32	0.8070	0.0734
TH01	8	24	0.7580	0.0784
D16S539	9	28	0.7825	0.0784
D13S317	8	29	0.7655	0.0812
D10S1248	12	39	0.7825	0.0837
D2S441	14	41	0.7772	0.0855
D3S1358	11	30	0.7569	0.0873
D22S1045	11	42	0.7697	0.0933
CSF1PO	9	30	0.7537	0.1071
D5S818	9	34	0.7164	0.1192
TPOX	9	28	0.6983	0.1283

Better for mixtures (more alleles seen)

361 Caucasians
341 African Americans
236 Hispanics

There are several loci more polymorphic than the **current CODIS 13 STRs**

N = 938
(only unrelated samples used)

Better for kinship (low mutation rate)

*Penta D & Penta E run on subset (N = 658)

Determination of Additional CODIS Core Loci

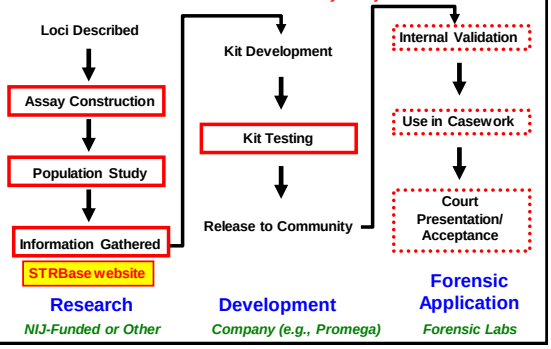
D.R. Hares (2012) Expanding the CODIS Core Loci in the United States. *Forensic Sci. Int. Genet.* 6: e52-e54
Addendum to expanding the CODIS core loci in the United States, Forensic Sci. Int. Genet. (2012) doi:10.1016/j.fsigen.2012.01.003

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-implementation-of-additional-codis-core-loci

Steps in Adopting Genetic Markers

Role of the NIST Human Identity Project Team



Recent Court Decision Impacting Sale of STR Typing Kits

Disclaimer: The information contained herein is only as accurate as my understanding of the information available to me at the time this presentation was given. ***Things are still evolving with this case…***

http://www.appliedbiosystems.com

Notice on ABI STR Kits

IMPORTANT NOTICE

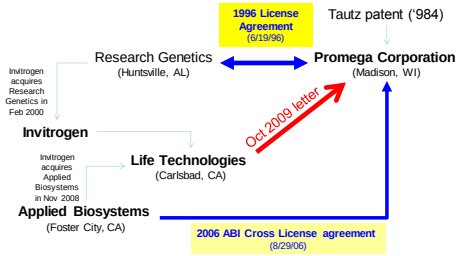
The UNITED STATES DISTRICT COURT FOR THE WESTERN DISTRICT OF WISCONSIN ruled that certain products (listed below) sold by Life Technologies Corporation ("Life") can only be used by customers for forensic and paternity uses ("Licensed Use"). Specifically, the Court held that the license Life holds from Promega Corporation ("Promega") does not include the following applications: (1) chimerism (which involves determining the relative amount present of two different types of DNA); (2) classifying molar specimens (which involves determining whether a mole is present and what type it is); (3) cell line authentication (which involves a determination of whether two cell lines are unique); (4) determination of fetal sex;(5) cancer analysis; (6) genetic research; (7) non-casework-related forensic applications such as general research in forensics or teaching and training of persons not employed in a forensic laboratory; (8) maternal cell contamination; and (9) sample tracking. Accordingly, this notice replaces any other label license or use statement for the listed products only as those labels or statements relate to the use of such products under the Promega license. Any other restrictions, such as regulatory restrictions, related to the use of these products are not affected by this notice. If a customer has any question regarding whether their intended use is within or outside the Licensed Use, please contact LicenseQuery@lifetech.com .

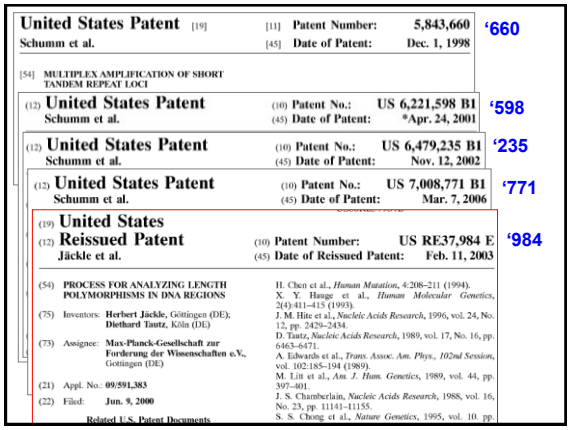
The following products are subject to this notice:
4322288 AmpFℓSTR® Identifiler® PCR Amplification Kit …

The following products are subject to this notice:

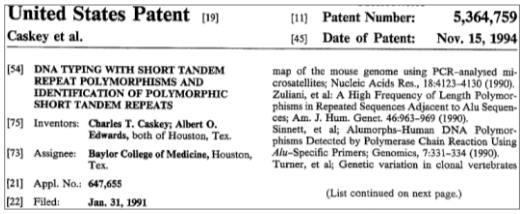
4322288 AmpFℓSTR® Identifiler® PCR Amplification Kit
4408580 AmpFℓSTR® Identifiler® Direct PCR Amplification Kit (1000 tests)
4467831 AmpFℓSTR® Identifiler® Direct PCR Amplification Kit (200 tests)
4427368 AmpFℓSTR® Identifiler® Plus PCR Amplification Kit
4373872 AmpFℓSTR® MiniFiler™ PCR Amplification Kit
4415021 AmpFℓSTR® NGM™ PCR Amplification Kit (1000 rxn)
4415020 AmpFℓSTR® NGM™ PCR Amplification Kit (200 rxn)
4457890 AmpFℓSTR® NGM SElect™ PCR Amplification Kit (1000 rxn)
4457889 AmpFℓSTR® NGM SElect™ PCR Amplification Kit (200 rxn)
403038 AmpFℓSTR® Profiler® PCR Amplification Kit
4303326 AmpFℓSTR® Profiler Plus® PCR Amplification Kit
4330284 AmpFℓSTR® Profiler Plus® ID PCR Amplification Kit
4305246 AmpFℓSTR® COfiler® PCR Amplification Kit
4307133 AmpFℓSTR® SGM Plus® PCR Amplification Kit
4382699 AmpFℓSTR® SEfiler Plus™ PCR Amplification Kit
4305979 AmpFℓSTR® Profiler Plus® and AmpFLSTR® Cofiler® Kits
4330621 AmpFℓSTR® Profiler Plus® ID Kit and AmpFLSTR® Cofiler ® Kit
4359513 AmpFℓSTR® Yfiler® PCR Amplification Kit
4382306 AmpFℓSTR® Sinofiler™ PCR Amplification Kit
4382324 AmpFℓSTR® Sinofiler™ PCR Amplification Kit Primer Set

Summary of the Facts from My Understanding of Court Documents





This Patent was Previously Licensed to Both Promega and Applied Biosystems



United States Patent [19]
Caskey et al.

[11] **Patent Number: 5,364,759**
[45] **Date of Patent: Nov. 15, 1994**

[54] **DNA TYPING WITH SHORT TANDEM REPEAT POLYMORPHISMS AND IDENTIFICATION OF POLYMORPHIC SHORT TANDEM REPEATS**

[75] Inventors: **Charles T. Caskey; Albert O. Edwards,** both of Houston, Tex.

[73] Assignee: **Baylor College of Medicine,** Houston, Tex.

[21] Appl. No.: **647,655**

[22] Filed: **Jan. 31, 1991**

map of the mouse genome using PCR-analysed microsatellites; Nucleic Acids Res., 18:4123-4130 (1990).
Zuliani, et al: A High Frequency of Length Polymorphisms in Repeated Sequences Adjacent to Alu Sequences; Am. J. Hum. Genet. 46:963-969 (1990).
Sinnett, et al; Alumorphs-Human DNA Polymorphisms Detected by Polymerase Chain Reaction Using *Alu*-Specific Primers; Genomics, 7:331-334 (1990).
Turner, et al; Genetic variation in clonal vertebrates

(List continued on next page.)

Patent expired after 17 years
on November 15, 2011

Timeline to Court Case

- On **October 20, 2009**, Life Technologies (LTI = ABI) sent a letter to Promega asserting new interpretation of the 1996 License Agreement which would have required Promega to pay >$50M within 60 days of demand (>20X what has previously been paid)
- During January 2010 meeting, Promega and ABI agreed to conduct audits about royalty payments
- In a February 10, 2010 letter, LTI conceded it had no documentary evidence to support its novel claim
- In a May 4, 2010 letter, LTI demanded arbitration of a supposed royalty BUT ABI had breached the 1996 agreement
- In a July 7, 2010 follow-up letter, Promega sought a declaration that LTI and ABI have **willfully infringed 5 patents by selling outside permitted fields** (in clinical diagnostics, clinical research, & research markets)

Trial Dates and Results

- February 6, 7, 8, 9, 10, 13, 14, 15 (2012)
- Jury verdict on February 15, 2012
- **Judgment on February 23, 2012**
- **Promega received $52,009,941 from Life Technologies (Applied Biosystems)**

Jury Verdict on February 15, 2012

IN THE UNITED STATES DISTRICT COURT

FOR THE WESTERN DISTRICT OF WISCONSIN

PROMEGA CORPORATION,

Plaintiff,

v.

LIFE TECHNOLOGIES CORPORATION, INVITROGEN IP HOLDINGS, INC. and APPLIED BIOSYSTEMS, LLC,

Defendants.

SPECIAL VERDICT

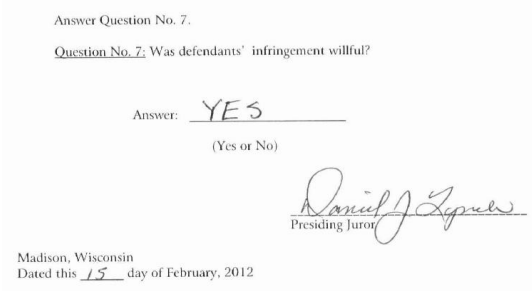
10-cv-281-bbc

- Question No.1: What is the **total dollar amount of worldwide STR kit sales made between August 29, 2006 through the end of January 2012** by defendants Life Technologies Corporation, Invitrogen IP Holdings. Inc. and Applied Biosystems, LLC?
- Answer: **$ 707,618,247**

Answer Question No. 5.

Question No. 5: What profits, if any, did plaintiff lose as a result of defendants' sales that you found in Question No.4?

Answer: $ 52,009,941



Answer Question No. 7.

Question No. 7: Was defendants' infringement willful?

Answer: YES

(Yes or No)

Presiding Juror

Madison, Wisconsin
Dated this 15 day of February, 2012

Forensic DNA Labs

- Forensic & paternity testing DNA laboratories performing casework **should not be directly impacted by this court ruling** because ABI has a license to sell for casework applications

Potential Impact on NIST

- Judge has narrowly defined that ***only forensic labs and paternity labs may be sold ABI kits*** **– NOT universities or other research labs**
- I have spoken with lawyers from both Promega and Life Technologies (Applied Biosystems)
- The initial plan was for Promega to work with LTI/ABI to develop a permitted purchase list institution by institution
  - Promega wants to take over cell line authentication market and other clinical DNA applications
- Purchase of ABI STR kits for forensic research and training may not be permitted in the future
- Both companies would like to keep their customers happy…

New STR Kits

Commercially Available STR Kits

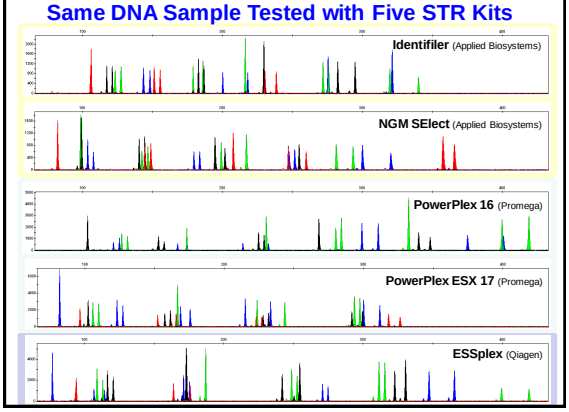
Applied Biosystems (17)
- ~~AmpFlSTR Blue (1996)~~
- ~~AmpFlSTR Green I (1997)~~
- Profiler (1997)
- Profiler Plus (1997)
- COfiler (1998)
- SGM Plus (1999)
- **Identifiler** (2001)
- Profiler Plus ID (2001)
- ~~SEfiler (2002)~~
- Yfiler (2004)
- MiniFiler (2007)
- SEfiler Plus (2007)
- Sinofiler (2008) – China only
- **Identifiler Direct** (2009)
- NGM (2009)
- **Identifiler Plus** (2010)
- NGM SElect (2010)

Promega Corporation (15)
- PowerPlex 1.1 (1997)
- PowerPlex 1.2 (1998)
- PowerPlex 2.1 (1999)
- **PowerPlex 16** (2000)
- PowerPlex ES (2002)
- PowerPlex Y (2003)
- PowerPlex S5 (2007)
- **PowerPlex 16 HS** (2009)
- PowerPlex ESX 16 (2009)
- PowerPlex ESX 17 (2009)
- PowerPlex ESI 16 (2009)
- PowerPlex ESI 17 (2009)
- PowerPlex 18D (2011)
- PowerPlex 21 (2012)
- PowerPlex ESI 17 Pro (2012)

Qiagen (2010)
Primarily selling kits in Europe
Due to patent restrictions cannot sell in U.S.
- ESSplex
- ESSplex SE
- Decaplex SE
- IDplex
- Nonaplex ESS
- Hexaplex ESS
- HD (Chimera)
- Argus X-12
- Argus Y-12
- DIPlex (30 InDels)

~1/3 of all STR kits were released in the last three years

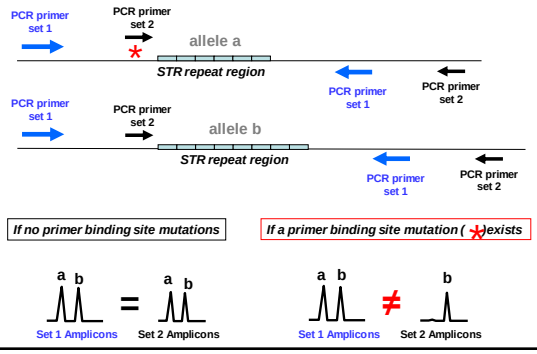
Same DNA Sample Tested with Five STR Kits

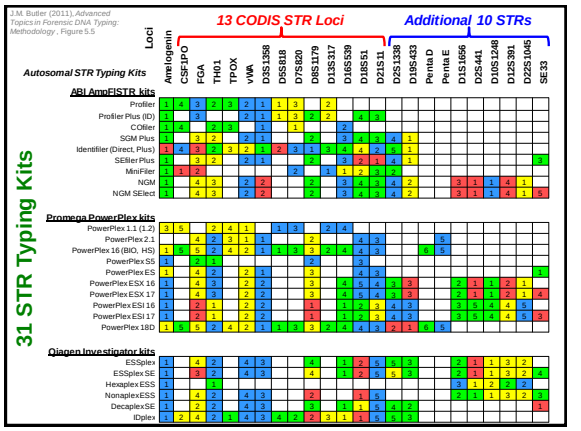


STR Kit Concordance Testing

- Many of these STR kits have different primer sequences for amplifying the same STR locus
- Need to analyze the same DNA samples with different STR typing kits looking for differences
- In some rare cases, allele dropout may occur due to mutations in primer binding regions

STR Kit Concordance Testing





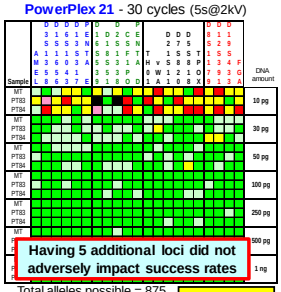
Data courtesy of Becky Hill (NIST)

STR Kit Performance

21plex (PowerPlex 21) vs 16plex (Identifiler Plus)

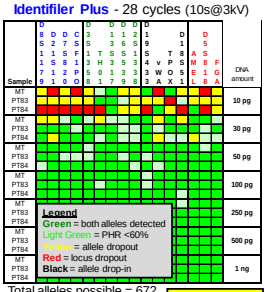
Three fully heterozygous (except PT83 at Penta D) pristine DNA samples were examined in a dilution series with PowerPlex 21 and Identifiler Plus. Results are ordered by amplicon size and dye color.

PowerPlex 21 - 30 cycles (5s@2kV)



Total alleles possible = 875
Total alleles present = 805
92% detected

Identifiler Plus - 28 cycles (10s@3kV)



Total alleles possible = 672
Total alleles present = 619
92% detected

STR Kit Comparisons Searching for Primer Binding Site Mutations

Becky Hill

Kits compared	Samples	Loci compared	Comparisons	# Differences	Concordance (%)
SGM-ID	1436	11	15,796	1	99.994
ID-ProPlus	1427	10	14,270	1	99.993
ID-IDplex	669	16	10,704	19	99.822
ID-PP16	662	14	9,268	4	99.957
ID-MiniFiler	1308	9	11,772	27	99.771
SGM-NGM	1436	11	15,796	4	99.975
ID-NGM	1449				
ProPlus-NGM	1427				
SGM-ESI	1436				
ProPlus-ESX	1427				
ESI-ESX	1455				
ESI-ESSplex	1445				
ESX-ESSplex	1445				
ESI-NGMSElect	715				

128 kit-to-kit comparisons
1,104,031 allele comparisons
1224 differences observed
~99.9% concordance
(many corrected now)

*Kits (except Identifiler) were kindly provided by **Applied Biosystems, Promega, and Qiagen** for concordance testing performed at NIST*

Random Match Probability for Various Combinations (assuming unrelated individuals)

STR Marker Combinations	RMP*	1 in …
13 CODIS STRs	**6.0E-16**	**1.7E+15**
15 STRs (+D2S1338, D19S433)	7.3E-19	1.4E+18
18 STRs (+D2S441, D10S1248, D22S1045)	4.9E-22	2.0E+21
20 STRs (+D1S1656, D12S391)	2.8E-25	3.6E+24
23 STRs (+SE33, Penta D, Penta E)	**1.2E-30**	**8.4E+29**

14 orders of magnitude improvement

**RMP values calculated by combining Probability of Identity values for each locus*

More Loci are Useful in Situations Involving Relatives

- **Missing Persons** and Disaster Victim Identification (kinship analysis)
- Immigration Testing (often limited references)
  - Recommendations for 25 STR loci
- Deficient Parentage Testing
  - often needed if only one parent and child are tested

Relationship testing labs are being pushed to answer more difficult genetic questions…and **we want to make sure the right tools are in place**

In February 25, 2011 issue of *Forensic Science International*…

Forensic Science International 205 (2011) 64–68

Contents lists available at ScienceDirect

Forensic Science International

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

Examples of kinship analysis where Profiler Plus™ was not discriminatory enough for the identification of victims using DNA identification☆

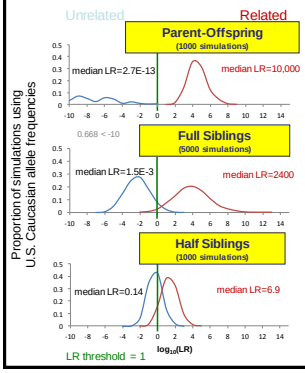
D. Hartman[a,b,*], L. Benton[a], L. Morenos[a], J. Beyer[a], M. Spiden[a], A. Stock[a]

[a] Victorian Institute of Forensic Medicine, 57-83 Kavanagh St, Southbank, VIC 3006, Australia
[b] Department of Forensic Medicine, Monash University, 57-83 Kavanagh St, Southbank, VIC 3006, Australia

Disaster victim identification from the 2009 Victorian bushfires relied on DNA (82% involved kinship associations rather than direct matching)

They advocate additional autosomal STR loci to aid kinship associations

How do 13 loci perform for kinship analysis?



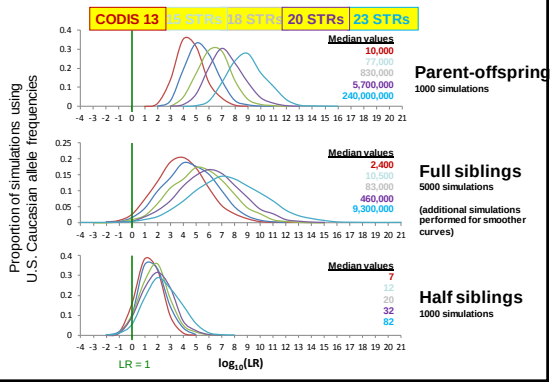
The degree of overlap corresponds with possible values for false positive or false negative results.

Parent-offspring comparisons:
No overlap between unrelated and related LR distributions

Full sibling comparisons:
False positive rate = 0.027
False negative rate = 0.033

Half sibling comparisons:
False positive rate = 0.155
False negative rate = 0.168

Do additional loci improve kinship determination?



Summary

- Additional autosomal STR loci exist in new STR kits and are being studied at NIST in U.S. population sample sets
- To avoid potential adventitious matches with large DNA databases, enable greater international data sharing, and aid missing persons applications, it is highly likely that additional loci will be added to the U.S. core in the future

Thank you for your attention

Acknowledgments: NIJ & FBI Funding

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