NIJ National Forensic Science Technology Center
President's DNA Initiative - Workshops

Validation Workshop

Developmental Validation

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Presentation Outline

Introductions: Presenters and Participants

Day #1
- Validation Overview (John)
- Introduction to DAB Standards (Robyn & John)
- Developmental Validation (John)

Day #2
- Inconsistency in Validation between Labs (John)
- Internal Validation (Robyn)
- Method Modifications and Performance Checks (Robyn)

Day #3
- Practical Exercises (Robyn)

Overview of This Section

- Why is developmental validation different from internal validation?
- Who performs developmental validation and why?
- What types of studies must be performed?
- For genetic markers, how do you address inheritance, detection of polymorphisms, species specificity, accuracy, sensitivity, stability, reproducibility, optimization of reactions, stochastic effects, multiplexes, product detection, population studies and statistical analysis, and mixture analysis?
- What are some factors that impact reliability of DNA typing and should be carefully examined?

DNA Advisory Board Quality Assurance Standards

Section 2. Definitions

- (ff) Validation is a process by which a procedure is evaluated to determine its **efficacy and reliability** for forensic casework analysis *(DNA analysis)* and includes:

Manufacturer

  - (1) Developmental validation is the acquisition of test data and determination of conditions and limitations of a new or novel DNA methodology for use on forensic samples;
  - (2) Internal validation is an accumulation of test data within the laboratory to demonstrate that established methods and procedures perform as expected in the laboratory.

Forensic Lab

Differences between Developmental and Internal Validation

- Detail of the studies
- Peer-reviewed publication
  - journals do not consider internal validation studies novel and are not likely to publish them

Who Performs Developmental Validation?

- Who? (SWGDAM Revised Validation Guidelines 1.2.1)
  - Manufacturer
  - Technical Organization
  - Academic Institution
  - Government Laboratory
  - Other Party (examples?)
- Are there potential conflicts of interest with any of these groups performing developmental validation?

SWGDAM Revised Validation Guidelines
http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm

When Should Developmental Validation Be Performed?

1.2.1 Developmental validation **must precede the use of a novel methodology** for forensic DNA analysis.

1.2.1.1 Peer-reviewed publication of the underlying scientific principle(s) of a technology is required.

What are examples of underlying principles for STR typing?

1.2.1.2 **Peer-reviewed publication of the results of developmental validation studies is encouraged**. However, technologies or procedures **may be implemented without peer-reviewed publication** if the results of developmental studies have been disseminated to the scientific community… such as … publication in a technical manual.

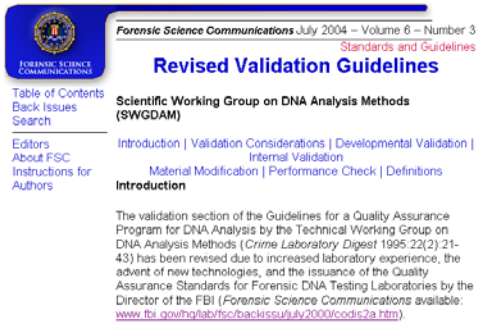
SWGDAM Revised Validation Guidelines
http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm

Examples of Delay in Publication

- ProfilerPlus/COfiler
  - Kits released in Dec 1997/May 1998 with technical manuals
  - Publication in Jan 2002 of developmental validation (submitted in July 2000)
- Identifiler
  - Kit released in July 2001 with technical manual
  - Publication in Nov 2004 of developmental validation (submitted in June 2002)
- Quantifiler
  - Kit released in Nov 2003 with technical manual
  - Publication in July 2005 of developmental validation
- PowerPlex 16
  - Kit released in May 2000 following presentations at meetings *(technical manual does not describe studies performed)*
  - Publication in July 2002 of developmental validation

Revised SWGDAM Validation Guidelines
(July 2004)

http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm



The document provides validation guidelines and definitions approved by SWGDAM July 10, 2003.

SWGDAM Revised Validation Guidelines

- The validation process identifies aspects of a procedure that are **critical** and must be carefully **controlled and monitored**.
- What are some critical aspects of STR typing?
  - Ask for responses from participants
- What factors need to be controlled and monitored in order to obtain reliable STR results?
  - Write down and see if validation studies address these factors…

SWGDAM Revised Validation Guidelines
http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm

1.2.1 Developmental validation is the **demonstration of the accuracy, precision, and reproducibility of a procedure** by the manufacturer, technical organization, academic institution, government laboratory, or other party. **Developmental validation must precede the use of a novel methodology for forensic DNA analysis**.

- What are some potential problems if developmental validation studies have not been performed or published prior to their use in forensic DNA analysis?

SWGDAM Revised Validation Guidelines
http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm

Publication Required

1.2.1.1 Peer-reviewed publication of the underlying scientific principle(s) of a technology is required.

- What are some of the underlying scientific principles for STR typing?
  - DNA extraction
  - PCR
  - Fluorescent dye labels
  - Capillary electrophoresis
  - Run-to-run precision that enables comparison to allelic ladders

SWGDAM Revised Validation Guidelines
http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm

Documentation for Developmental Validation Studies

1.2.1.2 **Peer-reviewed publication of the results of developmental validation studies is encouraged**. However, technologies or procedures may be implemented without peer-reviewed publication if the results of developmental studies have been disseminated to the scientific community for review and evaluation through multiple ways, such as presentation at a scientific meeting or publication in a technical manual.

- Is a presentation at a scientific meeting sufficient? What are some challenges with this form of reporting on validation studies?
- Is information from a technical manual sufficient (e.g., Quantifiler manual)?

SWGDAM Revised Validation Guidelines
http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm

Overview of Developmental Validation Studies

2. Developmental Validation: The developmental validation process may include the studies detailed below. Some studies may not be necessary for a particular method.

Examples where studies are not necessary?

2.1 Characterization of genetic markers
2.2 Species specificity
2.3 Sensitivity studies
2.4 Stability studies
2.5 Reproducibility
2.6 Case-type samples
2.7 Population studies
2.8 Mixture studies
2.9 Precision and accuracy
2.10 PCR-based procedures

SWGDAM Revised Validation Guidelines
http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm

Overview of Internal Validation Studies

3. Internal Validation: The internal validation process should include the studies detailed below encompassing **a total of at least 50 samples**. Some studies may not be necessary due to the method itself.

Examples where studies are not necessary?

3.1 Known and nonprobative evidence samples
3.2 Reproducibility and precision
3.3 Match criteria
3.4 Sensitivity and stochastic studies
3.5 Mixture studies
3.6 Contamination
3.7 Qualifying test

SWGDAM Revised Validation Guidelines
http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm

2.1 Characterization of genetic markers

2.1 Characterization of genetic markers: The basic characteristics (described below) of a genetic marker must be determined and documented.

2.1.1 **Inheritance**: The mode of inheritance of DNA markers demonstrated through family studies.

2.1.2 **Mapping**: The chromosomal location of the genetic marker (submitted to or recorded with the Nomenclature Committee of the Human Genome Organization).

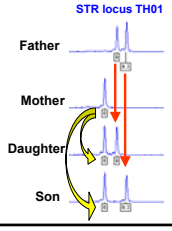
2.1.3 **Detection**: Technological basis for identifying the genetic marker.

2.1.4 **Polymorphism**: Type of variation analyzed.

SWGDAM Revised Validation Guidelines
http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm

2.1.1 **Inheritance**

- The mode of inheritance of DNA markers demonstrated through family studies.
- Examination of a CEPH family looking for Mendelian inheritance patterns…



Illustrate parental allele transfer with D13S317 F, M, S1, S2, D1, S5—all possible combinations seen

CEPH Utah Pedigree 13293

Marker	PGF	PGM	F	S1	S2	D1	D2	S3	S4	S5	M	MGF	MGM
CSF1PO	11,12	10,10	10,12	12,13	12,12	10,12	10,12	12,13	12,12	12,12	12,13	12,13	10,13
FGA	20,22	20,21	20,20	20,21	20,24	20,24	20,24	20,24	20,24	20,21	21,24	21,24	21,22
TH01	9.3,9.3	7,9	9,9.3	8,9	8,9.3	8,9	8,9	8,9	8,9	8,9	8,8	6,8	7,8
TPOX	8,8	8,8	8,8	8,8	8,8	8,8	8,8	8,8	8,8	8,8	8,8	8,8	8,9
VWA	16,16	17,19	16,17	16,17	16,17	17,17	16,16	16,17	17,17	16,17	16,17	16,16	16,17
D3S1358	14,15	17,18	14,18	15,18	16,18	14,15	14,15	15,18	15,18	16,18	15,16	15,16	15,17
D5S818	10,12	10,12	10,12	11,12	12,13	10,13	10,11	12,13	11,12	12,13	11,13	11,12	9,13
D7S820	13,13	9,11	9,13	9,12	9,9	9,9	9,13	9,12	12,13	12,13	9,12	9,11	9,12
D8S1179	12,13	11,13	13,13	13,13	10,13	10,13	13,13	10,13	13,13	13,13	10,13	10,13	13,13
D13S317	9,13	9,10	9,10	10,12	10,11	9,12	10,11	10,11	10,12	9,11	11,12	11,12	11,12
D16S539	12,13	12,13	13,13	12,13	13,13	12,13	13,13	12,13	13,13	13,13	12,13	9,13	12,12
D18S51	13,13	13,14	13,13	12,13	13,13	13,13	12,13	13,13	13,13	13,13	12,13	13,17	12,12
D21S11	29, 29	28, 29	29, 29	29, 32.2	29, 32.2	29, 32.2	29, 32.2	29, 32.2	29, 32.2	29, 32.2	32.2, 32.2	30, 32.2	28, 32.2
AMEL	X,Y	X,X	X,Y	X,Y	X,Y	X,X	X,X	X,Y	X,Y	X,Y	X,X	X,Y	X,X

From APPENDIX 2 in J.M. Butler (2001) Forensic DNA Typing (1st edition)

Mutation Rates for Common STR Loci

J.M. Butler (2005) *J. Forensic Sci., in press*

http://www.aabb.org/About_the_AABB/Stds_and_Accred/planrpt03.pdf, Appendix 2

STR System	Maternal Meioses (%)	Paternal Meioses (%)	Number from either	Total Number of Mutations	Mutation Rate
CSF1PO	95/304,307 (0.03)	982/643,118 (0.15)	410	1,487/947,425	**0.16%**
FGA	205/408,230 (0.05)	2,210/692,776 (0.32)	710	3,125/1,101,006	**0.28%**
TH01	31/327,172 (0.009)	41/452,382 (0.009)	28	100/779,554	**0.01%**
TPOX	18/400,061 (0.004)	54/457,420 (0.012)	28	100/857,481	**0.01%**
VWA	184/564,398 (0.03)	1,482/873,547 (0.17)	814	2,480/1,437,945	**0.17%**
D3S1358	60/405,452 (0.015)	713/558,836 (0.13)	379	1,152/964,288	**0.12%**
D5S818	111/451,736 (0.025)	763/655,603 (0.12)	385	1,259/1,107,339	**0.11%**
D7S820	59/440,562 (0.013)	745/644,743 (0.12)	285	1,089/1,085,305	**0.10%**
D8S1179	96/409,869 (0.02)	779/489,968 (0.16)	364	1,239/899,837	**0.14%**
D13S317	192/482,136 (0.04)	881/621,146 (0.14)	485	1,558/1,103,282	**0.14%**
D16S539	129/467,774 (0.03)	540/494,465 (0.11)	372	1,041/962,239	**0.11%**
D18S51	186/296,244 (0.06)	1,094/494,098 (0.22)	466	1,746/790,342	**0.22%**
D21S11	464/435,388 (0.11)	772/526,708 (0.15)	580	1,816/962,096	**0.19%**
Penta D	12/18,701 (0.06)	21/22,501 (0.09)	24	57/41,202	**0.14%**
Penta E	29/44,311 (0.065)	75/55,719 (0.135)	59	163/100,030	**0.16%**
D2S1338	15/72,830 (0.021)	157/152,310 (0.10)	90	262/225,140	**0.12%**
D19S433	38/70,001 (0.05)	78/103,489 (0.075)	71	187/173,490	**0.11%**
SE33 (ACTBP2)	0/330 (<0.30)	330/51,610 (0.64)	None reported	330/51,940	**0.64%**

2.1.2 **Mapping**

The chromosomal location of the genetic marker (submitted to or recorded with the Nomenclature Committee of the Human Genome Organization).

- Not a major concern for standard STR loci since they have been well-defined…

Locus Name	Chromosomal Location	Physical Position[a]
CSF1PO	5q33.1 c-fms proto-oncogene, 6th intron	Chr 5 149.484 Mb
FGA	4q31.3 alpha fibrinogen, 3rd intron	Chr 4 156.086 Mb
TH01	11p15.5 tyrosine hydroxylase, 1st intron	Chr 11 2.156 Mb
TPOX	2p25.3 thyroid peroxidase, 10th intron	Chr 2 1.436 Mb
VWA	12p13.31 von Willebrand Factor, 40th intron	Chr 12 19.826 Mb
D3S1358	3p21.31	Chr 3 45.543 Mb
D5S818	5q23.2	Chr 5 123.187 Mb
D7S820	7q21.11	Chr 7 83.401 Mb
D8S1179	8q24.13	Chr 8 125.863 Mb
D13S317	13q31.1	Chr 13 80.52 Mb
D16S539	16q24.1	Chr 16 86.168 Mb
D18S51	18q21.33	Chr 18 59.098 Mb
D21S11	21q21.1	Chr 21 19.476 Mb

Position of Each CODIS STR Locus in Human Genome

Review article on core STR loci genetics and genomics to be published this fall

From Table 5.2, *Forensic DNA Typing*, 2nd Edition, p. 96 (J.M. Butler, 2005)

2.1.3 **Detection**

Technological basis for identifying the genetic marker.

2.1.4 **Polymorphism**

Type of variation analyzed.

2.2 Species specificity

- 2.2 Species specificity: For techniques designed to type human DNA, **the potential to detect DNA from forensically relevant nonhuman species should be evaluated**. For techniques in which a species other than human is targeted for DNA analysis, the ability to detect DNA profiles from nontargeted species should be determined. The presence of an amplification product in the nontargeted species does not necessarily invalidate the use of the assay.
- Why is this important?
- Examples of non-human PCR products?
  - amelogenin

SWGDAM Revised Validation Guidelines
http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm

2.3 Sensitivity studies

2.3 Sensitivity studies: When appropriate, the range of DNA quantities able to produce reliable typing results should be determined.

- What dilutions should be attempted?

SWGDAM Revised Validation Guidelines
http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm

2.4 Stability studies

2.4 Stability studies: The ability to obtain results from DNA recovered from biological samples deposited on various substrates and subjected to various environmental and chemical insults has been extensively documented. **In most instances, assessment of the effects of these factors on new forensic DNA procedures is not required.** However, if substrates and/or environmental and/or chemical insults could potentially affect the analytical process, then the process should be evaluated using known samples to determine the effects of such factors.

SWGDAM Revised Validation Guidelines
http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm

2.5 Reproducibility

2.5 Reproducibility: The technique should be evaluated in the laboratory and among different laboratories to ensure the consistency of results. Specimens obtained from donors of known types should be evaluated.

SWGDAM Revised Validation Guidelines
http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm

2.6 Case-type samples

2.6 Case-type samples: The ability to obtain reliable results should be evaluated using samples that are representative of those typically encountered by the testing laboratory. When possible, consistency of typing results should be demonstrated by comparing results from the previous procedures to those obtained using the new procedure.

SWGDAM Revised Validation Guidelines
http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm

2.7 Population studies

2.7 Population studies: The distribution of genetic markers in populations should be determined in relevant population groups. When appropriate, databases should be tested for independence expectations.

- How many samples are required in a population study?
- What statistical tests need to be performed?

SWGDAM Revised Validation Guidelines
http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm

Population Data Comparison with OmniPop

- **OmniPop** (Excel macro created by Brian Burritt of the San Diego Police Department) – compares allele frequencies across published population data
- **http://www.cstl.nist.gov/biotech/strbase/populationdata.htm**

STR Locus	Profile Computed	Number of Populations Used	Cumulative Profile Frequency Range (1 in …)	Cumulative Profile Frequency against U.S. Caucasians (Appendix II)
D3S1358	**16,17**	166	5.24 to 62.6	9.19
VWA	**17,18**	166	37.6 to 1,080	81.8
FGA	**21,22**	166	737 to 119,000	1,010
D8S1179	**12,14**	166	8,980 to 5,430,000	16,400
D21S11	**28,30**	166	165,000 to 248,000,000	186,000
D18S51	**14,16**	166	3.85×10^6 to 2.68×10^{10}	4.88×10^6
D5S818	**12,13**	166	2.28×10^7 to 4.22×10^{11}	4.51×10^7
D13S317	**11,14**	166	4.32×10^8 to 1.69×10^{13}	1.38×10^9
D7S820	**9,9**	166	1.17×10^{10} to 2.98×10^{16}	4.22×10^{10}
D16S539	**9,11**	97	4.06×10^{11} to 1.11×10^{18}	5.82×10^{11}
TH01	**6,6**	97	9.30×10^{12} to 1.45×10^{19}	1.05×10^{13}
TPOX	**8,8**	97	3.33×10^{13} to 1.54×10^{20}	3.63×10^{13}
CSF1PO	**10,10**	97	3.43×10^{14} to 2.65×10^{21}	7.43×10^{14}

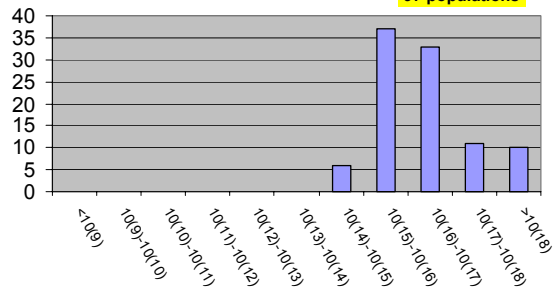
Profile with 13 STRs

OmniPop 150.4.2 Brian Burnett - San Diego Police Department (619) 531-2215 bburnett@pd.sandiego.gov

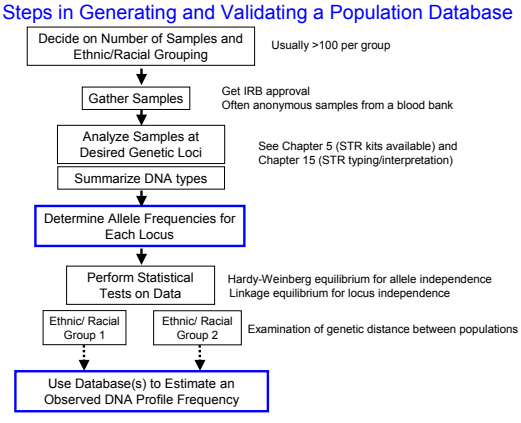
Distribution of Profile Frequencies

OmniPop 150.4.2

97 populations



Steps in Generating and Validating a Population Database



From Figure 20.1, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

Allele Frequency Tables

Allele frequencies denoted with an asterisk (*) are **below the 5/2N minimum allele threshold** recommended by the National Research Council report (NRCII) *The Evaluation of Forensic DNA Evidence* published in 1996.

D3S1358

Allele	Butler *et al.* (2003) *JFS* 48(4):908-911 Caucasian N= 302	Einum *et al.* (2004) *JFS* 49(6): 1381-1385 Caucasian N= 7,636	Allele	African American N=258	African American N= 7,602
11	0.0017*	0.0009	11	--	0.0003*
12	0.0017*	0.0007	12	--	0.0045
13	--	0.0031	13	0.0019*	0.0077
14	0.1027	0.1240	14	0.0892	0.0905
15	**0.2616**	**0.2690**	15	0.3023	0.2920
15.2	--	--	15.2	0.0019*	0.0010
16	0.2533	0.2430	16	**0.3353**	**0.3300**
17	0.2152	0.2000	17	0.2054	0.2070
18	0.15232	0.1460	18	0.0601	0.0630
19	0.01160	0.0125	19	0.0039*	0.0048
20	0.0017*	0.0001*	20		

Most common allele

J.M. Butler (2005) *J. Forensic Sci., in press*

STR Locus	Number Reported	**264 variant alleles** reported as of Apr 2005 on STRBase
CSF1PO	11	5, 7.3, 8.3, 9.1, 9.3, 10.1, 10.2, 10.3, 11.1, 12.1, 16
FGA	69	12.2, 13.2, 14, 14.3, 15, 15.3, 16, 16.1, 16.2, "<17", 17, 17.2, 18.2, 19.1, 19.2, 19.3, 20.1, 20.2, 20.3, 21.1, 21.2, 21.3, 22.1, 22.2, 22.3, 23.1, 23.2, 23.3, 24.1, 24.2, 24.3, 25.1, 25.2, 25.3, 26.1, 26.2, 26.3, 27.3, 29.2, 30.2, 31, 31.2, 32.1, 32.2, 33.1, 34.1, 34.2, 35.2, 41.1, 41.2, 42.1, 42.2, 43.1, 43.2, 44, 44.1, 44.2, 44.3, 45.1, 45.2, 46.1, 46.2, 47.2, 48.2, 49, 49.1, 49.2, 50.2, 50.3
TH01	7	4, 7.3, 8.3, 9.1, 10.3, 11, 13.3
TPOX	7	4, 5, 7.3, 13.1, 14, 15, 16
VWA	6	16.1, 18.3, 22, 23, 24, 25
D3S1358	18	8, 8.3, 9, 10, 11, 15.1, 15.2, 15.3, 16.2, 17.1, 17.2, 18.1, 18.2, 18.3, ">19", 20, 20.1, 21.1
D5S818	5	10.1, 11.1, 12.3, 17, 18
D7S820	22	5, 5.2, 6.3, 7.1, 7.3, 8.1, 8.2, 8.3, 9.1, 9.2, 9.3, 10.1, 10.3, 11.1, 11.3, 12.1, 12.2, 12.3, 13.1, 14.1, 15, 16
D8S1179	4	7, 15.3, 18, 20
D13S317	10	5, 6, 7, 7.1, 8.1, 11.1, 11.3, 13.3, 14.3, 16
D16S539	10	6, 7, 9.3, 11.3, 12.1, 12.2, 13.1, 13.3, 14.3, 16
D18S51	30	7, 8, 9, 11.2, 12.2, 12.3, 13.1, 13.3, 14.2, 15.1, 15.2, 16.1, 16.2, 16.3, 17.2, 17.3, 18.1, 18.2, 19.2, 20.1, 20.2, 21.2, 22.1, 22.2, 23.2, 24.2, 27, 28.1, 28.3, 40
D21S11	24	24.3, 25.1, 25.2, 25.3, 26.2, 27.1, 27.2, 28.1, 28.3, 29.1, 29.3, 30.3, 31.1, 31.3, 32.1, 33.1, 34.1, 34.3, 35.1, 36.1, 36.2, 37, 37.2, 39
Penta D	14	6, 6.4, 7.1, 7.4, 9.4, 10.3, 11.1, 11.2, 12.2, 12.4, 13.2, 13.4, 14.1, 14.4
Penta E	13	9.4, 11.4, 12.1, 12.2, 13.2, 14.4, 15.2, 15.4, 16.4, 17.4, 18.4, 19.4, 23.4
D2S1338	3	13, 23.2, 23.3
D19S433	11	6.2, 7, 8, "<9", 11.1, 12.1, 13.2, 18, 18.2, 19.2, 20
SE33	0	None reported yet in STRBase

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

J.M. Butler (2005) *J. Forensic Sci., in press*

STR Locus	Number Reported	**62 tri-allelic patterns** reported as of April 2005 on STRBase
CSF1PO	2	9/11/12; 10/11/12
FGA	10	19/20/21; 19/22/23; 19/24/25; 20/21/22; 20/21/24; 20/23/24; 21/22/23; 21/25/26; 22/24/25; 22.2/23/23.2
TH01	1	7/8/9
TPOX	13	6/8/10; 6/9/10; 6/10/11; 6/10/12; 7/9/10; 7/10/11; 8/9/10; 8/10/11; 8/10/12; 8/11/12; 9/10/11; 9/10/12; 10/11/12
VWA	8	11/16/17; 12/18/19; 14/15/17; 14/15/18; 14/16/18; 14/17/18; 15/16/17; 18/19/20
D3S1358	4	15/16/17; 15/17/18; 16/17/19; 17/18/19
D5S818	2	10/11/12; 11/12/13
D7S820	2	8/9/12; 8/10/11
D8S1179	5	10/12/13; 10/12/15; 12/13/14; 12/13/15; 13/15/16
D13S317	3	8/11/12; 10/11/12; 10/12/13
D16S539	1	12/13/14
D18S51	7	12/13/15; 12/14/15; 12/16/17; 14/15/22; 15/16/20; 16/17/20; 19/22.2/23.2
D21S11	4	28/29/30; 28/30.2/31.2; 29/31/32; 30/30.2/31
Penta D	0	None reported yet in STRBase
Penta E	0	None reported yet in STRBase
D2S1338	0	None reported yet in STRBase
D19S433	0	None reported yet in STRBase
SE33	0	None reported yet in STRBase

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

2.8 Mixture studies

2.8 Mixture studies: The ability to obtain reliable results from mixed source samples should be determined.

- How many mixtures should be evaluated?
- What mixture ratios should be tested?
- What allele combinations should be examined?

SWGDAM Revised Validation Guidelines
http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm

2.9 Precision and accuracy

2.9 Precision and accuracy: The extent to which a given set of measurements of the same sample agree with their mean and the extent to which these measurements match the actual values being measured should be determined.

- How many samples should be examined in a precision study?

SWGDAM Revised Validation Guidelines
http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm

2.10 PCR-based procedures

2.10 PCR-based procedures: **Publication of the sequence of individual primers is not required** in order to appropriately demonstrate the accuracy, precision, reproducibility, and limitations of PCR-based technologies.

- *Single biggest change in the revised validation guidelines…*
- What are advantages of having the primer sequences?

SWGDAM Revised Validation Guidelines
http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm

2.10.1 The reaction conditions needed to provide the required degree of specificity and robustness must be determined. These include thermocycling parameters, the concentration of primers, magnesium chloride, DNA polymerase, and other critical reagents.

2.10.2 The potential for differential amplification among loci, preferential amplification of alleles in a locus, and stochastic amplification must be assessed.

2.10.3 When more than one locus is coamplified, the effects of coamplification must be assessed (e.g., presence of artifacts).

2.10.4 Positive and negative controls must be validated for use.

SWGDAM Revised Validation Guidelines
http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm

2.10.5 Detection of PCR product

2.10.5.1 Characterization without hybridization

2.10.5.1.1 When PCR product is characterized directly, appropriate measurement standards (qualitative and/or quantitative) for characterizing the alleles or resulting DNA product must be established.

2.10.5.1.2 When PCR product is characterized by DNA sequencing, appropriate standards for characterizing the sequence data must be established.

2.10.5.2 Characterization with hybridization

2.10.5.2.1 Hybridization and wash conditions necessary to provide the required degree of specificity must be determined.

2.10.5.2.2 For assays in which the probe is bound to the matrix, a mechanism must be employed to demonstrate whether adequate amplified DNA is present in the sample (e.g., a probe that reacts with an amplified allele(s) or a product yield gel).

SWGDAM Revised Validation Guidelines
http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm

What is the goal of validation studies involving a new STR typing kit

- Stutter product amounts
  - Why?: aids in mixture interpretation guidelines (how often does your laboratory call peaks below 15% of an adjacent allele?)
- Precision studies
  - Why?: aids in defining allele bin windows (in reality does anyone ever change to ±0.5 bp from the Genotyper macro?)
- Sensitivity studies
  - Why?: aids in defining lower and upper limits
- Mixture studies
  - Why?: aids in demonstrating the limits of detecting the minor component
- Concordance studies
  - Why?: to confirm that new primer sets get the same results as original primer sets – potential of polymorphism causing allele dropout…
- Peak height ratio studies

Appropriate Documentation…

- Publications in the Peer-Reviewed Literature
  - How to find them…
  - How to read and critic them…
- In terms of documentation, is the community doing too much? Too little?
  - Discuss benefit of STRBase Validation homepage
- Should we be requesting more information from the manufacturers of commercial kits in terms of developmental validation studies?

FBI DNA Quality Assurance Audit Developmental Validation Scorecard

Discussion

Developmental validation must precede the introduction of a novel methodology for forensic DNA analysis. A novel methodology may include an existing technology or testing procedure that has been developed for a specific technology (e.g., medical testing, genetic analysis) that is not currently applied to forensic DNA analysis. Citations in peer-reviewed scientific journals that provide the underlying scientific basis for a novel methodology should be available.

		Yes	No	N/A
8.1.2	Have novel forensic or database DNA methodologies used by the laboratory undergone developmental validation to ensure the accuracy, precision, and reproducibility of the procedure?	___	___	___
8.1.2.1	Is there documentation and is it available that defines and characterizes each locus?	___	___	___
8.1.2.2(FO)	Have species' specificity, sensitivity, stability, and mixture studies been conducted?	___	___	___
8.1.2.3(FO)	Does the laboratory have access to a population database that is documented and available for use in population statistics?	___	___	___
8.1.2.3.1(FO-a)	Where appropriate, has the database been tested for independence expectations?	___	___	___
8.1.2.3.1(FO-b)	Does the database information include allele and frequency distributions for the locus or loci obtained from relevant populations?	___	___	___

Example of Work Performed for Developmental Validation

ABI Kit Validation Papers

J. Forensic Sci. 2002; 47(1): 66-96

Cydne L. Holt,[1] Ph.D.; Martin Buoncristiani,[2] M.P.H.; Jeanette M. Wallin,[1] M.P.H.; Theresa Nguyen,[1] B.S.; Katherine D. Lazaruk,[1] Ph.D.; and P. S. Walsh,[1] M.P.H.

TWGDAM Validation of AmpF*ℓ*STR™ PCR Amplification Kits for Forensic DNA Casework

J. Forensic Sci. 2004; 49(6): 1265-1277

Patrick J. Collins,[1] B.A.; Lori K. Hennessy,[1] Ph.D.; Craig S. Leibelt,[1] A.B.; Rhonda K. Roby,[1,] M.P.H.; Dennis J. Reeder,[1] Ph.D.; and Paul A. Foxall,[1] Ph.D.

Developmental Validation of a Single-Tube Amplification of the 13 CODIS STR Loci, D2S1338, D19S433, and Amelogenin: The AmpF*ℓ*STR® Identifiler® PCR Amplification Kit

AmpF*ℓ*STR® Identifiler™
PCR Amplification Kit
User's Manual

Example with Identifiler STR Kit

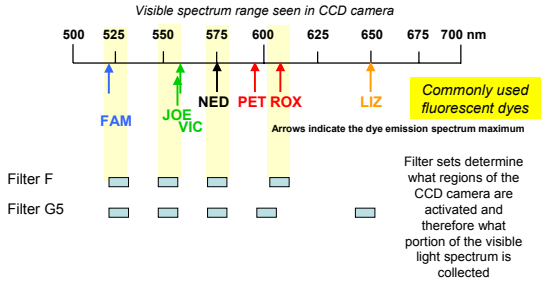
- **Your lab is currently running ProfilerPlus/COfiler and wants to switch to Identifiler**. What is needed for your internal validation?
- **What is different between Identifiler and ProfilerPlus/COfiler**?
  - Two new STR loci: D19S433 and D2S1338
  - Different fluorescent dyes
  - Additional fluorescent dye (5-dye vs 4-dye)
  - Different dye on internal size standard
  - More loci being amplified in the multiplex
  - Mobility modifiers to move allele sizes
- PCR primer sequences are the same so potential allele discordance due to primer binding site mutations should not be an issue
- **What has been reported in terms of developmental validation for Identifiler?**

Population Studies with D2S1338 and D19S433

- These STR loci are part of the widely used SGM Plus kit
- Included in profile frequency calculator using 24 European populations and 5,700 individuals: http://www.str-base.org/calc.php

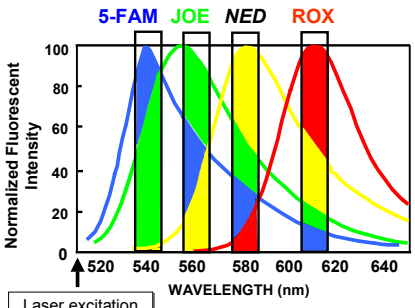
- Budowle, B. (2001) Genotype profiles for five population groups at the short tandem repeat loci D2S1338 and D19S433. *Forensic Sci. Comm.* 3(3); available at http://www.fbi.gov/hq/lab/fsc/backissu/july2001/budowle1.htm
- Budowle, B., *et al.* (2001) Population data on the STR loci D2S1338 and D19S433. *Forensic Sci. Comm.* 3(3); available at http://www.fbi.gov/hq/lab/fsc/backissu/july2001/budowle2.htm
- Butler, J.M., *et al.* (2003) Allele frequencies for 15 autosomal STR loci on U.S. Caucasian, African American, and Hispanic populations. *J. Forensic Sci.* 48(4):908-911; genotypes available at http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm

Different Fluorescent Dyes



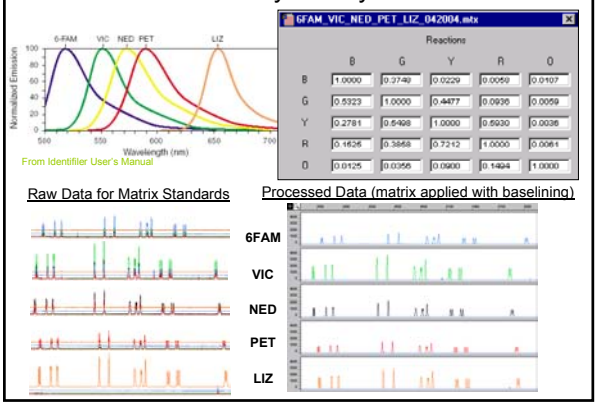
	Blue	Green	Yellow	Red	Orange	Used with These Kits
Filter F	5FAM	JOE	NED	ROX		Profiler Plus
Filter G5	6FAM	VIC	NED	PET	LIZ	Identifiler

Fluorescent Emission Spectra for ABI Dyes

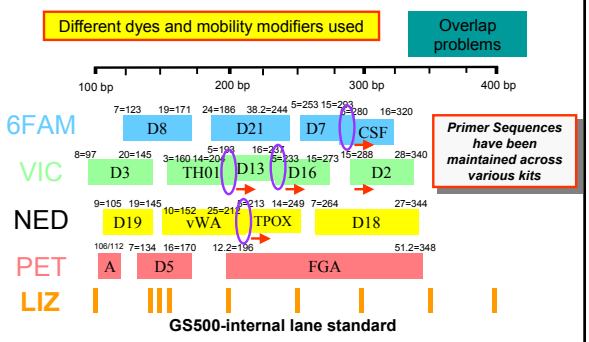


Butler, J.M. (2001) *Forensic DNA Typing*, Figure 10.4, ©Academic Press

5 x 5 matrix for 5-dye analysis on ABI 310



AmpFISTR® Identifiler™



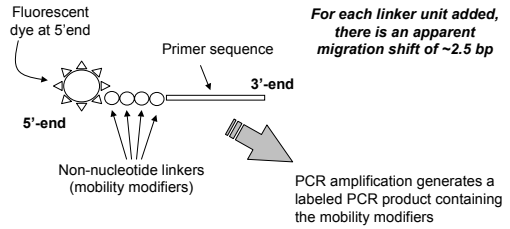


Figure 5.7, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

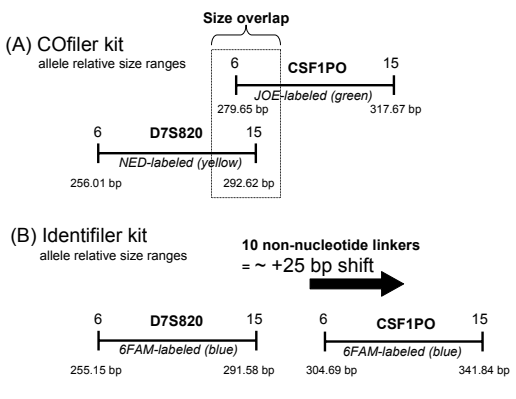


Figure 5.8, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

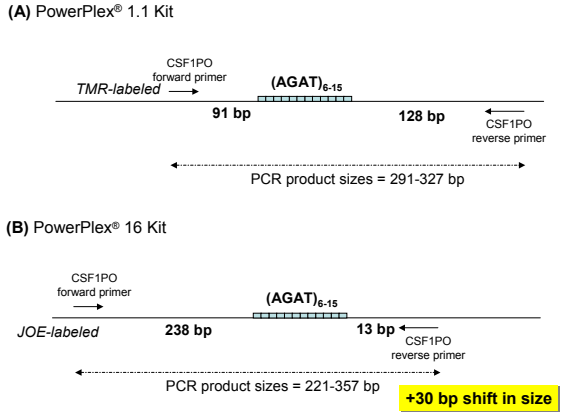


Figure 5.9, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

Changes in Promega Primer Sequences

STR loci included in each kit	D3S1358	TH01	D21S11	D18S51	Penta E	D5S818	D13S317	D7S820	D16S539	CSF1PO	Penta D	Amelogenin	VWA	D8S1179	TPOX	FGA
PowerPlex 1.1		A				A	A	A	A	A		A	A		A	
PowerPlex 1.2		A				A	B	A	B	A		B	A		A	
PowerPlex 2.1	A	B	A	A	A								A	A	B	A
PowerPlex 16	A	B	A	A	A	A	B	A	B	B	A	C	A	A	B	A

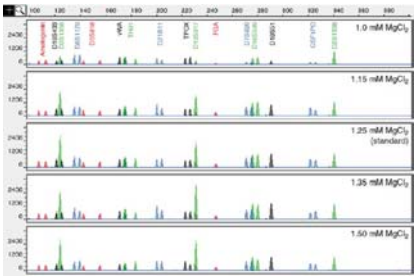
Examination of PCR Components

- Assay robustness (ruggedness) determined by testing multiple concentrations around the final optimized concentration of each component

- AmpliTaq Gold DNA Polymerase—1.5, 2, **2.25**, 2.5, 3, and 4.5 U per reaction
- $MgCl_2$—0.8, 1.0, 1.15, **1.25**, 1.35, 1.50, 1.75, and 2.0 mM
- dNTP mixture (equimolar dATP, dCTP, dGTP and dTTP)—600, 720, **800**, 880, and 1000 uM
- KCl—25, 40, **50**, 60, and 75 mM
- BSA—0, 2, 3.2, **4**, 4.8, 8, and 16 ug per reaction
- Sodium azide—0%, **0.05%**, 0.10%, 0.15%, and 0.20%

$MgCl_2$ Titration

Identifiler STR Kit Developmental Validation



Collins PJ, Hennessy LK, Leibelt CS, Roby RK, Reeder DJ, Foxall PA. Developmental validation of a single-tube amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: the AmpFISTR Identifiler PCR amplification kit. *J. Forensic Sci.* 2004; 49(6): 1265-1277.

Mobility Shift with Non-nucleotide Linker

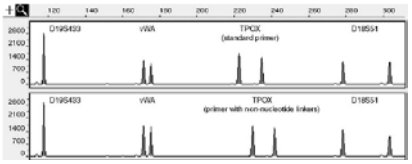


FIG. 1—*NED dye labeled loci from two amplifications of a single sample using TPOX primers both with and without non-nucleotide linkers. The X-axis indicates base pair size and the Y-axes RFU. The top panel depicts the amplification without non-nucleotide linkers. Sizes for the TPOX alleles for this panel were 222.93 and 234.81 bp. Sizes for the TPOX alleles in the amplification using the modified primer, depicted in the bottom panel, were 229.85 and 241.71 bp, indicating an average shift of 6.91 bp. Peaks heights, intralocus balance, and intracolor balance were similar in both amplifications.*

Collins PJ, Hennessy LK, Leibelt CS, Roby RK, Reeder DJ, Foxall PA. Developmental validation of a single-tube amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: the AmpFISTR Identifiler PCR amplification kit. *J. Forensic Sci.* 2004; 49(6): 1265-1277.

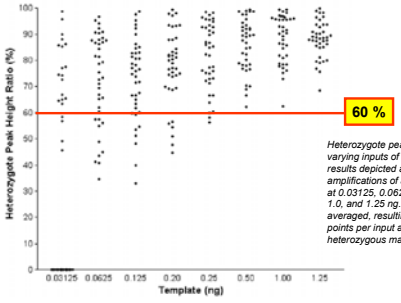
Sizing Precision with Non-nucleotide Linkers

TABLE 1—*Sizing shift and sizing precision data for loci incorporating non-nucleotide linkers on the ABI* PRISM *310 Genetic Analyzer.*

Locus	Increase in Detected Size (bp)	Range of Standard Deviation of Alleles for Identifiler Kit (bp)	Range of Standard Deviation of Alleles Previous Kit (bp)
CSF1PO	26	0.08–0.13*	0.03–0.10[‡]
D2S1338	16	0.05–0.12*	0.02–0.15[‡]
D13S317	12	0.05–0.09*	0.02–0.09[§]
D16S539	23	0.06–0.09*	0.01–0.08[‡]
TPOX	7	0.03–0.08*	0.02–0.07[†]

Heterozygote Peak Height Ratios

Identifiler STR Kit Developmental Validation

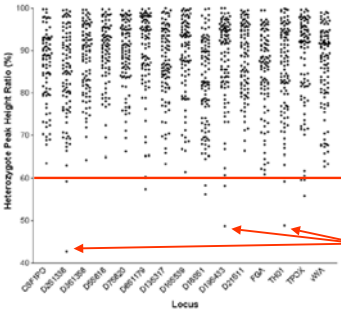


Heterozygote peak height ratios with varying inputs of template DNA. The results depicted are from three amplifications of a single genomic DNA at 0.03125, 0.0625, 0.125, 0.2, 0.25, 0.5, 1.0, and 1.25 ng. Multiple injections were averaged, resulting in a total of 39 data points per input amount (13 heterozygous markers × 3 repetitions).

Collins PJ, Hennessy LK, Leibelt CS, Roby RK, Reeder DJ, Foxall PA. Developmental validation of a single-tube amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: the AmpFISTR Identifiler PCR amplification kit. *J. Forensic Sci.* 2004; 49(6): 1265-1277.

Heterozygote Peak Height Ratios

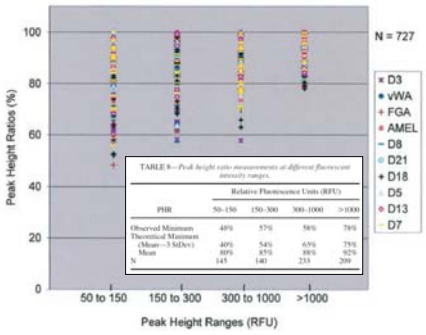
Identifiler STR Kit Developmental Validation



116 correctly genotyped population samples (n = 69–101, depending on locus). Template inputs varied from approximately 250 pg to greater than 3 ng

Collins PJ, Hennessy LK, Leibelt CS, Roby RK, Reeder DJ, Foxall PA. Developmental validation of a single-tube amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: the AmpFISTR Identifiler PCR amplification kit. *J. Forensic Sci.* 2004; 49(6): 1265-1277.

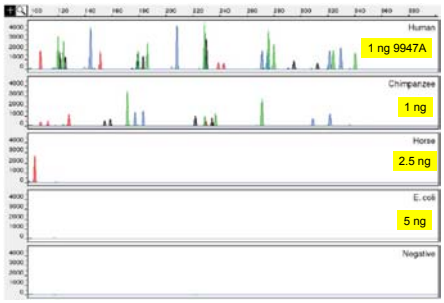
Heterozygote Peak Height Ratios



Holt CL, Buoncristiani M, Wallin JM, Nguyen T, Lazaruk KD, Walsh PS. TWGDAM validation of AmpFISTR PCR amplification kits for forensic DNA casework. *J Forensic Sci* 2002; 47(1): 66-96.

Non-Human Studies (Species Specificity)

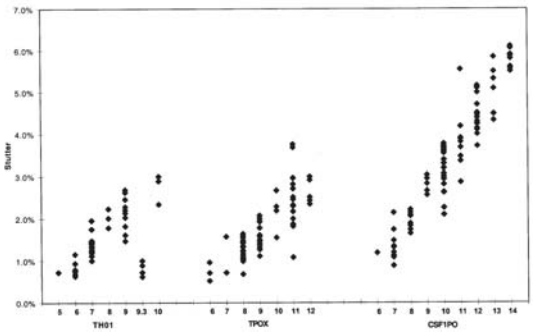
Identifiler STR Kit Developmental Validation



Collins PJ, Hennessy LK, Leibelt CS, Roby RK, Reeder DJ, Foxall PA. Developmental validation of a single-tube amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: the AmpFISTR Identifiler PCR amplification kit. *J. Forensic Sci.* 2004; 49(6): 1265-1277.

Measured Stutter Percentages

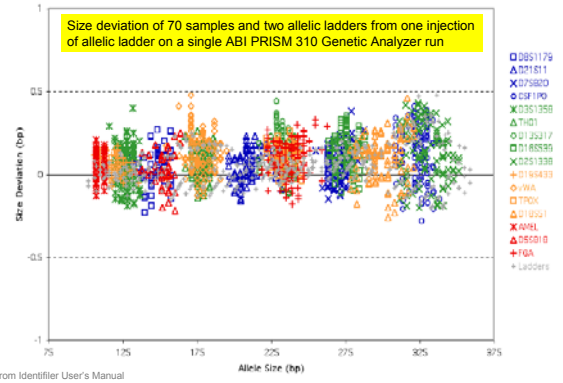
Variable by Allele Length and Composition



Holt CL, Buoncristiani M, Wallin JM, Nguyen T, Lazaruk KD, Walsh PS. TWGDAM validation of AmpFISTR PCR amplification kits for forensic DNA casework. *J Forensic Sci* 2002; 47(1): 66-96.

Precision from Run-to-Run on ABI 310

Size deviation of 70 samples and two allelic ladders from one injection of allelic ladder on a single ABI PRISM 310 Genetic Analyzer run



From Identifiler User's Manual

Practical Exercise #1

- Each class member to read one of the provided developmental validation articles
- Report to everyone on Friday morning
- Give a 5 min synopsis of the article (1-1.5 hours to complete)
- Answer a few questions such as
  - Does this study fully describe a developmental validation?
  - What would you have done differently?

Workshop Practical Exercise #1

Literature Summary

Reported Developmental Validation Efforts

Numbers of Samples Run in Developmental Validation Studies

Kit/System	Reference	Sensitivity	Precision	Stutter	Mixture	Peak Height Ratio	Non-Probative Cases
PP16	Krenke et al. (2002)						
Profiler Plus	Holt et al. (2002)						
Identifiler	Collins et al. (2004)						
SGM Plus	Cotton et al. (2000)						
Alu qPCR	Nicklas et al. (2003)						
Quantifiler	Green et al. (2005)						
mtDNA	Wilson et al. (1995)						
ABI 310	Lazaruk et al. (1998)						
ABI 377	Fregeau et al. (1999)						
ABI 3100	Koumi et al. (2004)						
TrueAllele	Kadash et al. (2004)						
PowerPlex Y	Krenke et al. (2005)						
Y-PLEX 12	Shewale et al. (2004)						
DNA IQ	Greenspoon et al. (2004)						

Validation Summary Sheet for PowerPlex Y

Study Completed (17 studies done)	Description of Samples Tested (performed in 7 labs and Promega)	# Run
Single Source (Concordance)	5 samples x 8 labs	40
Mixture Ratio (male:female)	6 labs x 2 M/F mixture series x 11 ratios (1:0,1:1,1:10,1:100,1:300,1:1000,0.5:300, 0.25:300,0.125:300, 0.0625:300, 0.03:300 ng M:F)	132
Mixture Ratio (male:male)	6 labs x 2 M/M mixtures series x 11 ratios (1:0, 19:1, 9:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:9, 1:19, 0:1)	132
Sensitivity	7 labs x 2 series x 6 amounts (1/0.5/0.25/0.125/0.06/0.03)	84
Non-Human	24 animals	24
NIST SRM	6 components of SRM 2395	6
Precision (ABI 3100 and ABI 377)	10 ladder replicates + 10 sample replicated + [8 ladders + 8 samples for 377]	36
Non-Probative Cases	65 cases with 102 samples	102
Stutter	412 males used	412
Peak Height Ratio	N/A (except for DYS385 but no studies were noted)	
Cycling Parameters	5 cycles (28/27/26/25/24) x 8 punch sizes x 2 samples	80
Annealing Temperature	5 labs x 5 temperatures (54/58/60/62/64) x 1 sample	25
Reaction volume	5 volumes (50/25/15/12.5/6.25) x [5 amounts + 5 concentrations]	50
Thermal cycler test	4 models (480/2400/9600/9700) x 1 sample + [3 models x 3 sets x 12 samples]	76
Male-specificity	2 females x 1 titration series (0-500 ng female DNA) x 5 amounts each	10
TaqGold polymerase titration	5 amounts (1.38/2.06/2.75/3.44/4.13 U) x 4 quantities (1/0.5/0.25/0.13 ng DNA)	20
Primer pair titration	5 amounts (0.5x/0.75x/1x/1.5x/2x) x 4 quantities (1/0.5/0.25/0.13 ng DNA)	20
Magnesium titration	5 amounts (1/1.25/1.5/1.75/2 mM Mg) x 4 quantities (1/0.5/0.25/0.13 ng DNA)	20
Krenke *et al.* (2005) *Forensic Sci. Int.* 148:1-14	TOTAL SAMPLES EXAMINED	1269

A Comparison to Y-PLEX 12 Validation

Shewale, J. G., Nasir, H., Schneida, E., Gross, A. M., Budowle, B., and Sinha, S. K. (2004) Y-chromosome STR system, Y-PLEX 12, for forensic casework: development and validation. *J Forensic Sci.* 49(6): 1278-1290.

Study Completed (26 experiments cited)	Description of Samples Tested	TOTAL
Single Source (Concordance)	[50 male + 30 female] mentioned in materials and methods; IPATIMUP,Humboldt shared samples	
Mixtures		
Mixture Ratio (male:female)	6 ratios (1:0/1:100/1:200/1:400/1:600/1:800) x 1 series (0.5 ng male with variable female DNA)	6
Mixture Ratio (male:male)	6 ratios (1:0/1:5/1:10/1:20/1:30/1:40) x 1 series (0.2 ng male-1 with increasing level of male-2)	6
Sensitivity	15 males x 5 amounts (0.05/0.1/0.2/0.5/1/2 ng)	75
Non-Human	9 mammals + 5 bacteria/virus	14
NIST SRM	6 components of SRM 2395	6
Precision (ABI 310, 377, 3100)	50 ladders (310) + 49 ladders (377) + 58 ladders (3100)	157
Non-Probative Cases	19 cases (comprising 45 samples by my calculations)	45
Stutter	34 males (part of another study?)	34
Peak Height Ratio	N/A	
Cycling Parameters	3 males x 4 cycles (28/30/32/34) x 1 amount (1 ng)	12
Annealing Temperature	1 sample x 5 temperatures (56/58/60/62/64) x 1 amount (1 ng)	5
Proficiency		
Substrate	*SEE Y-PLEX 6 and Y-PLEX 5 papers*	
Environment	*SEE Y-PLEX 6 and Y-PLEX 5 papers*	
Various tissues		
Reaction volume	3 volumes (12.5/25/50) x 4 males x 1 amount (1 ng)	12
Thermal cycler test	3 models (9600/9700/MJ PTC-200) x 1 sample	3
Male-specificity	46 unrelated female samples ranging up to 700 ng in amount	46
TaqGold polymerase titration	4 amounts (0.625/1.25/2.5/3.75 U) x 1 sample	4
Primer pair titration	3 amounts (0.25x/0.5x/1x) x 1 sample	3
Magnesium titration	at least 4 amounts (1.0/1.5/1.8/2.2 mM Mg) x 1 sample	4
	TOTAL SAMPLES EXAMINED	432

This Y-PLEX 12 developmental validation was performed in only one lab? (rather than 8) and had one-third the number of samples tested as the PowerPlex Y kit (432 vs. 1269). The study also shares two authors (Ann Marie Gross and Bruce Budowle) with the Krenke *et al.* (2005) PowerPlex Y study.

Validation Section of the DNA Advisory Board Standards

issued July 1998 (and April 1999); published in *Forensic Sci. Comm.* July 2000

STANDARD 8.1 The laboratory shall use validated methods and procedures for forensic casework analyses *(DNA analyses)*.

8.1.1 Developmental validation that is conducted **shall be appropriately documented**.

8.1.3 Internal validation **shall be performed and documented by the laboratory**.

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Practical Examples

- Switch from ABI 7000 to ABI 7500 for Quantifiler
  - What is needed from manufacturer?
- Switch from ABI 310 to ABI 3130
  - Developmental or internal validation?
  - How many samples should be run?

ABI 7500 Quantifiler Validation Documentation

http://www.appliedbiosystems.com

Applied Biosystems

an Applera Corporation Business

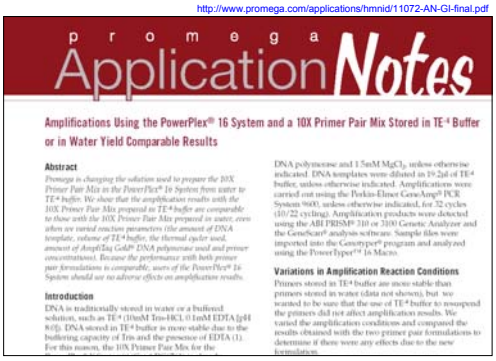
Experimental data supports that the 7500 system with v1.2.3 software provides consistent performance when compared to the ABI PRISM® 7000 Sequence Detection System previously validated for forensic applications. Therefore, the 7500 system can be sold to Human Identification customers at this time. Further guidance for specific operating conditions will follow.

Validation of the Applied Biosystems 7500 Real-Time PCR System with v1.2.3 Software

Applied Biosystems scientists have conducted experiments following the guidance provided by the DAB/SWGDAM to validate the Applied Biosystems 7500 Real-Time PCR System with v1.2.3 software ("7500 System") for use in forensic applications using the Quantifiler Human and Quantifiler Y kits. We are pleased to let you know that the Applied Biosystems 7500 Real-Time PCR System equipped with v1.2.3 software is now validated for use in forensic sample testing pursuant to these guidelines using the Quantifiler kits. We conducted experiments, reviewed data, and determined that the 7500 Real-Time PCR System provides results that are robust, reliable, reproducible and provide accurate results when used in conjunction with the Quantifiler™ Human and Quantifiler™ Y kits for the analysis of genomic DNA samples.

Promega Material Modification Reported
for PP16 Primer Mix Storage

http://www.promega.com/applications/hmnid/11072-AN-GI-final.pdf



promega
Application Notes

Amplifications Using the PowerPlex® 16 System and a 10X Primer Pair Mix Stored in TE⁻⁴ Buffer or in Water Yield Comparable Results

Abstract

Promega is changing the solution used to prepare the 10X Primer Pair Mix in the PowerPlex® 16 System from water to TE⁻⁴ buffer. We show that the amplification results with the 10X Primer Pair Mix prepared in TE⁻⁴ buffer are comparable to those with the 10X Primer Pair Mix prepared in water, even when we varied reaction parameters (the amount of DNA template, volume of TE⁻⁴ buffer, the thermal cycler used, amount of AmpliTaq Gold® DNA polymerase used and primer concentrations). Because the performance with both primer pair formulations is comparable, users of the PowerPlex® 16 System should see no adverse effects on amplification results.

Introduction

DNA is traditionally stored in water or a buffered solution, such as TE⁻⁴ (10mM Tris-HCl, 0.1mM EDTA, pH 8.0). DNA stored in TE⁻⁴ buffer is more stable due to the buffering capacity of Tris and the presence of EDTA (1). For this reason, the 10X Primer Pair Mix for the

DNA polymerase and 1.5mM $MgCl_2$, unless otherwise indicated. DNA templates were diluted in 1× 2µl of TE⁻⁴ buffer, unless otherwise indicated. Amplifications were carried out using the Perkin-Elmer GeneAmp® PCR System 9600, unless otherwise indicated, for 32 cycles (10/22 cycling). Amplification products were detected using the ABI PRISM® 310 or 3100 Genetic Analyzer and the GeneScan® analysis software. Sample files were imported into the Genotyper® program and analyzed using the PowerTyper™ 16 Macro.

Variations in Amplification Reaction Conditions

Primers stored in TE⁻⁴ buffer are more stable than primers stored in water (data not shown), but we wanted to be sure that the use of TE⁻⁴ buffer to resuspend the primers did not affect amplification results. We varied the amplification conditions and compared the results obtained with the two primer pair formulations to determine if there were any effects due to the new formulation.