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:
  - (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.

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?
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.

Examples of Delay in Publication

- **ProfilerPlus/COFlie**
  - Kits released in Dec 1997/May 1998 with technical manuals
  - Publication in Jan 2002 of developmental validation (submitted in July 2000)

- **Identifier**
  - Kit released in July 2001 with technical manual
  - Publication in Nov 2004 of developmental validation (submitted in June 2002)

- **Quantifier**
  - 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


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?

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
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)?

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.

- 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

Examples where studies are not necessary?

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.

- 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

Examples where studies are not necessary?

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...

[Diagram illustrating parental allele transfer with D13S317 F, M, S1, S2, D1, S5—all possible combinations seen]

<table>
<thead>
<tr>
<th>STR locus</th>
<th>TH01</th>
<th>FGA</th>
<th>CSF1PO</th>
<th>VWA</th>
<th>TPOX</th>
<th>TH01</th>
<th>FGA</th>
<th>CSF1PO</th>
<th>VWA</th>
<th>TPOX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father</td>
<td>21,22</td>
<td>21,24</td>
<td>21,24</td>
<td>8,9</td>
<td>6,6</td>
<td>21,22</td>
<td>21,24</td>
<td>21,24</td>
<td>8,9</td>
<td>6,6</td>
</tr>
<tr>
<td>Mother</td>
<td>20,22</td>
<td>20,24</td>
<td>20,24</td>
<td>8,9</td>
<td>6,6</td>
<td>20,22</td>
<td>20,24</td>
<td>20,24</td>
<td>8,9</td>
<td>6,6</td>
</tr>
<tr>
<td>Daughter</td>
<td>20,22</td>
<td>20,24</td>
<td>20,24</td>
<td>8,9</td>
<td>6,6</td>
<td>20,22</td>
<td>20,24</td>
<td>20,24</td>
<td>8,9</td>
<td>6,6</td>
</tr>
</tbody>
</table>

Example APPENDIX 2.1 from Butler (2005) Forensic DNA Typing (1st edition)
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...

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

From Table 5.2, Forensic DNA Typing, 2nd Edition, p. 90 (J.M. Butler, 2005)
Validation Workshop – Developmental Validation
Aug. 24, 2005 at NFSTC

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?

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.

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.

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.

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?

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

<table>
<thead>
<tr>
<th>STR Locus</th>
<th>Profile Computed</th>
<th>Number of Populations Used</th>
<th>Cumulative Profile Frequency Range (1 in …)</th>
<th>Cumulative Profile Frequency against U.S. Caucasians (Appendix II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3S1358</td>
<td>15.17</td>
<td>166</td>
<td>5.24 to 52.6</td>
<td>9.19</td>
</tr>
<tr>
<td>VNIA</td>
<td>17.10</td>
<td>166</td>
<td>37.6 to 1,006</td>
<td>81.8</td>
</tr>
<tr>
<td>FGA</td>
<td>21.22</td>
<td>166</td>
<td>737 to 119,300</td>
<td>2.01</td>
</tr>
<tr>
<td>D5S1103</td>
<td>12.14</td>
<td>166</td>
<td>6,860 to 54,400</td>
<td>18,400</td>
</tr>
<tr>
<td>D21S11</td>
<td>28.30</td>
<td>166</td>
<td>165,050 to 2,346,000</td>
<td>180,000</td>
</tr>
<tr>
<td>D16S51</td>
<td>14.16</td>
<td>166</td>
<td>7.85 x 1E5 to 2.65 x 10^6</td>
<td>4.88 x 10^6</td>
</tr>
<tr>
<td>D3S1137</td>
<td>15.14</td>
<td>166</td>
<td>4.32 x 10^10 to 1.69 x 10^10</td>
<td>3.38 x 10^10</td>
</tr>
<tr>
<td>D13S77</td>
<td>11.14</td>
<td>166</td>
<td>5.28 x 10^11 to 1.62 x 10^11</td>
<td>5.01 x 10^11</td>
</tr>
<tr>
<td>D7S820</td>
<td>9.01</td>
<td>97</td>
<td>3.06 x 10^11 to 1.15 x 10^11</td>
<td>1.05 x 10^11</td>
</tr>
<tr>
<td>THO1</td>
<td>4.6</td>
<td>97</td>
<td>3.26 x 10^11 to 1.45 x 10^11</td>
<td>3.63 x 10^11</td>
</tr>
<tr>
<td>D18S51</td>
<td>8.8</td>
<td>97</td>
<td>3.33 x 10^10 to 1.54 x 10^10</td>
<td>7.43 x 10^10</td>
</tr>
<tr>
<td>D8S1179</td>
<td>10.10</td>
<td>97</td>
<td>4.33 x 10^11 to 2.65 x 10^11</td>
<td>7.43 x 10^10</td>
</tr>
</tbody>
</table>

Prepared by John M. Butler
### Allele Frequency Tables

**Butler et al. (2003)**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Number Reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF1PO</td>
<td>2112/2112/2112</td>
</tr>
<tr>
<td>FGA</td>
<td>1023/2021/2021</td>
</tr>
<tr>
<td>TH01</td>
<td>121/121/121</td>
</tr>
<tr>
<td>TPOX</td>
<td>1020/1020/1020</td>
</tr>
<tr>
<td>VWA</td>
<td>2012/2012/2012</td>
</tr>
</tbody>
</table>

**Emun et al. (2004)**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Number Reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF1PO</td>
<td>2112/2112/2112</td>
</tr>
<tr>
<td>FGA</td>
<td>1023/2021/2021</td>
</tr>
<tr>
<td>TH01</td>
<td>121/121/121</td>
</tr>
<tr>
<td>TPOX</td>
<td>1020/1020/1020</td>
</tr>
<tr>
<td>VWA</td>
<td>2012/2012/2012</td>
</tr>
</tbody>
</table>

**African American**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Number Reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF1PO</td>
<td>2112/2112/2112</td>
</tr>
<tr>
<td>FGA</td>
<td>1023/2021/2021</td>
</tr>
<tr>
<td>TH01</td>
<td>121/121/121</td>
</tr>
<tr>
<td>TPOX</td>
<td>1020/1020/1020</td>
</tr>
<tr>
<td>VWA</td>
<td>2012/2012/2012</td>
</tr>
</tbody>
</table>

### Profile with 13 STRs

<table>
<thead>
<tr>
<th>Locus</th>
<th>Distribution of Profiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>OmniPop 150.4.2</td>
<td>97 populations</td>
</tr>
</tbody>
</table>

### Distribution of Profile Frequencies

<table>
<thead>
<tr>
<th>Allele Frequency</th>
<th>African American</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>0.0017*</td>
</tr>
<tr>
<td>12</td>
<td>0.0007</td>
</tr>
<tr>
<td>13</td>
<td>0.0031</td>
</tr>
<tr>
<td>14</td>
<td>0.1027</td>
</tr>
<tr>
<td>15</td>
<td>0.2616</td>
</tr>
<tr>
<td>16</td>
<td>0.2533</td>
</tr>
<tr>
<td>17</td>
<td>0.2152</td>
</tr>
<tr>
<td>18</td>
<td>0.1522</td>
</tr>
<tr>
<td>19</td>
<td>0.0160</td>
</tr>
<tr>
<td>20</td>
<td>0.0017*</td>
</tr>
</tbody>
</table>

**Most common allele**

<table>
<thead>
<tr>
<th>Allele Frequency</th>
<th>African American</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>0.0009</td>
</tr>
<tr>
<td>12</td>
<td>0.0007</td>
</tr>
<tr>
<td>13</td>
<td>0.0031</td>
</tr>
<tr>
<td>14</td>
<td>0.1240</td>
</tr>
<tr>
<td>15</td>
<td>0.2600</td>
</tr>
<tr>
<td>16</td>
<td>0.2430</td>
</tr>
<tr>
<td>17</td>
<td>0.2000</td>
</tr>
<tr>
<td>18</td>
<td>0.1460</td>
</tr>
<tr>
<td>19</td>
<td>0.0125</td>
</tr>
<tr>
<td>20</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

### 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?
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?

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?

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.

2.10.5 Detection of PCR product

2.10.5.1 Characterization without hybridization

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).

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?
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
- 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/calculator.php


Different Fluorescent Dyes

Visible spectrum range seen in CCD camera

- Commonly used fluorescent dyes
- Filter sets determine what regions of the CCD camera are activated and therefore what portion of the visible light spectrum is collected

<table>
<thead>
<tr>
<th>Filter F</th>
<th>Filter G5</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAM</td>
<td>FAM</td>
</tr>
<tr>
<td>JOE</td>
<td>JOE</td>
</tr>
<tr>
<td>VIC</td>
<td>VIC</td>
</tr>
<tr>
<td>NED</td>
<td>NED</td>
</tr>
<tr>
<td>PET</td>
<td>PET</td>
</tr>
<tr>
<td>ROX</td>
<td>ROX</td>
</tr>
<tr>
<td>LIZ</td>
<td>LIZ</td>
</tr>
</tbody>
</table>

Arrows indicate the dye emission spectrum maximum
Fluorescent Emission Spectra for ABI Dyes

![Fluorescent Emission Spectra](image)

Laser excitation (488, 514.5 nm)

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

AmpFISTR® Identifier™

Different dyes and mobility modifiers used

Overlap problems

![AmpFISTR Identifier](image)

Primer Sequences have been maintained across various kits

GS500-internal lane standard

Primer sequence

PCR amplification generates a labeled PCR product containing the mobility modifiers

For each linker unit added, there is an apparent migration shift of ~2.5 bp

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

Size overlap

6FAM

VIC

NED

PET

LIZ

6FAM-labeled (blue)

VIC-labeled (green)

NED-labeled (yellow)

PET-labeled (red)

LIZ-labeled (white)

Primer Sequences

PCR product sizes = 291-327 bp

+30 bp shift in size

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

(A) PowerPlex® 1.1 Kit

(B) PowerPlex® 16 Kit

PCR product sizes = 221-367 bp

+30 bp shift in size

Figure 5.8, J.M. Butler (2005) Forensic DNA Typing, 2nd Edition © 2005 Elsevier Academic Press
Changes in Promega Primer Sequences

<table>
<thead>
<tr>
<th>STR loci included in each kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>PowerPlex 1.1</td>
</tr>
<tr>
<td>PowerPlex 1.2</td>
</tr>
<tr>
<td>PowerPlex 2.1</td>
</tr>
<tr>
<td>PowerPlex 16</td>
</tr>
</tbody>
</table>

Examination of PCR Components

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

  \[ \text{CONC} \rightarrow 0.05, 0.1, 0.2, 0.5, 1, 2, \text{ and 4x per kit} \]

\[ \text{CONC} \rightarrow 0.05, 0.1, 0.2, 0.5, 1, 2, \text{ and 4x per kit} \]

Mobility Shift with Non-nucleotide Linker

Patients, controls, and in vitro controls were run in triplicate and the average data points were plotted to calculate the percentage of shift. The mobility shift depicted is the difference between the control run and the control run with the non-nucleotide linker.

Sizing Precision with Non-nucleotide Linkers

<table>
<thead>
<tr>
<th>Marker</th>
<th>Increase in</th>
<th>Range of Standard Deviation of Alleles for</th>
<th>Range of Standard Deviation of Alleles</th>
<th>Percentage of Sizing Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF1PO</td>
<td>1.1 bp</td>
<td>0.08 - 0.15²</td>
<td>0.15 - 0.20</td>
<td>99.99%</td>
</tr>
<tr>
<td>D21S118</td>
<td>1.0 bp</td>
<td>0.05 - 0.12⁻</td>
<td>0.12 - 0.15</td>
<td>99.99%</td>
</tr>
<tr>
<td>D16S539</td>
<td>1.0 bp</td>
<td>0.05 - 0.09⁻</td>
<td>0.09 - 0.16</td>
<td>99.99%</td>
</tr>
<tr>
<td>TPOX</td>
<td>1.0 bp</td>
<td>0.05 - 0.08⁻</td>
<td>0.08 - 0.12</td>
<td>99.99%</td>
</tr>
</tbody>
</table>

Heterozygote Peak Height Ratios

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Peak Height Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>90 %</td>
</tr>
<tr>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>

Prepared by John M. Butler
Validation Workshop – Developmental Validation
Aug. 24, 2005 at NFSTC

Heterozygote Peak Height Ratios

*Identifiler STR Kit Developmental Validation*

![Graph showing heterozygote peak height ratios.]

Low amount of input DNA (~250 pg)

60 %


Non-Human Studies (Species Specificity)

*Identifiler STR Kit Developmental Validation*

![Graph showing non-human studies.]


Measured Stutter Percentages

Variable by Allele Length and Composition


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


**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?
Validation Workshop – Developmental Validation

Aug. 24, 2005 at NFSTC

Validation Summary Sheet for PowerPlex Y

<table>
<thead>
<tr>
<th>Study Completed (# Male/ # Female)</th>
<th>Detection of Samples Taken per kit and Type of Panels</th>
<th>Runs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Source (Concordance)</td>
<td>6 samples x 6 Male</td>
<td>40</td>
</tr>
<tr>
<td>Mutation ratios (male/female)</td>
<td>10 ladder replicates + 1 samples replicated + [8 ladders + 8 samples enriched] for [27]</td>
<td>36</td>
</tr>
<tr>
<td>TaqGold polymerase titration</td>
<td>6 labs x 2 M/M mixtures series x 11 ratios (0.5:0.5, 0.5:1.0, 0.5:1.5, 0.5:2.0, 0.5:2.5, 0.5:3.0, 0.5:3.5, 0.5:4.0, 0.5:4.5, 0.5:5.0)</td>
<td>132</td>
</tr>
<tr>
<td>Thermal cycler test</td>
<td>6 labs x 2 M/M mixtures series x 11 ratios (1.0:1.0, 1.0:1.5, 1.0:2.0, 1.0:2.5, 1.0:3.0, 1.0:3.5, 1.0:4.0, 1.0:4.5, 1.0:5.0, 1.0:5.5)</td>
<td>132</td>
</tr>
<tr>
<td>Reaction volume</td>
<td>205 amounts (1/1.25/1.5/1.75/2 mM Mg) x 4 quantities (1/0.5/0.25/0.13 ng DNA)</td>
<td>205</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>6 labs x 2 M/M mixtures series x 11 ratios (0.05:0.05, 0.05:0.1, 0.05:0.15, 0.05:0.2, 0.05:0.25, 0.05:0.3, 0.05:0.35, 0.05:0.4, 0.05:0.45, 0.05:0.5)</td>
<td>65</td>
</tr>
<tr>
<td>Non-Human</td>
<td>6 laboratories x 5 amounts each (0-500 ng female DNA) x 5 amounts each</td>
<td>50</td>
</tr>
<tr>
<td>NIST SRM</td>
<td>6 substances of SRM 2395 NIST SRM</td>
<td>6</td>
</tr>
<tr>
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<td>6 labs x 2 M/M mixtures series x 11 ratios (0.05:0.05, 0.05:0.1, 0.05:0.15, 0.05:0.2, 0.05:0.25, 0.05:0.3, 0.05:0.35, 0.05:0.4, 0.05:0.45, 0.05:0.5)</td>
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<td>NIST SRM</td>
<td>6 substances of SRM 2395 NIST SRM</td>
<td>6</td>
</tr>
</tbody>
</table>

**A Comparison to Y-PLEX 12 Validation**


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 PowerPlex Y kit.

The study also shares two authors (Ann Marie Gross and Bruce Budowle) with the PowerPlex Y kit.

**Practical Examples**

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

**Validation Section of the DNA Advisory Board Standards**


**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.

**ABI 7500 Quantifier Validation Documentation**

Experimental data supports that the 7500 system with v1.2.3 software provides consistent performance when compared to the ABI PRISM® 7500 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

**Prepared by John M. Butler**

12
Promega Material Modification Reported for PP16 Primer Mix Storage


**Application Notes**

Amplifications Using the PowerPlex® 16 System and a 18S Primer Pair Mix Stored in TE Buffer or in Nuclease-FREE Water

Introduction

Promega recommends storing the reaction mix stored at 4°C to maintain the stability of the primers and dNTPs. The reagent mix should be refrigerated and stored at 4°C for all components. Theuse of this protocol may reduce precipitation of the primer mix. The use of the TE Buffer or nuclease-free water may help to reduce the risk of precipitation. The use of the TE Buffer or nuclease-free water may help to reduce the risk of precipitation.

Methodology

DNA is extracted from 10μL of undiluted blood from a boiled 10μL reaction mixture using the PowerPlex® 16 System. The reaction mixture is then used in the Amplification Kit to amplify the target DNA. The Amplification Kit is used to amplify the target DNA. The Amplification Kit is used to amplify the target DNA.

Validation

DNA from a known target is used in a 1:10 dilution with non-target DNA. The Amplification Kit is used to amplify the target DNA. The Amplification Kit is used to amplify the target DNA. The Amplification Kit is used to amplify the target DNA.

Conclusion

The Amplification Kit is used to amplify the target DNA. The Amplification Kit is used to amplify the target DNA. The Amplification Kit is used to amplify the target DNA.