PowerPlex® Fusion
Overview & Developmental Validation Preliminary Summary
Ann MacPhetridge, Marketing Manager
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PowerPlex® Fusion System
Webinar outline

- Expansion of CODIS
- Overview of the PowerPlex® Fusion System
- PowerPlex® Fusion Developmental Validation Data – a summary of work performed to-date by Promega
Expansion of Databases... Expansion of Multiplexes

- Need for common loci is recognized
- European Standard Set has expanded
- CODIS will expand in the coming years
- Goals:
  - More data sharing
  - Fewer adventitious matches
Supporting Expansion of Core Loci
Product Requirements

1. Include all current CODIS and ESS loci, plus Amelogenin
   • US SWGDAM: include DYS391

2. Support current CE instruments and software
   • 5-dye chemistry proven on the 31xx and 3500 Genetic Analyzer

3. Enable fast cycling

4. Create one kit for all labs to simplify validation, purchasing, etc.
   • Provide database (i.e. direct amp) and casework protocols
Development Approach
Building on Proven Technology

PowerPlex Fusion

- PowerPlex ESX/ESI
  - 5-Dye
  - New ladders
  - Primer design

- PowerPlex 18D
  - Direct amp
  - Fast cycling

- PowerPlex 16 HS
  - Hot start enzyme

- PowerPlex 21
  - Robust buffer
What is the PowerPlex® Fusion System?

• Analyzes 24 loci in a single reaction
  • CODIS + ESS + Pentas + D2S1338 + D19 +DYS391 + Amelogenin
  • Highest discrimination

• Uses rapid PCR technology (~85 min)

• Offers casework and database protocols in one kit
  • One kit to validate, purchase & QC

• Designed to work with 31xx CE
  • No 6-dye upgrade required
Most essential loci located below \( \sim \)400bp; nine under 220bp

- Excluding “extended” FGA alleles, the most informative required loci are <375bp
- All required “new CODIS loci” (“Panel A”)
- 2 of 3 “recommended” loci, TPOX and D22 (“Panel B”) -- no SE33
- Pentas
**Locus Selection**  
**Inclusion of Pentas**

- Straightforward inclusion in multiplexes because of “size range” (allele range)
- Commercially-available for more than a decade
- Easy mixture interpretation due to low stutter
- High discrimination added by two highly polymorphic loci
- Used routinely in Asia, Europe, North and South America
  - >1M samples in US NDIS contain Pentas
Enhanced allelic ladder

• 36 new alleles compared to PowerPlex® 16
• New virtual bins to increase genotyping efficiency
• Fewer inconclusive calls
Configuration and protocol
Product Overview—Configuration

Kit sizes

• 200 reactions (DC2402)
• 800 reactions (DC2408)

Components

• PRE-AMP: 5X Master Mix (w/ hot start Taq), 5X Primer Mix, 2800M Control DNA, Amplification Grade Water
• POST-AMP: Size standard (ILS 500), Allelic Ladder

Related Products

• 5-dye Matrix for AB 31xx & 3500 (DG4700)
• GeneMapper ID & ID-X panel/bin/stutter file downloads (free)
**Supported Instruments**

**Thermal Cycler:**
- GeneAmp 9700

**Capillary Electrophoresis:**
- 31xx Genetic Analyzer
- 3500 Genetic Analyzer
Usable Sample Types

- Extracted DNA
- Direct amplification from solid support materials
  - FTA® and Indicating FTA® cards
  - Swabs – Cotton, Omni Swab [treated with SwabSolution™]
  - Bode Buccal DNA Collector™ [treated with PunchSolution™]
  - S&S 903 Specimen Collection paper [treated with PunchSolution™]
PowerPlex® Fusion Protocol

PowerPlex® Fusion System

Extracted DNA
OR punch
OR swab extract

Reaction Assembly
• 5X Master Mix
• 5X Primer Mix
• 15µl volume available

Amplification: <1.5 hours

<table>
<thead>
<tr>
<th>1 cycle</th>
<th>96°C for 1 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 cycles:</td>
<td>94°C for 10 seconds</td>
</tr>
<tr>
<td></td>
<td>59°C for 1:00 min</td>
</tr>
<tr>
<td></td>
<td>72°C for 30 seconds</td>
</tr>
<tr>
<td></td>
<td>60°C for 10 minutes</td>
</tr>
<tr>
<td></td>
<td>4°C soak</td>
</tr>
</tbody>
</table>

Detection and Analysis
• 5 Dye Chemistry (G5)
• 3130/3130xl or 3500/3500xl
Protocols
Extracted DNA, FTA® punches, NonFTA punches, Swabs

Extracted DNA
- Extract DNA from sample
- Add up to 15µl of sample into reaction

FTA punches
- Punch FTA® into plate
- Add reaction mix

NonFTA Punches
- Punch card into plate, treat with PunchSolution™
- Add reaction mix to dried punch

Swabs
- SwabSolution™ incubation of swab
- Add 2µl of extract into reaction mix

Cycling time ~85 minutes for all applications
Extracted DNA
0.5ng

3130x1

3kv 5s
Example “Direct Amp” Data

Blood FTA® punch (Direct)

Buccal FTA® punches (Direct)

Buccal S&S903 punch (PunchSolution™ treated)

Swab (SwabSolution™ extract)
**Buccal Swab Workflow**

Add buccal swab to tube or plate*.

- Add 1ml of SwabSolution™ Reagent.
- Incubate 30 minutes at 70°C**.

 Transfer 2µl of swab extract.

Add to 23µl of PCR amplification mix‡.
Perform thermal cycling.

**Simple, rapid process for all swabs**

* For plate format, use 2.2mL deep well plate (V6781)
** Use new heat transfer block (A2661)
‡ For reactions other than PowerPlex® 18D and PowerPlex® 21, add AmpSolution™ Reagent
NonFTA® punch workflow
S&S903, Bode Buccal DNA Collector™ Device

**Punch** card into well.

**Add** 10µl of PunchSolution™ Reagent.

**Incubate** 30 minutes at 70°C (to dryness).

**Add** PCR amplification mix.

**Perform** thermal cycling.

Rapid, same-plate processing of non-FTA® punches
Performance
Sensitivity
Titration of Purified DNAs

![Bar graph showing sensitivity titration of purified DNAs. The x-axis represents different DNA concentrations (1 ng, 500 pg, 250 pg, 100 pg, 50 pg), and the y-axis represents the average height of the Het Peak (RFU). The graph indicates that higher DNA concentrations result in higher RFU values, demonstrating sensitivity.]
Sensitivity
100pg Amplification
PowerPlex® Fusion Inhibitor Titration

- **Tannic Acid**
  - 50ng/µl
  - 200ng/µl

- **Humic Acid**
  - 50ng/µl
  - 200ng/µl

- **Hematin**
  - 250mM
  - 1µM
  - 125mM
  - 500mM
Inhibitor Tolerance
Titration of Common Forensic PCR Inhibitors

500pg of DNA (n=3) was amplified with each kit in the presence of the listed inhibitors.

<table>
<thead>
<tr>
<th>Concentration (µM)</th>
<th>PowerPlex® Fusion</th>
<th>PowerPlex® 16 HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>250</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>500</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1000</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Humic Acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25ng/µl</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>50ng/µl</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>100ng/µl</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>200ng/µl</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Tannic Acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25ng/µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50ng/µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100ng/µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200ng/µl</td>
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<td></td>
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</tbody>
</table>

500pg of DNA (n=3) was amplified with each kit in the presence of the listed inhibitors.
PowerPlex® Fusion
Developmental Validation Data
Developmental Validation – Preliminary data
Reaction Volume – Solid Support Samples

% Alleles Called

<table>
<thead>
<tr>
<th>Reaction Volume</th>
<th>FTA 1 punch</th>
<th>Swab 2µl</th>
<th>Bode Collector 1 punch</th>
</tr>
</thead>
<tbody>
<tr>
<td>25µl</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>12.5µl</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>6.25µl</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

N = 3

Promega
Developmental Validation – Preliminary data
Reaction Volume – Extracted DNA

% Alleles Called

N = 3

- 25µl
- 12.5µl
- 6.25µl

500pg DNA
100pg DNA
Developmental Validation – Preliminary data
Precision Studies AB 3130xl

3130xl POP-4 Precision - 32 Ladders
Developmental Validation – Preliminary data
Precision Studies AB 3500xl

3500xl POP-4 Precision - 48 Ladders

Standard Deviation

Fragment Size (bp)
Developmental Validation
Population Study - NIST Concordance Testing

- PowerPlex® Fusion results compared to all other kits tested including: Sinofiler/NGM/Identifiler/Yfiler/IDplex/ESSplex/PP16/PP21 kits with 652 unrelated individuals (NIST U.S. population set)
- Fusion is fully concordant with NIST SRMs 2391b&c certified values
- No PP Fusion null alleles
- No PP Fusion discordance with other PowerPlex kits, discordance with ABI or Qiagen kits is on their end and are previously documented
## Developmental Validation
### Population Study - Probability of Identity Values

<table>
<thead>
<tr>
<th>STR Typing Kits (Locus Combinations)</th>
<th>Total (N=1036)</th>
<th>African Americans (N=342)</th>
<th>U.S. Caucasians (N=359)</th>
<th>U.S. Hispanics (N=238)</th>
<th>U.S. Asians (N=97)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CODIS 13</td>
<td>5.02E-16</td>
<td>1.13E-15</td>
<td>3.01E-15</td>
<td>1.39E-15</td>
<td>1.71E-14</td>
</tr>
<tr>
<td>Identifiler</td>
<td>6.17E-19</td>
<td>1.03E-18</td>
<td>6.94E-18</td>
<td>2.76E-18</td>
<td>5.30E-17</td>
</tr>
<tr>
<td>PowerPlex 16</td>
<td>2.82E-19</td>
<td>6.08E-19</td>
<td>4.22E-18</td>
<td>1.29E-18</td>
<td>2.54E-17</td>
</tr>
<tr>
<td>PowerPlex 18D</td>
<td>3.47E-22</td>
<td>5.55E-22</td>
<td>9.76E-21</td>
<td>2.58E-21</td>
<td>7.87E-20</td>
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<tr>
<td>ESS 12</td>
<td>2.99E-16</td>
<td>9.14E-16</td>
<td>9.68E-16</td>
<td>2.64E-15</td>
<td>3.41E-14</td>
</tr>
<tr>
<td>ESI 16 / ESX 16 / NGM</td>
<td>2.74E-20</td>
<td>6.02E-20</td>
<td>2.21E-19</td>
<td>4.03E-19</td>
<td>9.80E-18</td>
</tr>
<tr>
<td>ESI 17 / ESX 17 / NGM SElect</td>
<td>1.81E-22</td>
<td>6.44E-22</td>
<td>1.74E-21</td>
<td>3.99E-21</td>
<td>1.86E-19</td>
</tr>
<tr>
<td>PowerPlex 21</td>
<td>6.71E-27</td>
<td>2.08E-26</td>
<td>2.51E-25</td>
<td>7.96E-26</td>
<td>5.80E-24</td>
</tr>
<tr>
<td>CODIS 19 (-DYS391)</td>
<td>1.95E-23</td>
<td>1.08E-22</td>
<td>1.66E-22</td>
<td>1.93E-22</td>
<td>6.01E-21</td>
</tr>
<tr>
<td>GlobalFiler (-DYS391)</td>
<td>1.60E-27</td>
<td>5.63E-27</td>
<td>2.95E-26</td>
<td>5.10E-26</td>
<td>2.57E-24</td>
</tr>
<tr>
<td>PowerPlex FUSION (-DYS391)</td>
<td>1.36E-28</td>
<td>2.83E-28</td>
<td>5.25E-27</td>
<td>4.81E-27</td>
<td>2.01E-25</td>
</tr>
</tbody>
</table>

Slide kindly provided by John Butler (NIST) using data collected by Becky Hill & analysis tools developed by Dave Duewer
Developmental Validation – Preliminary data
Mixture Study

Percent Minor Alleles Called

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Percent Minor Alleles Called</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:19</td>
<td>70</td>
</tr>
<tr>
<td>1:9</td>
<td>90</td>
</tr>
<tr>
<td>1:5</td>
<td>90</td>
</tr>
<tr>
<td>1:2</td>
<td>90</td>
</tr>
<tr>
<td>1:1</td>
<td>90</td>
</tr>
<tr>
<td>2:1</td>
<td>90</td>
</tr>
<tr>
<td>5:1</td>
<td>90</td>
</tr>
<tr>
<td>9:1</td>
<td>90</td>
</tr>
<tr>
<td>19:1</td>
<td>70</td>
</tr>
</tbody>
</table>
Developmental Validation – Preliminary data
Inhibitors Study

Humic Acid

Hematin

N = 2
Developmental Validation – Preliminary data
Inhibitors Study

**Tannic Acid**

- 0% Alleles Called at 0ng/ul, 100ng/ul, 300ng/ul, and 500ng/ul.

**EDTA**

- 0% Alleles Called at 0mM, 0.4mM, 0.8mM, and 1.0mM. The graph shows a significant drop at 0.8mM.

N = 2
PowerPlex® Fusion System

• Provides a rapid thermal cycling protocol
• Sensitive and robust for casework
• Allows for direct amplification for swabs and punches in databasing
• Is fully compatible with current 31xx & 3500 series Genetic Analyzers
• Includes all core CODIS and ESS loci as well as a Pentas and DYS391
  • Most discrimination available
• Will allow searching of all autosomal STRs historically entered in NDIS
Questions?