Internal Validation of the
Promega® PowerPlex® Fusion System
with the
Applied Biosystems® 3130xl
Genetic Analyzer

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Overview

- What is DNA?
- Processing
- Extraction
- Quantification
- Amplification
- Capillary Electrophoresis
- Analysis
What is DNA?

Deoxyribo Nucleic Acid
Nucleoside triphosphate
Nucleotide

Purines
- Adenine
- Guanine

Pyrimidines
- Cytosine
- Uracil
- Thymine
2D Structure

http://diverge.hunter.cuny.edu/~weigang/images/02-16_dnastructure_f.jpg
3D Structure

- Chromosomes - 44 XY/XX

[Image of 3D chromosomes]
Alleles and Traits

• Chromosomes carry traits in forms of alleles
So......What is DNA????

- Blueprint
- User’s Manual
DNA in Forensics

- STR - Short Tandem Repeats
- Alleles identified by number of STR repeats
Extraction
Quantification

DAB Standards
Want You to Quant

http://1.bp.blogspot.com/-eDNT1iDtxSA/TZZCy5BZywI/AAAAAAAAAN4/6GPb1x4j1tg/s640/Poster3.I+WANT+YOU.jpg
PCR: Polymerase Chain Reaction

30 cycles of 3 steps:

1. Denaturation
2. Annealing
3. Extension

- Primers
- Forward and reverse primers
- Only dNTPs

(Andy Vierstraete 1999)
The first 4 cycles of PCR in detail

- **wanted gene**

- **template DNA**

<table>
<thead>
<tr>
<th></th>
<th>1st cycle</th>
<th>2nd cycle</th>
<th>3rd cycle</th>
<th>4th cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of double strands with the right length:</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>

(Andy Vierstraete 2001)
Capillary Electrophoresis

- Sample Injection
- Mixture of dye-labeled PCR products from multiplex PCR reaction

- Size Separation
- Argon ion laser (488 nm)

- Fluorescence
- ABI Prism spectrograph

- Color Separation
- CCD Panel (with virtual filters)

- Sample Detection
Analysis

GeneMapper ID v3.2.1 sorts the peaks

Overview Validation

- Overview of the Chemistry
- Validation Work
- Troubleshooting
- Future Studies
- Conclusion
Promega® PowerPlex® Fusion System

• 24 loci – 5 Dye Chemistry
• Core 13 CODIS loci
  (CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317,
   D16S539, D18S51, and D21S11)
• 12 European Standard Set loci
  (TH01, vWA, GA, D21S11, D3S1358, D8S1179, D18S51, D10S1248, D22S1045,
   D2S441, D1S1656, and D12S391)
• Penta E, Penta D, D2S1338, D19S433 and
  Amelogenin and a Y-STR loci (DYS391)
Promega® PowerPlex® Fusion System (cont’d)

- Amplifies on 9700 at Max Ramp Speed
- Works on ABI® 310, 3130, 3130 xl, 3500, 3500 xl
- No upgrades necessary
- Only simple software upgrades for GeneMapper® ID v3.2.1 present free online
Preliminary Testing – 2800M Raw Data
Validation Work

1. Cycle Number, DNA Target, Injection Time Study
2. Threshold Study
3. Precision Study
4. Peak Height Ratio Study
5. Concordance Study
6. Contamination Study
7. Stutter Study
8. Mixture Study
9. Inhibition Study
10. Non-Probative Samples Study
Cycle Number, DNA Target, Injection Time

- 30 cycles vs. 31 cycles
- Serial Dilution in triplicates

<table>
<thead>
<tr>
<th>Concentration (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 ng</td>
</tr>
<tr>
<td>5 ng</td>
</tr>
<tr>
<td>2.5 ng</td>
</tr>
<tr>
<td>1 ng</td>
</tr>
<tr>
<td>0.5 ng</td>
</tr>
<tr>
<td>0.25 ng</td>
</tr>
<tr>
<td>0.125 ng</td>
</tr>
<tr>
<td>0.0625 ng</td>
</tr>
<tr>
<td>0.0313 ng</td>
</tr>
<tr>
<td>0.0156 ng</td>
</tr>
</tbody>
</table>
Cycle Number, DNA Target, Injection Time (cont’d)

• Injection at 3, 5, 10, 15 seconds at 3kV
• Total Injection Time changed from 1500 seconds to 1700 seconds
Threshold Study

- Reagent Blanks ran in 5 replicates and injected 5 times
- Results analyzed at 1 rfu
Analytical Threshold

- Method 1: IUPAC

\[ AT = Y_{bl} + kS_{bl} \]

- \( AT \) = Analytical Threshold
- \( Y_{bl} \) = Average black RFU signal
- \( k = 3 \)
- \( S_{bl} \) = Standard Deviation of the blank signal
Analytical Threshold

• Method 2: SWGDAM

\[
AT = 2(Y_{\text{max}} - Y_{\text{min}})
\]

AT = Analytical Threshold

\(Y_{\text{max}}\) = Highest peak within instrumental noise

\(Y_{\text{min}}\) = signal of the lowest trough
## Results

*Table 1: Analytical Threshold- Method 1*

<table>
<thead>
<tr>
<th>Dye</th>
<th>Average Height</th>
<th>Standard Deviation Height</th>
<th>Minimum Height</th>
<th>Maximum Height</th>
<th>Analytical Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue</td>
<td>4.63</td>
<td>1.56</td>
<td>1</td>
<td>18</td>
<td>9.31</td>
</tr>
<tr>
<td>Green</td>
<td>6.03</td>
<td>1.87</td>
<td>1</td>
<td>20</td>
<td>11.63</td>
</tr>
<tr>
<td>Yellow</td>
<td>8.37</td>
<td>2.46</td>
<td>2</td>
<td>27</td>
<td>15.75</td>
</tr>
<tr>
<td>Red</td>
<td>6.34</td>
<td>1.86</td>
<td>2</td>
<td>35</td>
<td>11.93</td>
</tr>
</tbody>
</table>

*Table 2: Analytical Threshold- Method 2*

<table>
<thead>
<tr>
<th>Dye</th>
<th>Average Height</th>
<th>Standard Deviation Height</th>
<th>Minimum Height</th>
<th>Maximum Height</th>
<th>Analytical Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue</td>
<td>4.63</td>
<td>1.56</td>
<td>1</td>
<td>18</td>
<td>34</td>
</tr>
<tr>
<td>Green</td>
<td>6.03</td>
<td>1.87</td>
<td>1</td>
<td>20</td>
<td>38</td>
</tr>
<tr>
<td>Yellow</td>
<td>8.37</td>
<td>2.46</td>
<td>2</td>
<td>27</td>
<td>52</td>
</tr>
<tr>
<td>Red</td>
<td>6.34</td>
<td>1.86</td>
<td>2</td>
<td>35</td>
<td>68</td>
</tr>
</tbody>
</table>
Troubleshooting

- Artifact present in Reagent Blanks but not in samples
- Consistent with Qiagen® EZ1 DNA Investigator kit contaminant profile
- Re-amplified- not replicated
- Eliminated because not reproducible.
LOD and LOQ

- LOD = Average noise signal + 3 * Std
- LOQ = Average noise signal + 10 * Std
## Results

<table>
<thead>
<tr>
<th>Dye</th>
<th>Average Height</th>
<th>Standard Deviation Height</th>
<th>Minimum Height</th>
<th>Maximum Height</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue</td>
<td>4.63</td>
<td>1.56</td>
<td>1</td>
<td>18</td>
<td>9.31</td>
<td>20.21</td>
</tr>
<tr>
<td>Green</td>
<td>6.03</td>
<td>1.87</td>
<td>1</td>
<td>20</td>
<td>11.63</td>
<td>24.72</td>
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<tr>
<td>Yellow</td>
<td>8.37</td>
<td>2.46</td>
<td>2</td>
<td>27</td>
<td>15.75</td>
<td>32.96</td>
</tr>
<tr>
<td>Red</td>
<td>6.34</td>
<td>1.86</td>
<td>2</td>
<td>35</td>
<td>11.93</td>
<td>24.96</td>
</tr>
</tbody>
</table>
Stochastic Threshold

\[ ST = \left( \frac{1}{ \text{Average PHR} - 3 \times \text{STD}} \right) \times AT \]
## Results

<table>
<thead>
<tr>
<th>Dye</th>
<th>AVG PHR</th>
<th>STD PHR</th>
<th>AT-M1</th>
<th>AT-M2</th>
<th>ST-M1</th>
<th>ST-M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue</td>
<td>0.8191</td>
<td>0.0883</td>
<td>9.31</td>
<td>34</td>
<td>16.8044</td>
<td>61.3696</td>
</tr>
<tr>
<td>Green</td>
<td>0.8023</td>
<td>0.0666</td>
<td>11.63</td>
<td>38</td>
<td>19.3093</td>
<td>63.0915</td>
</tr>
<tr>
<td>Yellow</td>
<td>0.7874</td>
<td>0.1107</td>
<td>15.75</td>
<td>52</td>
<td>34.603</td>
<td>114.245</td>
</tr>
<tr>
<td>Red</td>
<td>0.7611</td>
<td>0.1234</td>
<td>11.93</td>
<td>68</td>
<td>30.517</td>
<td>173.944</td>
</tr>
</tbody>
</table>
Results

Stochastic Threshold from SD of TF punches

APH

PHR
Precision Study

- 16 ladders injected 5 times
- TF punches ran previously
- 3 Standard Deviation must be less than 0.5bp
Results

• Highest 3*STD = 0.284 at allele 15 for D12S391 at 31 cycles and 5 second injection

• Lowest 3*STD = 0.069 at allele 12 for D16S539 at 31 cycles and 5 second injection
Peak Height Ratio

- 5 second injection of the TF punches was used
- Average PHR versus template DNA calculated
Results
Concordance

- 35 convicted offenders samples used
- Compared to the PowerPlex® 16 results
Results

• All samples were in concordance with the previous results except for 1 sample that had drop out
Contamination

• Ladder and Run Negative checkerboard plate was ran and injected with the initial plate at 3, 5, 10, 15 seconds
Results

• No cross contamination was present in the run negatives.
• One injection did show some peaks in the Run Negative, yet those peaks were not present in the other injections so they were not considered since they were not reproducible.
Stutter Study

• 35 Convicted offenders samples amplified at 0.125ng, 0.5 ng, 1.875 ng
• Macro from strbase.com
• Formula:

\[
\text{Stutter} = \text{Avg Stutter Ratio} + 3 \times \text{Stutter Ratio Std}
\]
Stutter example at $D10S1248 - D13S317$
Results
Results

![Graph showing allele peak height vs. Stutter/Allele Height Ratio for D10S1248](image)
Results

![Graph showing allele distribution for D10S1248](image-url)
<table>
<thead>
<tr>
<th>Locus</th>
<th>Min</th>
<th>Max</th>
<th>Avg PHR</th>
<th>STD PHR</th>
<th>Stutter (fx)</th>
<th>Stutter</th>
</tr>
</thead>
<tbody>
<tr>
<td>D10S1248</td>
<td>5.556</td>
<td>17.582</td>
<td>8.930</td>
<td>2.620</td>
<td>16.78936</td>
<td>17%</td>
</tr>
<tr>
<td>D12S391</td>
<td>4.178</td>
<td>20.619</td>
<td>10.120</td>
<td>3.455</td>
<td>20.48612</td>
<td>20%</td>
</tr>
<tr>
<td>D16S539</td>
<td>3.287</td>
<td><strong>9.050/14.925</strong></td>
<td>5.986</td>
<td>1.950</td>
<td>11.83729</td>
<td><strong>15%</strong></td>
</tr>
<tr>
<td>D18S51</td>
<td>3.752</td>
<td>22.472</td>
<td>9.281</td>
<td>3.408</td>
<td>19.50568</td>
<td>22%</td>
</tr>
<tr>
<td>D19S433</td>
<td>3.241</td>
<td>13.359</td>
<td>7.479</td>
<td>2.171</td>
<td>13.99052</td>
<td>14%</td>
</tr>
<tr>
<td>D1S1656</td>
<td>4.247</td>
<td>19.802</td>
<td>9.093</td>
<td>2.813</td>
<td>17.5322</td>
<td>20%</td>
</tr>
<tr>
<td>D21S11</td>
<td>5.395</td>
<td><strong>15.301/22.115</strong></td>
<td>9.191</td>
<td>2.533</td>
<td>16.7903</td>
<td><strong>22%</strong></td>
</tr>
<tr>
<td>D22S1045</td>
<td>2.581</td>
<td>17.857</td>
<td>10.922</td>
<td>2.992</td>
<td>19.89792</td>
<td>20%</td>
</tr>
<tr>
<td>D2S1338</td>
<td>5.040</td>
<td>14.043</td>
<td>8.781</td>
<td>2.061</td>
<td>14.96469</td>
<td>15%</td>
</tr>
<tr>
<td>D2S441</td>
<td>2.164</td>
<td>10.313</td>
<td>5.514</td>
<td>1.661</td>
<td>10.49599</td>
<td>10%</td>
</tr>
<tr>
<td>D3S1358</td>
<td>5.726</td>
<td>13.043</td>
<td>8.681</td>
<td>2.007</td>
<td>14.70219</td>
<td>15%</td>
</tr>
<tr>
<td>D7S820</td>
<td>2.379</td>
<td><strong>18.537/24.528</strong></td>
<td>6.600</td>
<td>3.578</td>
<td>17.33509</td>
<td><strong>24%</strong></td>
</tr>
<tr>
<td>D8S1179</td>
<td>3.414</td>
<td>13.873</td>
<td>7.656</td>
<td>2.020</td>
<td>13.71633</td>
<td>14%</td>
</tr>
<tr>
<td>DYS391</td>
<td>5.157</td>
<td>15.302</td>
<td>8.069</td>
<td>2.056</td>
<td>14.23779</td>
<td>15%</td>
</tr>
<tr>
<td>TH01</td>
<td>1.266</td>
<td>7.014</td>
<td>2.851</td>
<td>1.519</td>
<td>7.407025</td>
<td>7%</td>
</tr>
<tr>
<td>CSF1PO</td>
<td>2.558</td>
<td>11.607</td>
<td>7.018</td>
<td>1.747</td>
<td>12.25882</td>
<td>12%</td>
</tr>
<tr>
<td>TPOX</td>
<td>1.808</td>
<td>7.962</td>
<td>3.570</td>
<td>1.353</td>
<td>7.628283</td>
<td>7%</td>
</tr>
</tbody>
</table>
Mixtures

- Female:Male (19:1, 9:1, 4:1, 1:1, 1:4, 1:9, 1:19)
- Male:Male (19:1, 9:1, 4:1, 1:1, 1:4, 1:9, 1:19)
- Male:Male:Male (1:1:1)
Results - Female:Male 19:1
Results - Male:Male 19:1
Results Male:Male:Male 1:1:1
Results Male:Male:Male 1:1:1
Inhibition

- Humic acid
  (100 ng/µl, 150 ng/µl, 200 ng/µl, 250 ng/µl, 300 ng/µl)
- EDTA
  (0.4 mM, 0.5 mM, 0.6 mM, 0.7 mM, 0.8 mM)
- Blue Denim Dye
  (1:10, 1:20, 1:50, 1:100, 1:500)
- 0.5 ng DNA target
Results

• *Humic Acid*- complete inhibition
• *EDTA*- no difference the total peak heights ranging from 26819 rfu at 0.7mM to 25,134 rfu at 0.8mM
  (minus A artifacts in red channel below 250bp)
Results

• *Denim Dye*:  
  • 1:10 – complete drop out  
  • 1:20 - drop out in loci greater than 250bp- (Ski Slope seen)  
  • 1:50, 1:100, 1:500 – complete profiles with TPH of 20,564, 26,498, 27,572 rfu respectively.
Results - Humic Acid 200ng/ul.
Results - Dye 1:20
Non-probative Samples

- 25 samples:
  1. 2 Buccal Swabs
  2. 5 Touch evidence
  3. 5 Differentials
  4. 2 Gum
  5. 5 Cigarette Butts
  6. 2 Hair Samples
  7. 4 Phone Swabs
Results
Results
Troubleshooting

- Total Peak height was showing slope similar to degradation with the lowest ratio of smallest to largest peak being 6% and the highest ratio was 40%.
- Tried 29 cycles
- Tried 0.25, 0.5 and 1 ng loads
- 2800M positive control showed better ratios than TF
- Study of TF versus Buccal Swab with 3 different Extraction Methods (*EZ1* water elution, *EZ1* TE elution, Organic Extraction)
Results of TPH at 40%

TPH 2800M 0.5ng- 30cycles MAX
Results of TPH at 6%
Future Studies

- Mixture Study
- Inhibition Study
- Cross Loci Calling
- Finish the Total Peak Height Sloping Issue
- Non-specific STR calling especially from microbial DNA
- Quantification through the Amplification Kit
Conclusion

- PowerPlex® Fusion provided accurate profiles and a wide range of input target DNA with low amount of artifacts.
- The use of the Promega® PowerPlex® Fusion amplification kit is recommended for the use in future casework samples.
Acknowledgement

- Marshall University Forensic Science Center
- Jason Chute
- Josh Stewart
- Christopher William Thatch
- Jennifer Hayden
- Season Seferyn
- Heather Harrah-Lea
- Amanda Hoffman
- Promega®’s Tech Services
References

• Pfosser, K. and Owen S. Evaluation of the PowerPlex® Fusion System for Use on the ABI PRISM® 310 Genetic Analyzer.
Thank You

Questions?