Development and Validation of the 3500 Series Genetic Analyzer for Human Identification

Jeff Sailus
Forensic Science Applications Group
Development of a Next Generation Genetic Analyzer
A Whole System Approach

**Consumables**
- Redesigned Consumables with Tracking
- Real-time System and Consumable Information

**Instrument Hardware**
- Improved Temperature Control
- Shorter Run Times
- New Laser and Polymer Pump Block

**Data Collection**
- Predefined Protocols and HID Templates
- Optional Signal Normalization

**QC Analysis**
- Data Analysis
- QC Tools and Flags
- Simplified Reinjection Scheme

**GMID-X v1.2**
- ID-X 1.1.1 Upgrade
- Supports Windows XP and Vista
- Analyzes .fsa & New .hid File Format
- Consumable Data Tracking
Improved Data Quality and Sample Throughput

- Faster Run Time
- Higher Throughput
- More Consistent Migration for Better Sizing Precision
- Smaller Oven
- Detection cell heater
- Flat Oven Door Seal and New Locking Mechanism
Instrument Setup and Performance

- Elimination of Lower Polymer Block and Polymer Delivery Tubing
- Redesigned Array Port
- Minimized Potential for Bubbles and Leaks
- Direct Channels Promote Efficient Polymer Flow
- Reduced Polymer Waste
Instrument Setup and Performance

• Pre-Filled, Quality-Controlled Reagents

• Information Recorded via RFID
  • Lot numbers
  • Part Numbers
  • Serial numbers
  • Dates (expiration and installation)
  • Capacity/Usage

• Per Sample Running Cost Comparable to 31xx
Data Collection:
QC Analysis Tools for Preliminary Data Analysis

Review data quality flags, electropherograms and sizing tables - Select samples for reinjection
3500 Developmental Validation Studies
Developmental Validation Scope
Hardware and Reagents

• Instruments
  • (3) 8 Capillary Instruments (3500)
  • (3) 24 Capillary Instruments (3500xL)

• Size Standard
  • GeneScan™ 600 LIZ® Size Standard v2.0
  • GeneScan™ 500 ROX™ Size Standard
    • 4 Dye Amplification Kits do not use the normalization functionality

• Polymer and Array
  • POP-4™ and 36 cm Capillary Array
## Developmental Validation Scope

### Sample Summary

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Samples</th>
<th>Input</th>
<th>Replicates</th>
</tr>
</thead>
</table>
| Genotype Concordance and Reproducibility | • 40 male and 42 female gDNA samples  
• 4 racial groups  
• Controls | 1ng total DNA                                                            | • 1 replicate per sample  
• 2 injections                                      |
| Sizing Precision and Accuracy          | Allelic ladder                                                          | 1 µL per well                              | • See below                                       |
| Sensitivity and Normalization          | • 007 control DNA  
• 3 gDNA samples                                                        | Kit dependent 0.125ng, 0.25ng, 0.5ng, 0.75ng, 1ng, 6ng | • 5 replicates for DNA samples  
• 3 replicates for NTC  
• 4 injections                                      |
| Mixture Analysis                       | • 4 pairs of male and female gDNA  
• Mixture ratios  
• 1:0, 1:1, 1:3, 1:5, 1:9, and 0:1                                      | • Recommended concentrations per kit       | 1 replicate per sample                              |
| Resolution                             | • Allelic Ladder  
• Research material                                                       | • 1 µL per well                             | 24 replicates                                    |
Validation Scope
AmpFlSTR® Kits

- Identifiler®,
- Minifiler™
- SGM Plus®,
- Yfiler®,
- Sinofiler™
- SEfiler Plus™
- Profiler Plus®,
- Cofiler®,
- Identifiler® Direct
- Identifiler® Plus
- NGM™
Precision and Accuracy Studies
3500 Sizing Precision Study:
Within an Injection of Identifiler Allelic Ladder
3500 Sizing Precision Study: Across Multiple Injections of Identifiler Allelic Ladder Samples
Resolution and Baseline Evaluation
Resolution

- SEfiler Plus™ (SE33 triplet) and Identifiler® (TH01 9.3/10) Allelic Ladders
- 160 injections per instrument on 6 instruments
- Research STR markers greater than 300 base pairs were also developed with single base pair differences (data not shown)
Baseline Noise - Developmental Evaluation
G5 Average Noise (PAT=Avg Noise+10x SD)

Data generated during the Sensitivity Studies were then used to confirm the level of pull up and an optimal peak amplitude threshold setting.
Introduction to Normalization
Normalization: What is it?

A method to attenuate signal variations associated with instrument, capillary array, sample salt load, and injection variability

- **Factory Standardization:** Hardware-based calibration to help enable more consistent instrument to instrument performance

- **(Optional) Internal Standard Normalization:** Chemistry and software based method to help enable more consistent performance across injections and instruments
  - GeneScan™ 600 LIZ® Size Standard v2.0
  - 3500 Data Collection and GMID-X v1.2
Sources of Variation by Normalization Method

Instrument to Instrument largest source of variation

Factory + Internal Standard Normalization greatest reduction in variation

%CV

Levels of Normalization

- No Norm
- Factory only
- Int Std only
- Factor+IntStd

Sources of Variation
Sensitivity and Internal Standard Normalization Studies
Sensitivity Study:
Average Peak Height across a Range of DNA Input Amounts
AmpFlSTR® MiniFiler™ PCR Amplification Kit

Normalization Method
- Factory Only
- Factory + Internal Std
Size Standard Peak Height Consistency Study:

Five AmpF™-STR® PCR Amplification Kits
GeneScan™ 600 LIZ® Size Standard v2.0

![Box plot showing peak height consistency across different lots and kit types.](image)

GS600 Peak Height

- **Identifiler®**
- **Identifiler® Direct**
- **Identifiler® Plus**
- **Minifiler™**
- **NGM™**
Size Standard Peak Height Consistency Study:
Injection to Injection Peak Height Consistency

Size Standard Peak Height Consistency · 6 instruments · 3 lots of size standard
Size Standard Peak Height Consistency Study:

%CV for 5 Different AmpFtSTR® Kits
GeneScan™ 600 LIZ® Size Standard v2.0

Factory + Internal Std Normalization · 6 instruments · 3 lots of size standard
Additional Developmental Validation Studies
Additional Developmental Studies

- Genotype Concordance
  - 100% genotype concordance with samples run on the 3130xL

- GeneMapper® ID-X v1.2 Verification
  - 100% Concordant with historical data set collected from legacy CE instruments using a variety of AmpFSTR® kits analyzed and GeneMapper® ID-X v1.1 software versions (including mixtures)
  - Normalization and RFID documentation functionality performed as expected
In Summary

• 100% genotype concordance between the 3500(xl) and 3130xl instrument and GMID-X software

• Average Sizing precision data with GeneScan™ 600 LIZ® Size Standard v2.0 was 0.05 or less within an injections and across injections

• Linear relationship between input amounts and peak heights, with average peak heights ~6,000 rfu for 1ng input reactions Identifiler® Plus and NGM™

• Internal Standard Normalization significantly reduced injection-to-injection and instrument-to-instrument variation, but not the absolute values of the allele peak heights

• No reproducible PCR artifacts were observed within the read regions - spectral pull up of less than 5% was observed
FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

©2009 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners.

For those who require IVD-marked devices, the 3500 Dx and 3500xL Dx Genetic Analyzers and system accessories meet the requirements of the In Vitro Diagnostic Medical Devices Directive (98/79/EC). The 3500 Dx and 3500xL Dx systems are for distribution and use in specific European countries only. For more information about the 3500 Dx Series Systems, contact your Applied Biosystems representative.
Thank you!

www.appliedbiosystems.com/3500HID