ABI 3130xl Upgrade Validation Experiments Performed at NIST

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ABI Seminar Series
Future Trends in Forensic DNA Technology
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http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

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National Institute of Justice
The Research, Development, and Evaluation Agency of the U.S. Department of Justice

Current Areas of NIST Effort with Forensic DNA

- **Standards**
  - Standard Reference Materials
  - Standard Information Resources (STRBase website)
  - Interlaboratory Studies
- **Technology**
  - Research programs in SNPs, miniSTRs, Y-STRs, mtDNA, qPCR
  - Assay and software development
- **Training Materials**
  - Review articles and workshops on STRs, CE, validation
  - PowerPoint and pdf files available for download

History of Genetic Analyzer Usage at NIST

- ABI 310 x 2 (originally with Mac, then NT)
  - 1st was purchased in 1996
  - 2nd was purchased in June 2002
- ABI 3100 (Data collection v1.0.1)
  - Purchased in June 2002
  - Original data collection software retained
- ABI 3130xl upgrade (Data collection v3.0)
  - Purchased in April 2001
  - Upgraded in September 2005
  - Located in a different room

Our Use of the ABI 3100

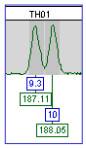
- Data collection software, version 1.0.1
- **POP-6** with 36 cm capillary array

- STR kits and in-house assays for autosomal STRs, Y-STRs, and miniSTRs
- SNaPshot assays for mtDNA SNPs, Y-SNPs, and autosomal SNPs
- DNA sequencing for mtDNA and STR repeat sequencing

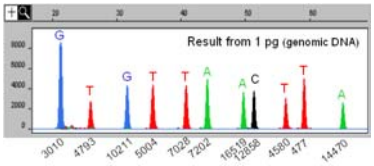
We can routinely get more than 400 runs per capillary array by not changing the polymer between applications

NIST ABI 3100 Analysis Using POP-6 Polymer

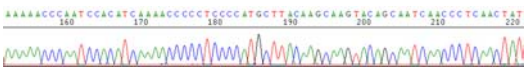
High Resolution STR Typing



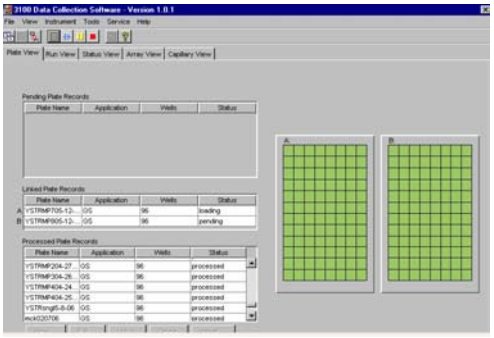
SNaPshot SNP Typing
(Coding Region mtSNP 11plex minisequencing assay)



mtDNA Sequencing (HV1)



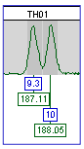
3100 Data Collection Software v1.0.1



Requirements for Reliable STR Typing

Butler *et al.* (2004) Electrophoresis 25: 1397-1412

- Reliable sizing over a 75-500 bp size region
- High run-to-run precision between processed samples to permit comparison of allelic ladders to sequentially processed STR samples
- Effective color separations of different dye sets used to avoid bleed through between 4 or 5 different colors
- Resolution of at least 1 bp to >350 bp to permit reliable detection of microvariant alleles

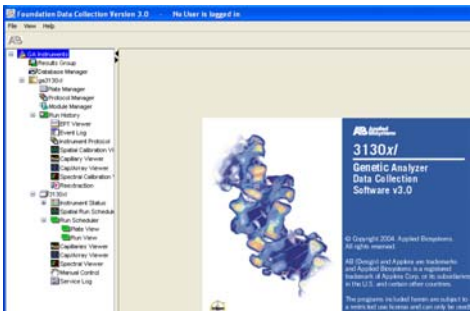


ABI 3130xl Upgrade Package

- Total price for the upgrade: $32,000
- **Both sequence analysis and genotyping capabilities** (different from the HID upgrade)
- Data collection version 1.1 was upgraded to version 3.0
- Other software included: GeneMapper ID, version 3.2 and Sequence Analysis software, version 5.2

This is a community instrument at NIST, used by other groups besides the HID project team

3130xl Data Collection Software v3.0

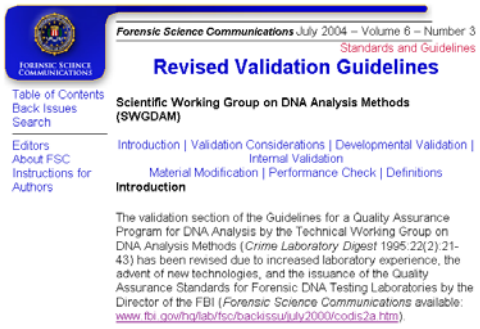


Validation Experiments for 3130xl Upgrade vs. Original 3100

- Relative Sensitivity of the 2 instruments
  - Are different injection parameters necessary to obtain similar peak heights?
- Precision
  - Instruments are located in different rooms with separate environmental conditions
- Genotyping accuracy (concordance)
  - Are the same typing results observed between both instruments?

Revised SWGDAM Validation Guidelines
(July 2004)

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



Forensic Science Communications July 2004 – Volume 6 – Number 3
Standards and Guidelines

Revised Validation Guidelines

Table of Contents
Back Issues
Search
Editors
About FSC
Instructions for Authors

Scientific Working Group on DNA Analysis Methods (SWGDAM)

Introduction | Validation Considerations | Developmental Validation | Internal Validation
Material Modification | Performance Check | Definitions

Introduction

The validation section of the Guidelines for a Quality Assurance Program for DNA Analysis by the Technical Working Group on DNA Analysis Methods (*Crime Laboratory Digest* 1995:22(2):21-43) has been revised due to increased laboratory experience, the advent of new technologies, and the issuance of the Quality Assurance Standards for Forensic DNA Testing Laboratories by the Director of the FBI (*Forensic Science Communications* available: www.fbi.gov/hq/lab/fsc/backissu/july2000/codis2a.htm).

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

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

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

General Steps for Internal Validation

- Review literature and learn the technique
- Obtain equipment/reagents, if necessary
- Determine necessary validation studies (there can be overlap and you only need to run a total of 50 samples)
- Collect/obtain samples, if necessary
- Perform validation studies, maintaining all documentation
- Summarize the studies and submit for approval to Technical Leader
- Write-up the analytical procedure(s). Include quality assurance (controls, standards, critical reagents and equipment) and data interpretation, as applicable
- Determine required training and design training module(s)
- Design qualifying or competency test

From Robyn Ragsdale (FDLE), Validation Workshop (Aug 24-26, 2005 at NFSTC)
http://www.cstl.nist.gov/biotech/strbase/validation/validationworkshop.htm

Instrument/Software Upgrades or Modifications

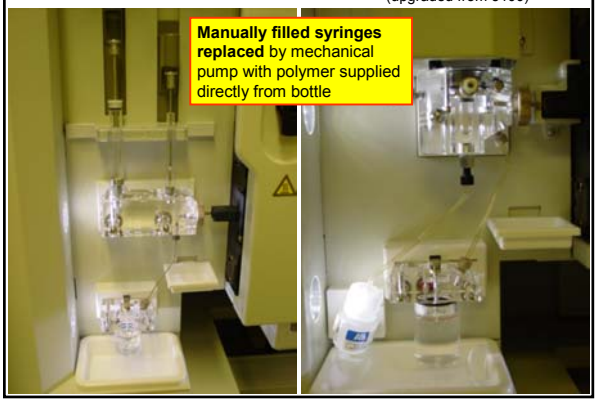
- What should be done to "validate" new upgrade?
  - ABI 7000 to ABI 7500
  - ABI 3100 to ABI 3130xl
  - GeneScan/Genotyper to GeneMapper*ID*
- Try to understand what is different with the new instrument or software program compared to the one you are currently using (e.g., ask other labs who may have made the switch)
- If possible, try to retain your current configuration for comparison purposes for the validation period

Run the same plate of samples on the original instrument/software and the new one

ABI 3100 and 3130xl Differences

- Polymer Block
  - No more manually filled syringes for the 3130xl
- Polymer solution
  - POP-7 vs. POP-4 and POP-6
- Data Collection software
  - New, user-friendly features in the upgraded software
  - Compensation for the red dye channel (variable binning – not present in v1.0.1)

ABI 3100 | ABI 3130xl (upgraded from 3100)



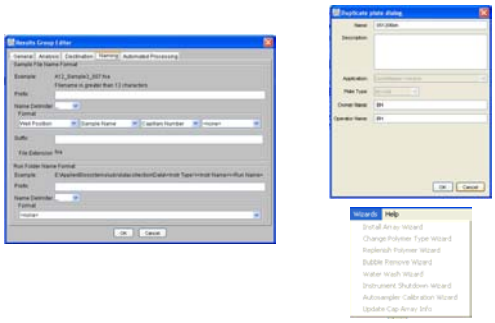
Benefits of the 3130xl Upgrade
(Compared to the original 3100, Data Collection 1.0.1)

- Takes much less time to change the polymer
- User-friendly wizards to install capillary arrays and change polymer
- **Can easily duplicate plate templates**
- Creation of results group to determine the format of how the data is saved
- Can easily import data, analysis methods, bins and panels, and size standard info into GeneMapper ID
- Data can be analyzed in GeneScan/Genotyper with "GeneMapper Generic" application setting

GeneMapper Generic Setting from Data Collection v3.0

We always choose the "GeneMapper – Generic" setting as the **Application** in our plate templates so we can use both types of data analysis software - allows more flexibility

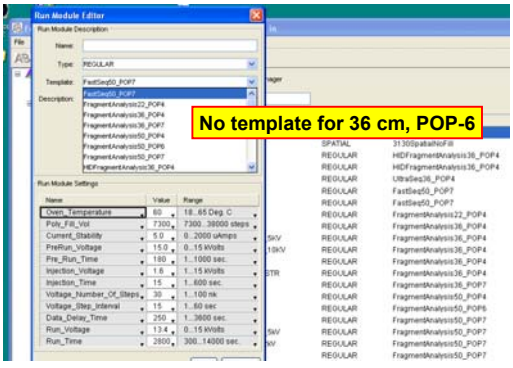
Benefit features of ABI 3130xl Data Collection Software v3.0



Some Problems Encountered with the ABI 3130xl

- The templates for the run modules are pre-set. If there is not a template for the necessary conditions of interest, this is a problem because you cannot create a new template
  - Currently, there is no template for Fragment Analysis 36 cm capillary array with POP-6 polymer
- Glitch in software: Plate is linked, but the green start button is unavailable to begin analysis
  - Easy fix for this problem: delete PUBSUB and PTP folders in E:/Applied Biosystems/Service/JMS/Bin and restart the 3130xl and computer

Pre-Set Templates cannot be changed



Sample Sets for Comparison

- Identifiler – 375 (cell line study)
  - Different injections and polymer
- Profiler Plus – 95 (father-son pairs)
  - Different polymer
- miniSTR – 665 (NIST US pop)
  - experienced room temperature problems, ran an allelic ladder in each run to accommodate for this problem

Summary of All Comparisons Performed to Date

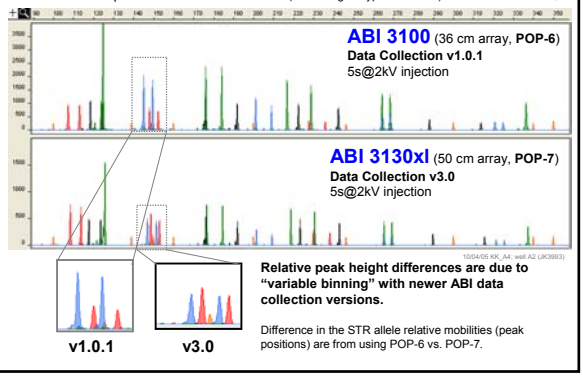
ABI 3130xl vs ABI 3100

- Ran plates of samples on both instruments with same injection and separation parameters and compared results **(Examined various STR kits, SNaPshot products, and mtDNA sequencing data)**
- Data Collection version 1.0.1 (3100) vs 3.0 (3130xl)
- POP-6 (3100) vs POP-7 (3130xl), **POP-4 (3100 and 3130xl)**
- **36 cm array (3100 and 3130xl)** vs 50 or 80 cm array (3130xl)
- Injection parameters: 10 sec @ 3 kV, 5 sec @ 2 kV

Environmental conditions may change over time so original validation is no longer valid…

Comparison of ABI 3100 Data Collection Versions

Same DNA sample run with Identifiler STR kit (identical genotypes obtained) GeneScan display



Relative peak height differences are due to "variable binning" with newer ABI data collection versions.

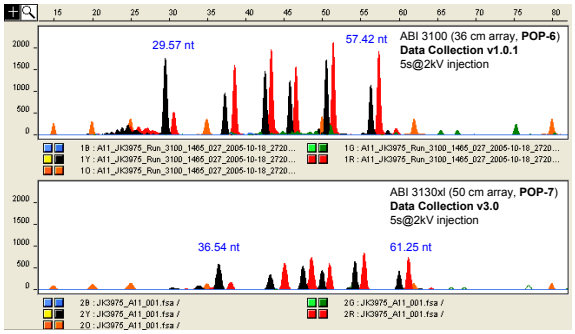
Difference in the STR allele relative mobilities (peak positions) are from using POP-6 vs. POP-7.

POP-7 Observations

- POP-7 is included in the 3130xl upgrade package
- Shorter run times compared to POP-6
- Similar resolution to POP-6
- Slightly lower precision compared to POP-6
- Mobility differences relative to POP-6, particularly for smaller DNA fragments used in SNaPshot assays

Comparison of SNaPshot Data

(Spectral Calibration, filter E5)

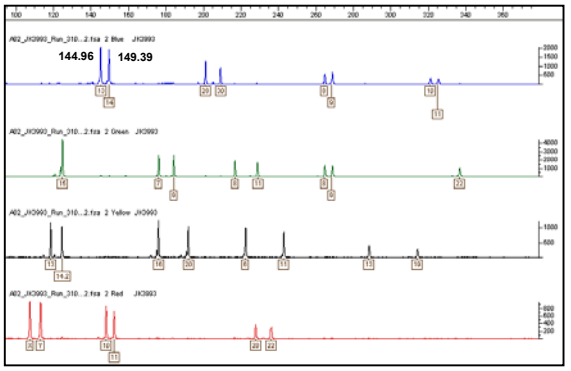


Due to drastic size differences with POP-7, we decided to continue using POP-6 for SNaPshot assays so we wouldn't have to rewrite the Genotyper macros

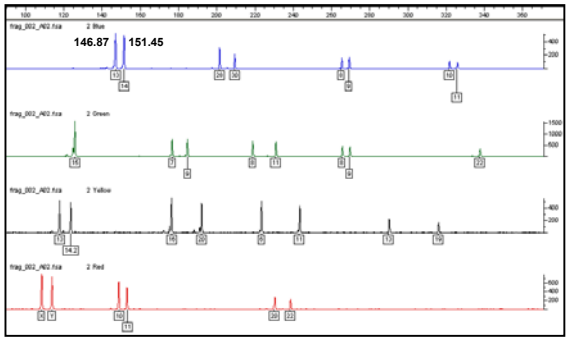
Results Obtained

- Precision
  - POP-4
  - POP-6
  - POP-7
- Sensitivity
- Concordance – same (correct) calls obtained in all instances

ABI 3100 Identifiler Data

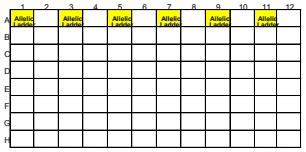


ABI 3130xl – Complete Concordance



Average Standard Deviation per Locus
ABI 3100 (POP-4) vs ABI 3130xl (POP-4)

Plate Template with Allelic Ladders



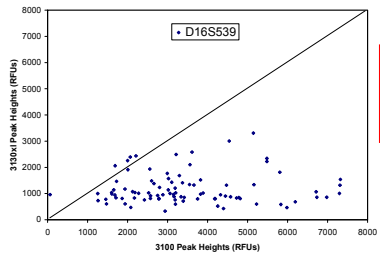
Same injection parameters

N = 6 allelic ladders

Allele	Avg St. Dev. 3100	Avg St. Dev. 3130xl
Amel	0.03	0.10
D13S317	0.03	0.12
D7S820	0.05	0.19
D2S1338	0.04	0.16
D21S11	0.06	0.24
D16S539	0.04	0.12
D18S51	0.05	0.23
CSF1PO	0.05	0.12
FGA	0.04	0.10

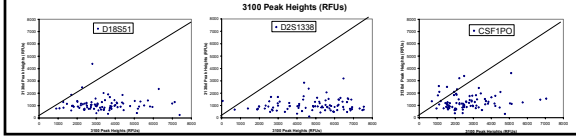
The st. dev. for the ladders run on the 3130xl are larger than those run on the 3100

Peak Height Comparisons
ABI 3100 vs ABI 3130xl

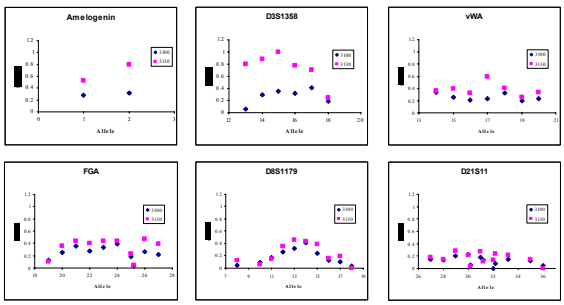


Our 3100 is more sensitive under equal injection parameters

N = 90 samples



Precision Comparison of Allele Sizing between ABI 3100 (POP-6) and ABI 3130xl (POP-7)



Conclusions and Recommendations for 3130xl Internal Validation

- We observed fully concordant allele calls between our original 3100 and 3130xl upgrade
- The 3130xl is more time efficient when changing polymer
- We experienced lower sensitivity using the same injection parameters with the 3130xl as compared to the 3100
  - Injections should be optimized based on empirical data for each instrument
- Run at least 50 unique samples on both instruments under the same conditions and analysis parameters to meet the SWGDAM Revised Validation Guidelines
  - Can be performed very quickly (less than a day)

Future Studies

- Correlation of room temperature with precision studies
- Examination of more samples using the 3130xl instrument
- Comparison of color balance between data collection software versions
- Further examination of POP-7

Acknowledgments

Funding from interagency agreement 2003-IJ-R-029 between NIJ and the NIST Office of Law Enforcement Standards

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Now at AFDIL

Our team publications and presentations are available at:
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

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