Status Update on ABI 3500 Open Letter

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SWGDAM

July 13, 2011

Open Letter Background and Response

- Last fall we were asked by NIJ to investigate the ABI 3500 Genetic Analyzer, which is the newest instrument from Applied Biosystems used for forensic (and clinical) DNA sequencing and genotyping. NIJ provided part of the funds used to purchase an instrument, which we obtained in November 2010.

- The federal government (through NIJ funds from Congress) is being requested by states to purchase the ABI 3500. We have conducted some validation studies and explored cost analysis at NIJ's request (see presentation given December 7, 2010 here at NIST: http://www.cstl.nist.gov/biotech/strbase/pub_pres/ForensicsNIST-ABI3500.pdf).

- I gave a presentation at the FBI's SWGDAM (Scientific Working Group on DNA Analysis Methods) meeting in January 2011 on what we have found with the ABI 3500. **A number of people requested that I follow up and something be written by myself or SWGDAM to Applied Biosystems to express our concerns.**

Background (cont)

- Jeff Sailus (ABI) visited NIST January 28 and provided helpful information on technical questions

- I was busy finishing my book and so did not write the letter until early March 2011. After getting approval of SWGDAM chair, I sent the letter to SWGDAM membership on March 14. I also sent the letter to about 900 forensic DNA scientists around the world invited their support. **Support for the letter has been overwhelming and 101 people agreed to sign on.** (a number of other people who found out about the letter after it had been sent to ABI have also expressed support)

- I sent the open letter to Applied Biosystems on March 31.

- ABI sent out a FAQ sheet (completed March 30) to customers with their responses.

- A letter addressed to me was sent April 4 from Lenny Klevan (ABI HID president). It has also been provided to many of their customers.

Background (cont)

- I gave a presentation at the ENFSI meeting in Brussels on April 8 discussing what had happened to that point.

- Because I was traveling and out of the office much of the time since early April, I was unable to connect with ABI until April 25 when I spoke with Lenny Klevan, the president of Applied Biosystems by phone for 45 minutes. He expressed desire to work with NIST to improve communication with the community and invited me to come to their headquarters in Foster City, CA or for several ABI scientists to come here to NIST to talk to us about the issues.

- Melissa Kotkin (ABI Field Application Specialist), who was already scheduled to come, came April 26 to work with Erica Butts and Becky Hill on our ABI 3500.

- **Lisa Calandro and Lori Hennessey (ABI) visited NIST May 11, 2011**

- **The bottom line is that communication has been improved with the forensic DNA community and that the open letter started this process.**

Open Letter to Applied Biosystems on Concerns with ABI 3500

- **3/14/11 - emailed ~900 forensic DNA scientists** (SWGDAM, forens-dna, ENFSI, EDNAP) inviting them to sign onto a letter that will be sent to Applied Biosystems expressing concern with ABI 3500
- **Very positive response with 101 who agreed to sign the letter**
- Letter was sent March 31 to the president of ABI and scientists involved with the ABI 3500
- **Community being notified of ABI's response**

Concerns Expressed in Open Letter

- RFID tags
- New .hid file structure requires new software
- Short shelf life of reagents – would like to see data for expiration times

Hopefully a change will result…

A desire for greater communication with the community – the 3500 FAQ sheet is a good start but does not directly address all of the concerns raised

Reagent Shelf Life Data

- What is the normal expiration data for buffer, polymer, arrays, and kits?
- What data is this based on?
- **They do not have any data (business decision)**

What was learned from the May 11 visit…

- RFID over-ride is possible (their R&D lab has instrument that can use “expired” reagents) – they are “considering” making this option available
- New software is required for 3500 .hid or .fsa files due to new file structure
- They do not have ANY data to support short shelf life of 3500 reagents
  - A business decision to set hard stops to keep labs from having failures that lead to ABI having to replace arrays
- ABI 31xx instruments have DEPRESSED signal (i.e., should have a lower analytical threshold)
- Normalization is not well worked out by ABI or really understood (although this has been a major selling point for the 3500)
- ABI was shocked that there were concerns with some of the feedback

Cost for the Forensic DNA Community to Switch from ABI 3100s to 3500s

1. **Instrument up-front cost**
   - Within the U.S. funding requests will likely come from federal grants

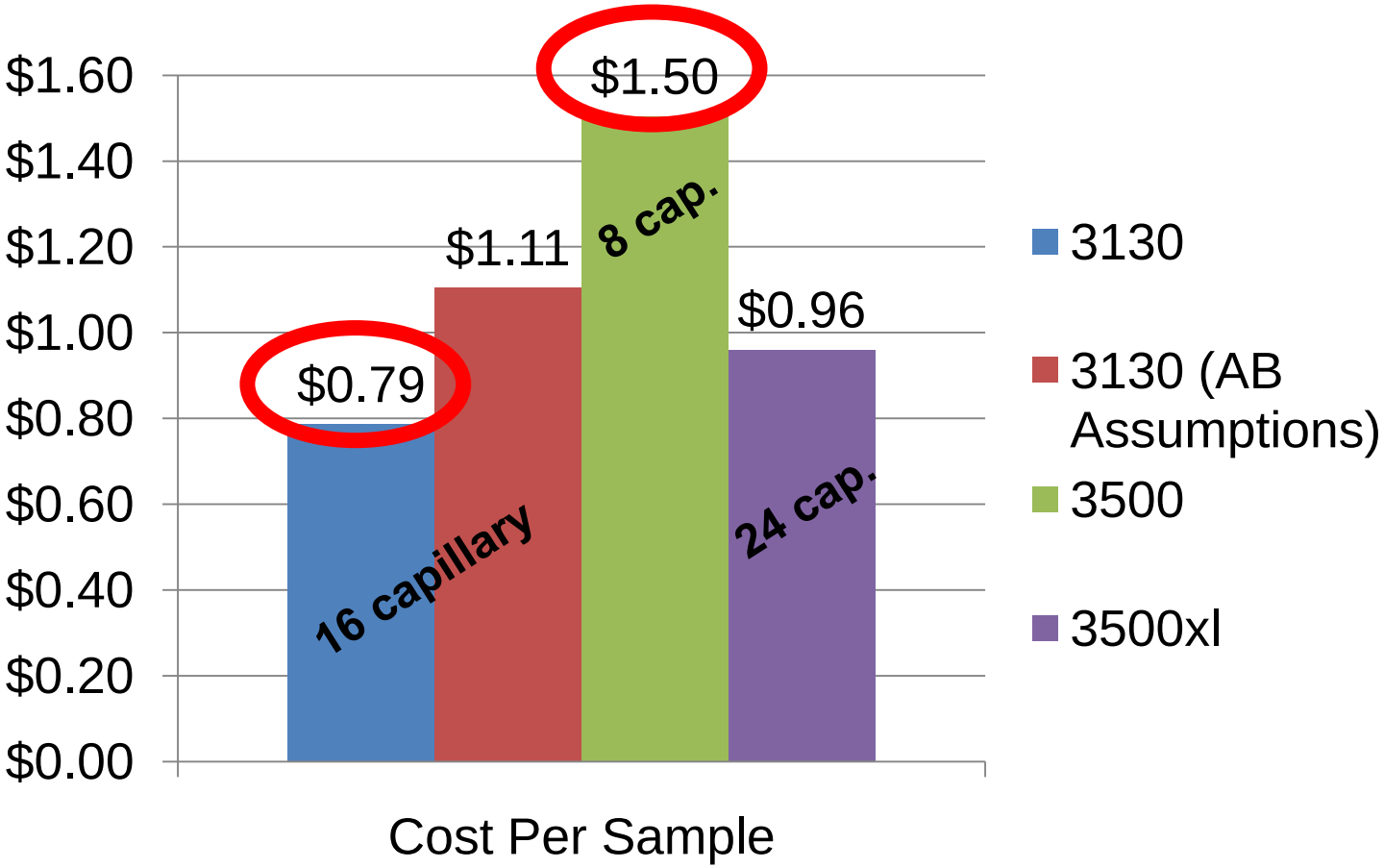
2. **New software purchase**
   - Will likely be requested from federal grant funds (NIJ)
   - new .hid file format will not work on current software (GMIDv3.2)
   - 3500 will not create .fsa files with 36cm arrays (HID applications)

3. **Validation time & expense**
   - Relative fluorescent scales are completely different…

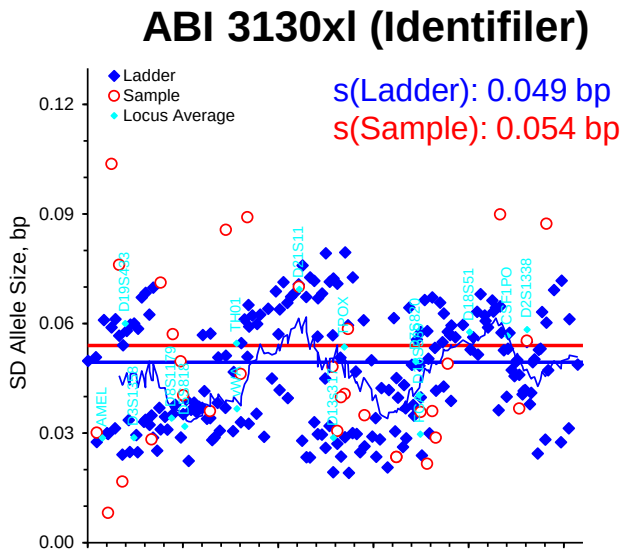
4. **Operational cost**
   - ABI claims that the running costs are equivalent to 3130s…

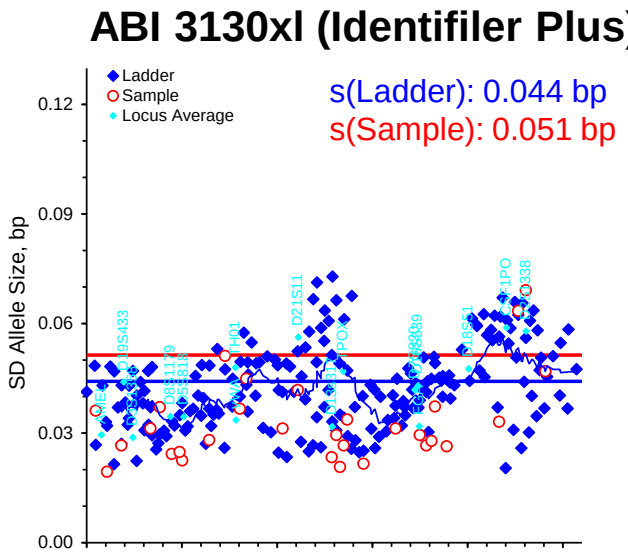
NIST Calculated Cost per Sample for ABI 3130xl vs. 3500 and 3500xl Reagents

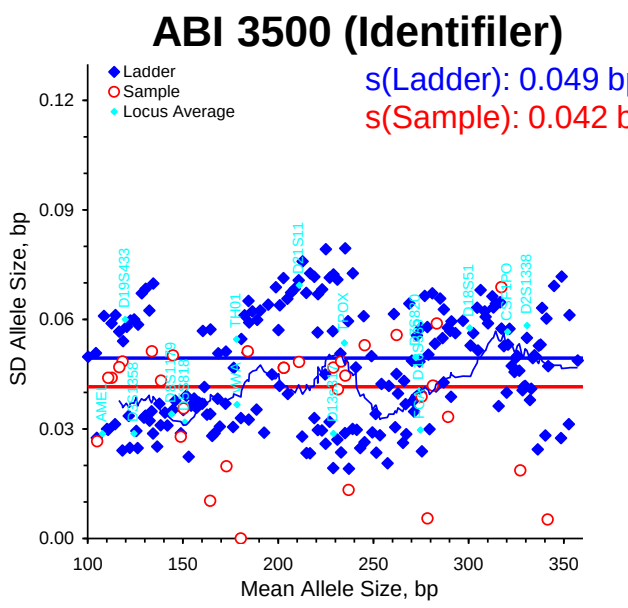
Running two plates per day (10 plates per week)

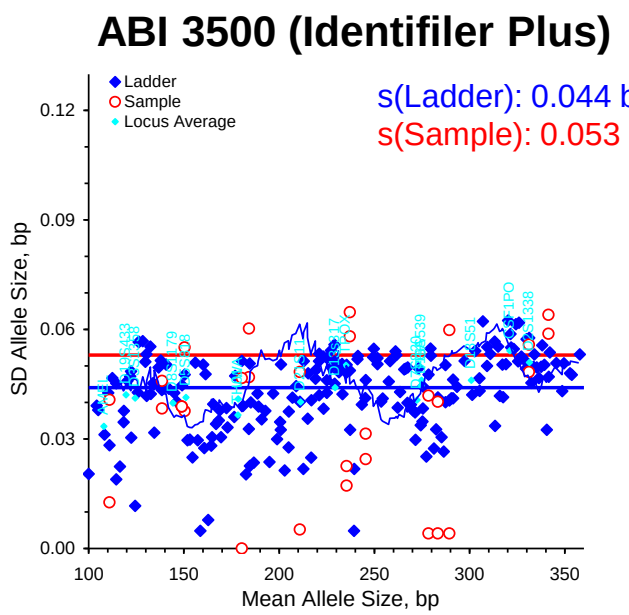


Precision Studies at NIST









No significant difference between the 3130*xl* and 3500 for precision

n=16 ladders
n=6 samples

A Sampling of Feedback I Have Received…

- **People did not just sign the letter but many have an opinion about the issues or concern about ABI customer support (I have received >100 emails – often with some very strong thoughts)**

- "I think that the AB3500 related issues most likely represent the beginning of a sea of problems, against which every independent lab must take arms. **It is not up to the manufacturer of a machine to decide the basic procedures of a lab - it is up to the lab**" (4/29/11)

- "I greatly appreciate your advocacy on behalf of our community. **Hopefully we will be heard**." (4/1/11)

Do the responders own 3500 instruments?

- "**We have the ABI3500 in our lab since October 2009** and use is for non-human biological trace analyses (both fragment analysis and sequencing). **We share the concerns you mention in the letter**." (3/28/11)

- "I would like to sign the letter- **We are currently in the process of validating a database-dedicated 3500 instrument** and agree with/support your suggestions." (3/18/11)

- "Thanks a lot for the initiative. **Although we recently bought two of the instruments and many licenses of the ID-X software I fully support your letter**. So please add my name to the signatory list." (3/18/11)

General Support for Open Letter and Concern for Economic Issues

- “I strongly believe that your initiative is excellent and actually we (i.e. the forensics community) should have done this long time ago especially in situations where we may have certain issues being “monopolized” in the market. Once I argued, during one of our ENFSI DNA WG meetings, that if we are not careful about certain things and while we are all trying to standardize our procedures etc we may find this difficult to accomplish because of non-scientific issues such as economic etc.” (3/21/11)

A Recent Example of Feedback

Sent: Monday, May 02, 2011 10:13 AM

Hi John,

Thank-you for the update as well as your ongoing efforts to support our community vis-à-vis Applied Biosystems instrumentation.

Our AB representative Dawn Waltman was here for a visit Monday. She followed up regarding your letter and AB response and **I advised their response had "missed the mark".** As a result she has scheduled a teleconference with her supervisor later this week. I will be echoing the same sentiments you have included in your letter and your follow up conversations with Lenny Klevan.

Regards,
Ray

Ray Wickenheiser
Laboratory Director
Montgomery County Police Crime Laboratory

Response from Dr. Robin Cotton
(shared with her permission)

Sent: Saturday, April 30, 2011 10:39 AM

Dear John,

Thank you for the information and the inclusion of the letter from Dr. Klevan. It is clear that **Dr. Klevan does not consider the substantial time and expense which will be required for each forensic laboratory for instrument and software validation.**

The other point which I feel is significant is the need for the additional software purchase. Since he states that the new software is compatible with .fsa files, I think **the company should make a software exchange available at low cost for any lab purchasing the 3500 instrument**. Many commercially available software companies make new versions available at reduced costs to individuals or groups already running an earlier versions. **Because of the increased number of technical changes the 3500 presents, the validation data may be more extensive than was required for previous instrument change-over and thus the validation time and cost to each laboratory will also be increased.**

Response from Dr. Robin Cotton
(shared with her permission)

It would also be relevant to ask Dr. Klevan to provide figures for the number of current 3500 users without the inclusion of paternity testing laboratories which are all commercial operations. While I am an advocate for private laboratories (both forensic and paternity), these facilities have the option to raise prices and accommodate the need for increased validation time and expense in other ways that do not require federal or other government support.

Additionally, in the Biomedical Forensic Science Masters program here at BU, we feel it is important to teach our students using current instrumentation and techniques. **Introduction of this new instrument will affect many forensic science teaching institutions, both undergraduate and graduate, as well as all current forensic DNA testing laboratories.** These institutions have significantly less access to NIJ funding for large equipment and software than the operating forensic DNA laboratories. Thus **the effect of changes reach into the educational institutions as well.**

Regards,
Robin W. Cotton, Ph.D.
Boston University

ABI Released Their Developmental Validation Study Last Friday

User Bulletin

Applied Biosystems 3500/3500xL Genetic Analyzer

June 2011

SUBJECT: **Protocols for the analysis of AmpFℓSTR® PCR Amplification Kit PCR products and validation summary**

This user bulletin describes protocols for processing AmpFℓSTR® PCR Amplification kit products on the Applied Biosystems 3500 Series Genetic Analyzers, using 3500 Data Collection software Version 1.0 and GeneMapper® *ID-X* Software Version 1.2 or later. In addition, comprehensive information regarding the validation of the 3500 Series Genetic Analyzers for use with the AmpFℓSTR® Kits is also included. This user bulletin is intended to be used in conjunction with related 3500 documentation. References to relevant supplemental documentation are provided where appropriate.

NIST Validation Studies Ongoing

- Erica Butts (MAAFS meeting, May 27, 2011)
  - http://www.cstl.nist.gov/biotech/strbase/pub_pres/MAAFS2011_3500validation.pdf
- Erica Butts (ABI Roadshow, July 12, 2011)
  - Will be posted on STRBase soon
- Erica Butts (ISFG talk, Aug 29 - Sept 2, 2011)

Going Forward…

- We like the data coming from the ABI 3500.
- How much further should we push on getting “expired” reagent data, RFIDs turned off, etc.?