


Forensics @ NIST
December 7, 2010 – Gaithersburg, MD

ABI 3500 Studies

Carolyn R. (Becky) Hill
John M. Butler



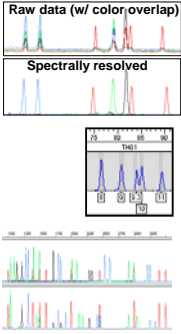
Overview

- Requirements for STR typing instruments
- History of instrumentation used by forensic DNA community
 - ABI 310 → ABI 3100 → ABI 3130xl → ABI 3500
- Instrumentation and experience at NIST
- Cost for the community of moving to 3500
 - Advantages? Disadvantages?

Analytical Requirements for STR Typing

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

- Fluorescent dyes must be **spectrally resolved** in order to distinguish different dye labels on PCR products
- PCR products must be **spatially resolved** – desirable to have single base resolution out to >350 bp in order to distinguish variant alleles
- High **run-to-run precision** – an internal sizing standard is used to calibrate each run in order to compare data over time

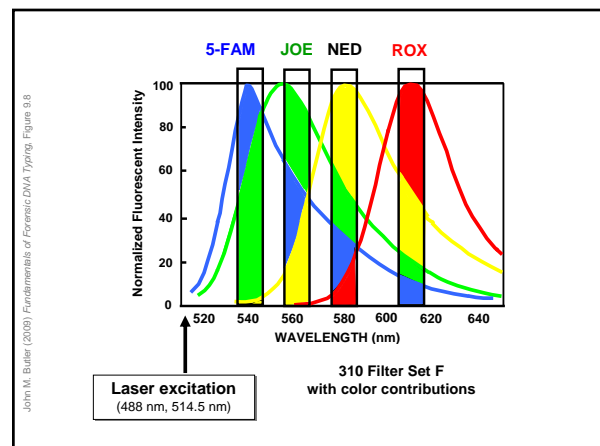
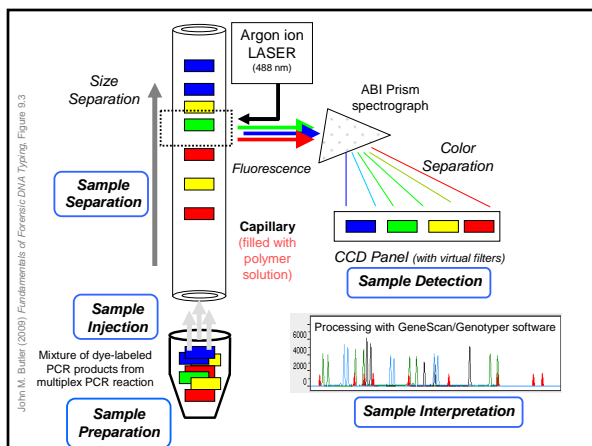


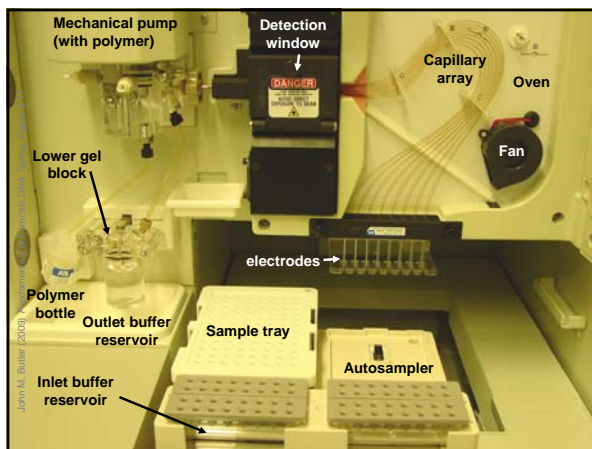
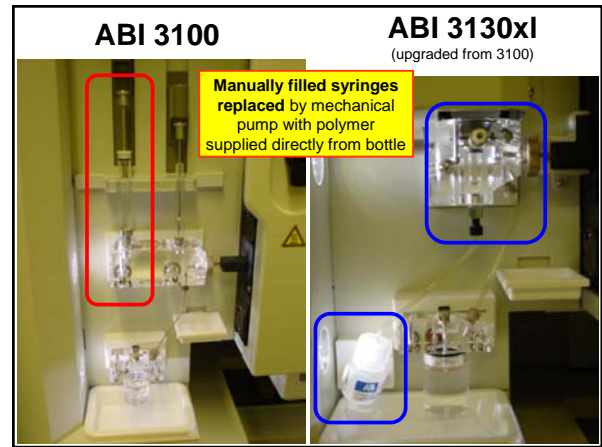
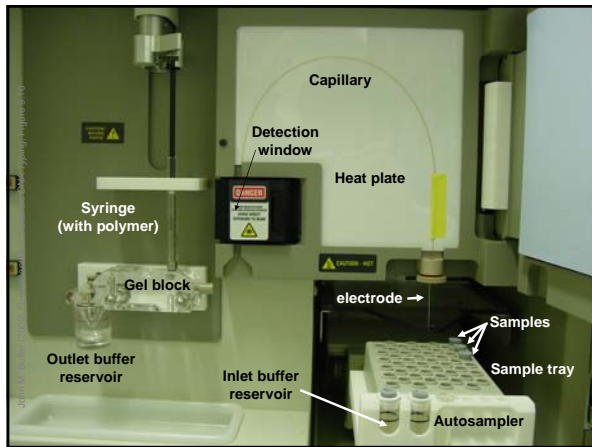
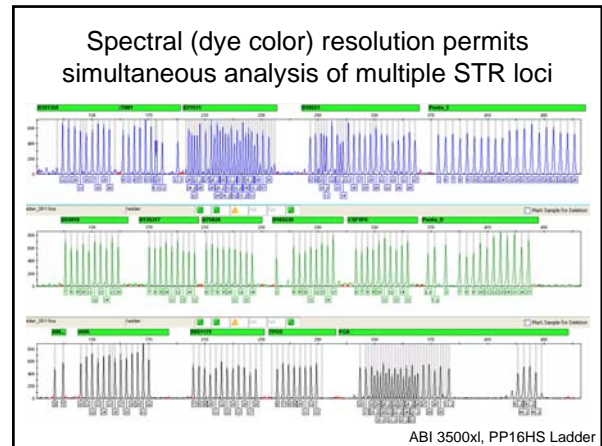
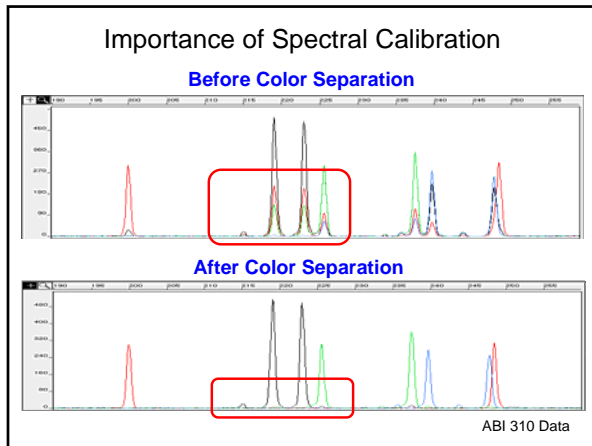
Capillary Electrophoresis (CE)

Steps Involved

- Collection
- Sample Storage
- Characterization
- Extraction
- Quantitation
- Amplification
- Separation/
Detection
- Data Interpretation
- Statistical Interpretation


- Since its introduction in 1995, the forensic DNA community has been using capillary electrophoresis instrumentation from Applied Biosystems to perform DNA separations, sizing, and genotyping
- With the conversion of the Virginia Department of Forensic Sciences from gels to CE in 2009, there is **now 100% dependence of the community on Applied Biosystems** instrumentation for STR typing



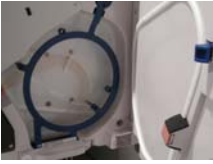


Details of the new ABI 3500


No lower pump block
(less polymer waste)




Improved sealing for better temperature control
(improved precision?)




Reagents prepackaged with RFID tags



Better seal around the detector



Array and Reagents Should Be Easier to Replace and the Software Easier to Use






ABI Genetic Analyzer Usage at NIST

(All instruments were purchased using NIJ funds)

ABI 310 Single capillary

- 1st was purchased in 1996 as Mac (A230, now B233)
- 2nd was purchased in June 2002 as NT (B261)

ABI 3100 → 3130xl 16 capillaries

- 1st purchased in April 2001 as ABI 3100
 - upgraded to 3130xl in Sept 2005
 - Located in a different room (A230, now B219)
- 2nd purchased in June 2002 as ABI 3100
 - Original data collection (v1.0.1) software retained
 - updated to 3130xl in Jan 2007 (B219, now B261)

ABI 3500 8 capillaries

- Purchased Nov 2010 (B233)

DNA Samples Run at NIST

we have processed >100,000 samples (from 1996-present)

- **STR kits**
 - Identifiler, PP16, PP16HS, Identifiler Plus, Identifiler Direct, Profiler Plus, Cofiler, SGM Plus, ESI/ESX 17, SE33 monoplex
- **Research & development on new assays**
 - **STRs:** Y-STR 20plex, MeowPlex, miniSTRs, 26plex
 - **SNPs:** SNaPshot assays: mtDNA (one 10plex), Y-SNPs (four 6plexes), Orchid SNPs (twelve 6plexes), ancestry SNPs (two 12plexes), SNPforID (one 29plex), SNPplex (one 48plex)
- **DNA sequencing**
 - Variant allele sequencing

We have a unique breadth and depth of experience with these instruments...

3500xl – 24 Capillary Array

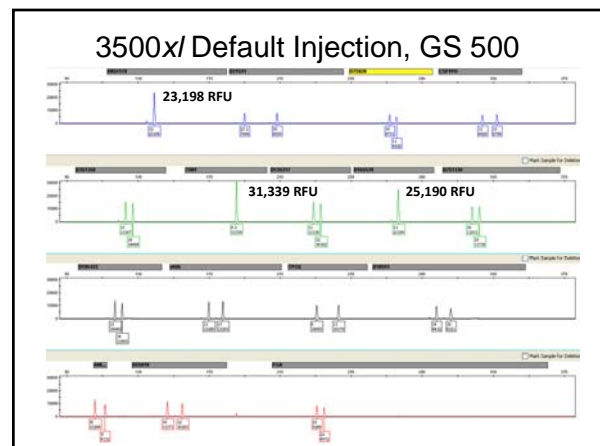
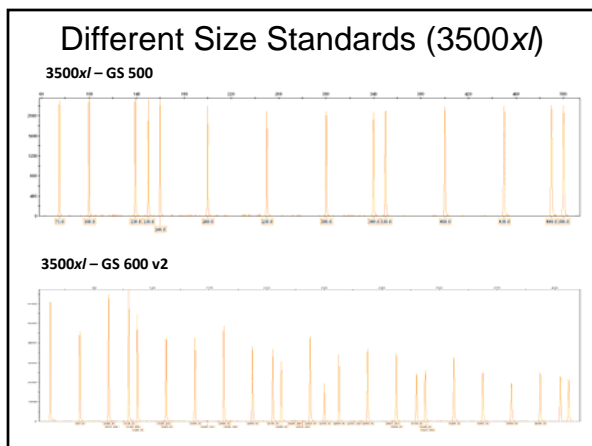
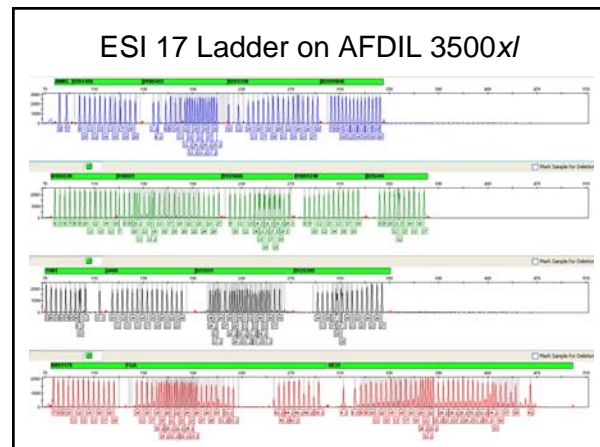
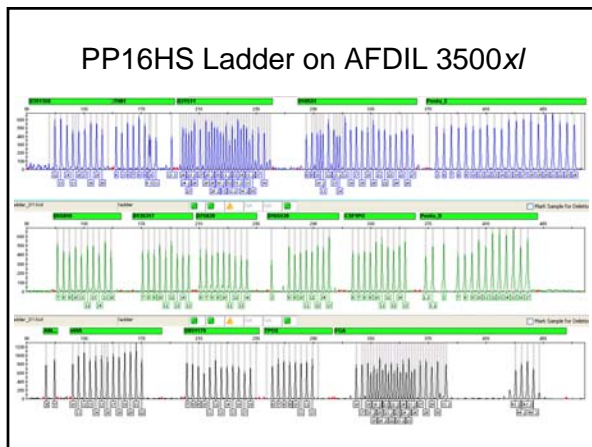
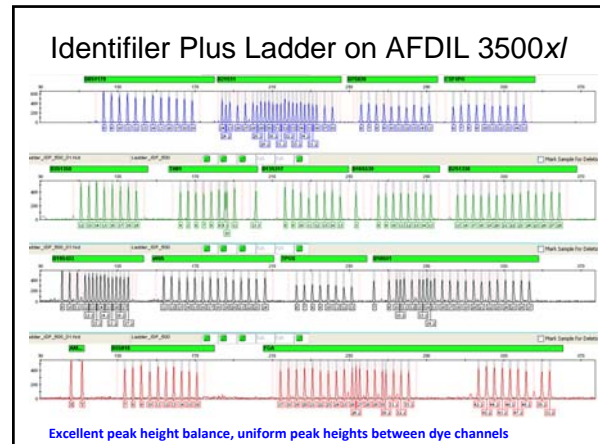


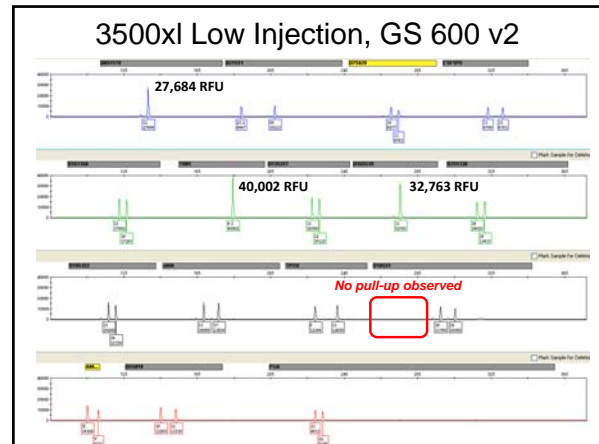
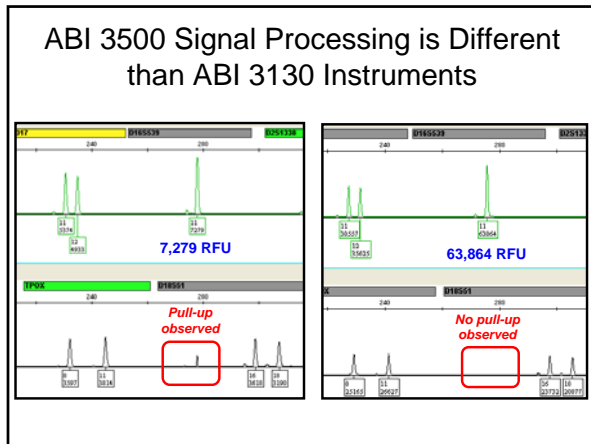
ABI 3500xl Testing at AFDIL

- **23 different samples (69 total)** were amplified using **Identifiler Plus, PP16HS, and ESI17**
 - Compared on NIST 3130xl and AFDIL 3500xl
- NIST GeneMapperID-X v1.1 upgraded to v1.2 in order to be able to read .hid files
 - Data files were supplied to SoftGenetics who has now developed GeneMarkerHID v1.95 to read .hid files
- **Promega products (PP16HS, ESI17) were tested** to confirm that they work on ABI 3500 series instruments – they do!

Experimental Design

- Identifiler Plus kit (full reactions)
 - 0.5 ng DNA, 28 cycles
 - GS 500 LIZ size standard
 - GS 600 LIZ v2 size standard (normalization)
- PowerPlex 16 HS (full reactions)
 - 0.5 ng DNA, 30 cycles
 - ILS 600 size standard
- PowerPlex ESI 17 23 samples + 1 Allelic Ladder
 - 0.5 ng DNA, 30 cycles
 - ILS 500 Orange size standard
- 3500x/ data compared to 3130x/ data
 - Default injection of 1.2 kV, 24 sec (3500x/)
 - Low injection of 1.2 kV, 10 sec (3500x/)
 - Default injection of 3 kV, 10 sec (3130x/)





- ### Summary of Data Observed
- The RFU scale for the 3500xl is different than the 3130xl (30000 RFU vs 8000 RFU).
 - The 3500xl instrument is more sensitive than the 3130xl – can adjust the injection time and voltage.
 - Identifiler Plus profiles on the 3500xl are well balanced (inter- and intra-locus and between dye channels).
 - The GS 600 v2 size standard is for the normalization of data between different instruments in the lab; the data is comparable to data using the GS 500 size standard.

Impact on the Community

Increased cost and increased backlogs?

ABI Genetic Analyzer	Years sold for forensic DNA	Number of Capillaries	Laser	Polymer delivery	Other features
373 (gel system)	1992-1997	--	Ar+	--	PMTs and color filter wheel for detection
377 (gel system)	1995-2003	--	Ar+	--	CCD camera
310	1995-	1	10 mW Ar+ (488/514nm)	syringe	Mac & Windows NT (later)
3100	2001-2007	16	25 mW Ar+ (488/514nm)	syringe	5-dye capability; variable binning to improve red channel; 220V power required
3100-Avant	2002-2007	4	25 mW Ar+ (488/514nm)	syringe	
3130	2003-2011	4	25 mW Ar+ (488/514nm)	pump	
3130xl	2003-2011	16	25 mW Ar+ (488/514nm)	pump	
3500	2010-	8	3500 features:		
3500xl	2010-	24	110V power; smaller footprint; new pump; RFID-tagged reagents ; 505 nm diode laser; improved temperature control; normalization possible between instruments; 6-dye capability		
3700	1997-2004	96		syringe	
3730	2005-	48		pump	
3730xl	2005-	96		pump	

ABI 3500 Evaluation

New Features of the ABI 3500 CE

- an improved polymer delivery pump design,
- ready-to-use consumables and containers,
- Radio Frequency Identification (RFID) consumable tracking,
- quality control software features for rapid identification and re-injection of failed samples,
- increased throughput,
- new laser technology,
- reduced power requirements,
- peak height normalization,
- intuitive user software, and integrated primary analysis software,
- improved peak height uniformity across capillaries, runs and instruments
- **6-dye channel capability**

- *NIJ will likely be requested to use federal grants to supply state and local labs with this new instrument*
- *It needs to be assessed for advantages and costs*




DNA Community Moving to ABI 3500s

<p>Advantages</p> <ul style="list-style-type: none"> Smaller footprint and 110V power requirement Better polymer delivery and temperature control <ul style="list-style-type: none"> Improved success rates? New capabilities <ul style="list-style-type: none"> between instrument normalization 6-dye detection (bigger kits with more loci) Simpler software 	<p>Disadvantages</p> <ul style="list-style-type: none"> Up-front cost of new instruments <ul style="list-style-type: none"> Federal government (NIJ) will likely be expected to foot the bill Generates .hid files <ul style="list-style-type: none"> Requires new analysis software Validation down-time <ul style="list-style-type: none"> New RFU thresholds Higher per run cost with RFID tags & limited expiration <ul style="list-style-type: none"> many labs cannot purchase reagents rapidly throughout the year Creating technicians not scientists <ul style="list-style-type: none"> Plug and play approach leading to loss of understanding for process Less flexible (impacts research with it)
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Cost for the Forensic DNA Community to Switch from ABI 3100s to 3500s

- Instrument up-front cost**
 - Will likely be requested from federal grant funds (NIJ)
- 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 .f5a files with 36cm arrays (HID applications)
- Validation time & expense**
 - Relative fluorescent scales are completely different...
- Operational cost**
 - ABI claims that the running costs are equivalent to 3130s...

Consumable Costs for the ABI 3500

<p>POP polymer pack with RFID tag</p>  <p>\$180 (384 samples) \$455 (960 samples)</p>	<p>Buffer pack with RFID tag</p>  <p>\$60 (\$25+\$35)</p>	<p>8-capillary array</p>  <p>\$1200 (160 injections)</p>
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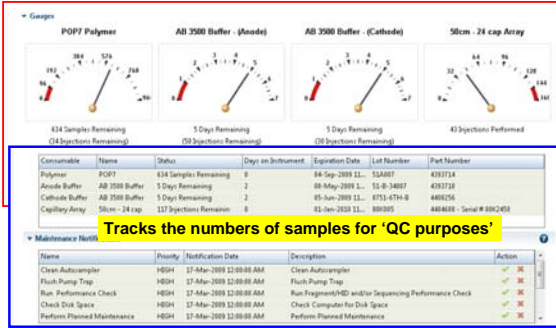
"Expires" after 1-week on the instrument

Thus, if you run 1 sample or 960 samples (or 384) in that week, the consumable cost will be the same...

Likely Cost Increase... and Backlog Increase?

- ABI 3500 reagents are RFID-tagged and made to work under very limited time windows (e.g., 1 week expiration for the polymer)
- If a lab is not running at full capacity, reagents will expire and add to the true cost of performing forensic DNA testing (i.e., can be a similar total cost whether running a few or a few hundred samples)
- Casework **throughput efficiencies are best when small batches are run frequently – to save money, will labs store samples to amass enough for a busy week of running samples through the 3500 instrument?**

ABI 3500 'Dash Board' Data Collection



Tracks the numbers of samples for 'QC purposes'

Consumable	Name	Status	Days on Instrument	Expiration Date	Lot Number	Part Number
Polymer	POPF	634 Samples Remaining (24 Injections Remaining)	0	04-Sep-2009 11:00 AM	SLAB07	4293714
Anode Buffer	AB 3500 Buffer	5 Days Remaining	7	08-May-2009 12:00 AM	13-B-14007	4293719
Cathode Buffer	AB 3500 Buffer	5 Days Remaining	7	05-Jun-2009 12:00 AM	1753-4794-B	4480276
Capillary Array	50cm - 24 cap	117 Injections Remaining	0	01-Jul-2009 12:00 AM	880095	4480409 - Serial # 0002458

Name	Priority	Notification Date	Description	Action
Clean Autosampler	HIGH	17-Mar-2009 12:00:00 AM	Clean Autosampler	✓ [X]
Flush Pump Trap	HIGH	17-Mar-2009 12:00:00 AM	Flush Pump Trap	✓ [X]
Run Performance Check	HIGH	17-Mar-2009 12:00:00 AM	Run Fragment/ID and/or Separating Performance Check	✓ [X]
Check Disk Space	HIGH	17-Mar-2009 12:00:00 AM	Check Computer for Disk Space	✓ [X]
Perform Planned Maintenance	HIGH	17-Mar-2009 12:00:00 AM	Perform Planned Maintenance	✓ [X]

https://www3.appliedbiosystems.com/cms/groups/portals/documents/web_content/cms_064299.jpg

Comparison to a New Car...

- Instrument cost** – like buying a new car with the latest features
- New software** – like installing a new navigational system and getting a new drivers license
- Validation time & expense** – like learning how to drive the new car which will handle differently
- Operational cost** – like paying for a more expensive high-grade gasoline

If the current car is already paid for and works fine (e.g., ABI 3130xl), then why are we going to purchase a new car (e.g., ABI 3500)?

Cost Comparison

ABI 3130xl (current)	ABI 3500 (new/future)
<ul style="list-style-type: none"> 16-capillary array <ul style="list-style-type: none"> – \$898 (for 100+ injections) POP4 polymer <ul style="list-style-type: none"> – \$468 (1760 samples) – “Expires” after 3 months – \$0.27 per sample Buffer <ul style="list-style-type: none"> – \$5 (for one week) 	<ul style="list-style-type: none"> 8-capillary array <ul style="list-style-type: none"> – \$1200 (for 160 injections) POP4 polymer <ul style="list-style-type: none"> – \$180 (384 samples) or \$455 (960 samples) – “Expires” after 1 week – \$0.47 per sample Buffer <ul style="list-style-type: none"> – \$60 (25+35) for one week

ABI 3500 Reagent Costs for 90 samples (90 samples +6 controls: allelic ladders, positive, negative)

Quantity Provided	Total Cost	
Capillary array	1 array of 8-capillaries (36 cm)	\$90
3500 POP-4 polymer	384 sample pouch (\$180)	\$45
ABC Buffer	4 pack (\$100)	\$3
CBC Buffer	4 pack (\$140)	\$4
Formamide, Hi-Di	25 mL (for \$25)	\$1
Pipet tips	960 tips for \$124	\$14
96well plate	10 plates for \$57 (not ABI)	\$12
Septa	20 septum	\$16
Identifier matrix standards DS-33	6FAM, VIC, NED, PET, LIZ	\$2
GS500 LIZ size standard	800 tests/pk	\$33
Identifer STR kit	200 tests/kit	\$1,715
	Subtotal	\$220
		\$1,715
		\$2.44 per sample
		25 µL PCR (full reaction)
		\$21.50 per sample

~90% of cost is the STR kit \$1,935

Reduced Inter-Instrument Variability Through Normalization Function

150 different ABI 310 instruments (2001 NIST interlab study)

3 different ABI 3500 instruments

More than 10 fold difference in observed signal across instruments

6-dye Detection Capability

No STR kits are available yet with 6 dyes

Enables 4 to 5 additional STR loci to be added

Summary

- The ABI 3500 and 3500xl instruments work fine for Applied Biosystems and Promega STR kits.
- The 3500 series instruments offer some improved capabilities for inter-instrument normalization and 6-dye detection. **Only time will tell how helpful these capabilities are...**
- The cost for the forensic DNA community to switch from ABI 3130xl vs ABI 3500/3500xl instruments will involve more than just the initial purchase price – **reagents are expensive.**

Questions?

becky.hill@nist.gov
301-975-4275

john.butler@nist.gov
301-975-4049

<http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm>