NIST Validation Studies on the 3500 Genetic Analyzer

O-04

https://products.appliedbiosystems.com/ab/en/US/htdocs/productMgr/images/3500_family_big.jpg

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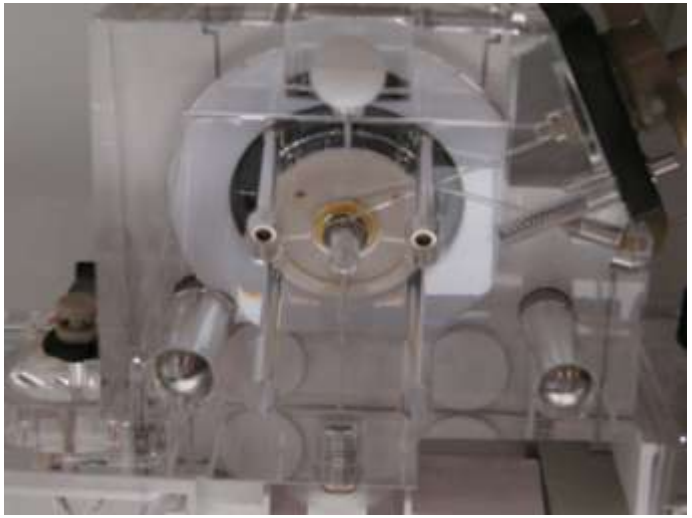
Outline

- Details of the ABI 3500 Genetic Analyzer
- Validation design and results with Identifiler and Identifiler Plus
  - Injection parameters and reaction setup
  - Precision and size standard comparison
  - Concordance and mixture evaluation
- Methodology of setting analytical and stochastic thresholds

Details of the ABI 3500

No lower pump block
(Fewer air bubbles)



Improved sealing for better temperature control

Primary Differences

	31xx Platforms	**3500 Platforms**
Laser	Argon ion (AR+) with 488/514 nm wavelength	Single-line 505 nm, solid-state, long-life laser
Power Requirement	220V	110V
File Generated	.fsa files	.hid files
Normalization	None	Instrument-to-instrument; only with AB kits
Optimal Signal Intensity	1500-3000 RFU	4x greater than 31xx platforms

What is Validation?

Section 1.1 (SWGDAM Revised Validation Guidelines) Validation is the process by which the scientific community acquires the necessary information to:

(a) Assess the ability of a procedure to obtain reliable results.

(b) Determine the conditions under which such results can be obtained.

(c) Define the limitations of the procedure.

The validation process identifies aspects of a procedure that are critical and must be carefully controlled and monitored.

Reliability, Reproducibility, Robustness

SWGDAM: FBI Laboratory's Scientific Working Group on DNA Analysis Methods

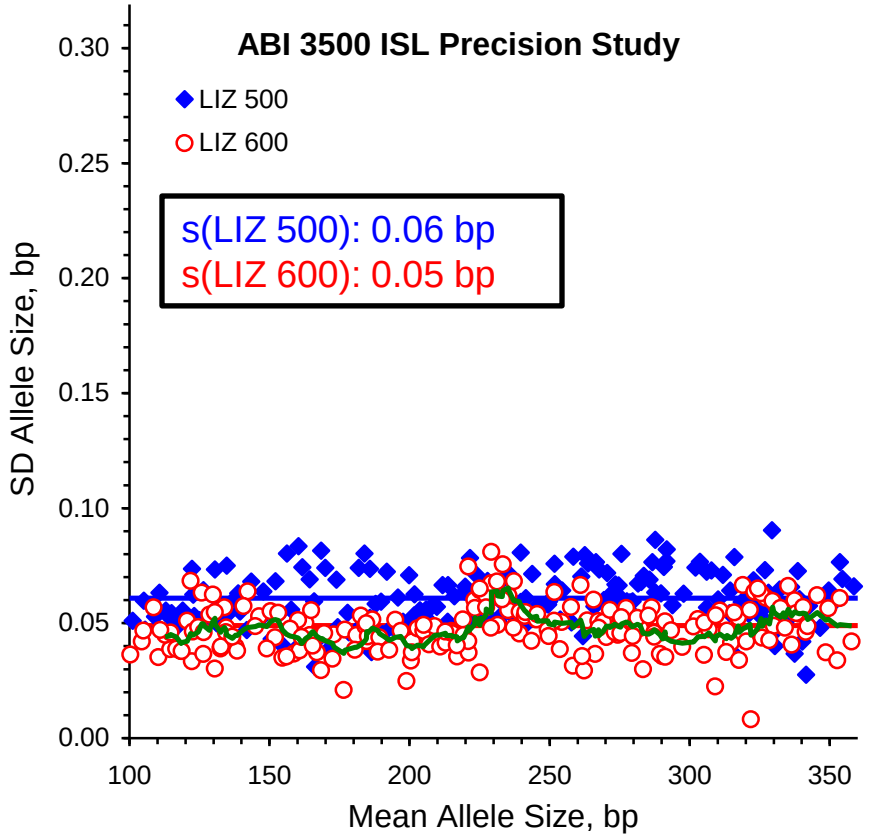
Experimental Summary

	Test	Types of Samples Used	Number Examined
Reliability	**Size Standard Comparison**	16 Allelic Ladders per size standard (LIZ 500 vs. LIZ 600 v2.0)	32
Reliability	**Injection Parameters**	3 samples heterozygous at 15 loci plus Amelogenin 1 ng DNA input	15 3 samples per injection
Reproducibility	**Precision**	Allelic Ladders	24
Reproducibility		3 samples heterozygous at all 15 loci plus Amelogenin	6
Reproducibility	**Concordance**	50 genomic DNA samples	60
Reproducibility		SRM 2391b: 10 genomic DNA samples	
Robustness	**Sensitivity**	Dilution series of 3 samples heterozygous at 15 loci plus Amelogenin	84 4 replicates of each dilution series
Robustness	**Mixtures**	Mixture dilution series of 2 samples heterozygous at 15 loci plus Amelogenin	28
		Total Number of Samples	**249**

Identical experiments for Identifiler and Identifiler Plus

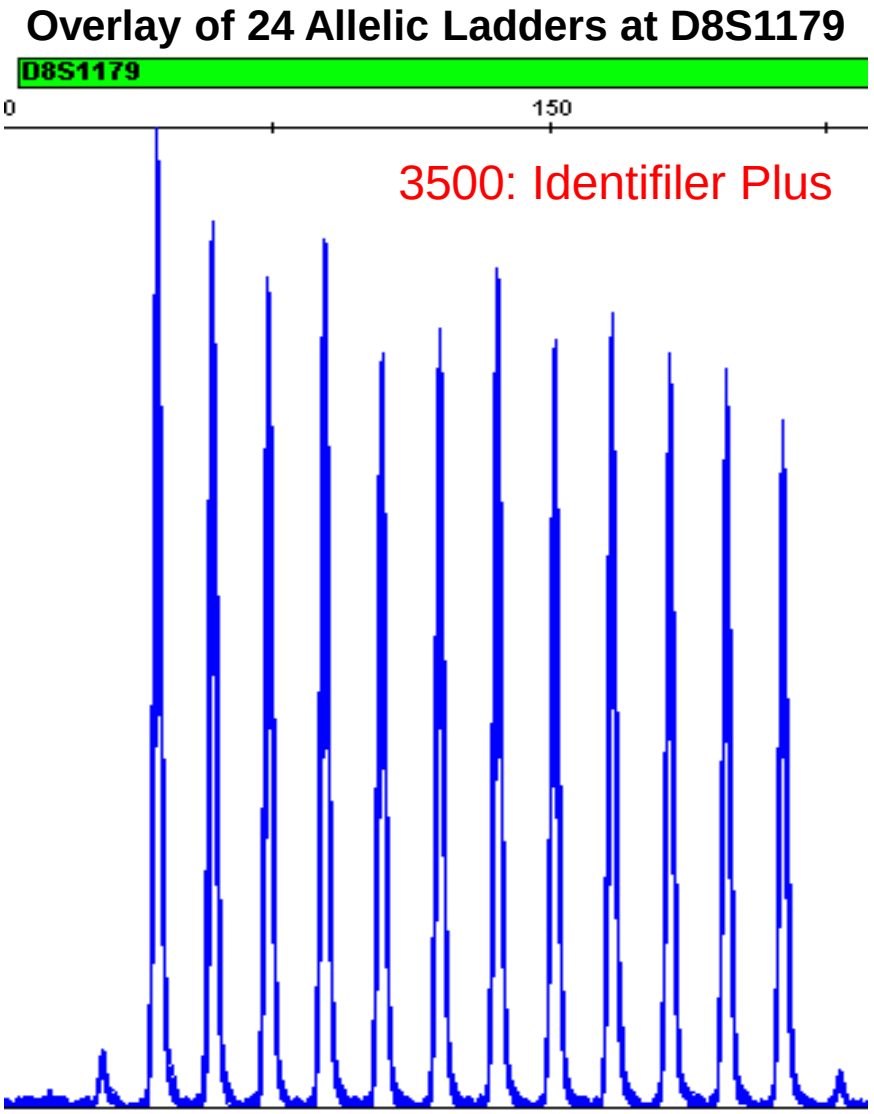
Validation Results: Reliability

- Injection parameters set for ½ PCR reactions at 28 cycles
  - Default: 1.2 kV for 15 s
  - Identifiler: 1.2 kV for 7 s
  - Identifiler Plus: 1.2 kV for 5 s
- No significant difference between the LIZ500 and LIZ600 v2.0 size standards



Validation Results: Reproducibility

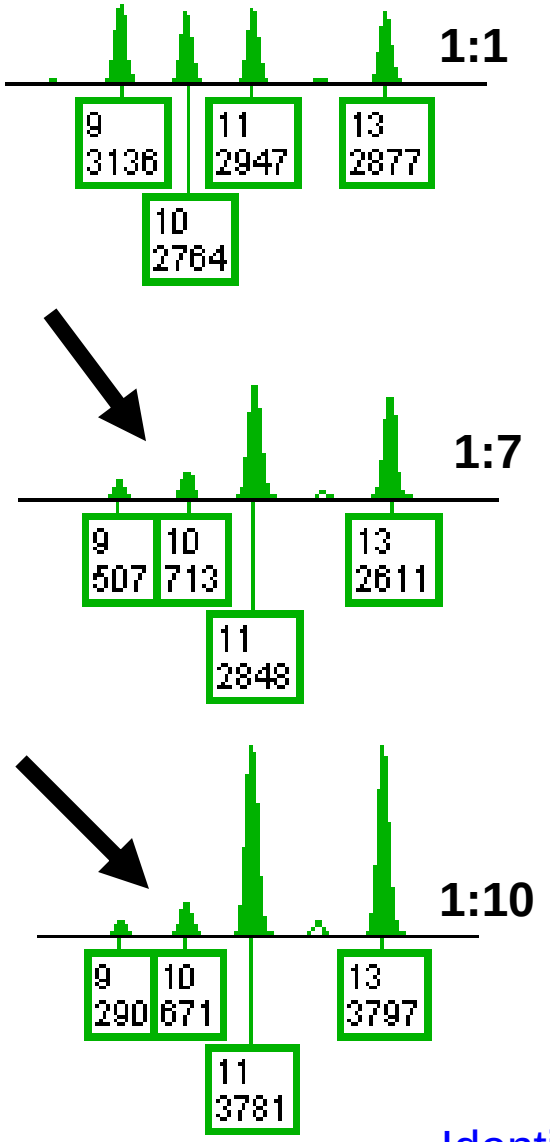
- 60 samples concordant between 3130*xl* and 3500
  - Total of 1689 alleles examined
- Precision of base pair sizing ±0.05 bp between allelic ladders and samples tested
  - No significant difference between the 3130*xl* and 3500
  - No significant difference between Identifiler and Identifiler Plus



Validation Results: Robustness

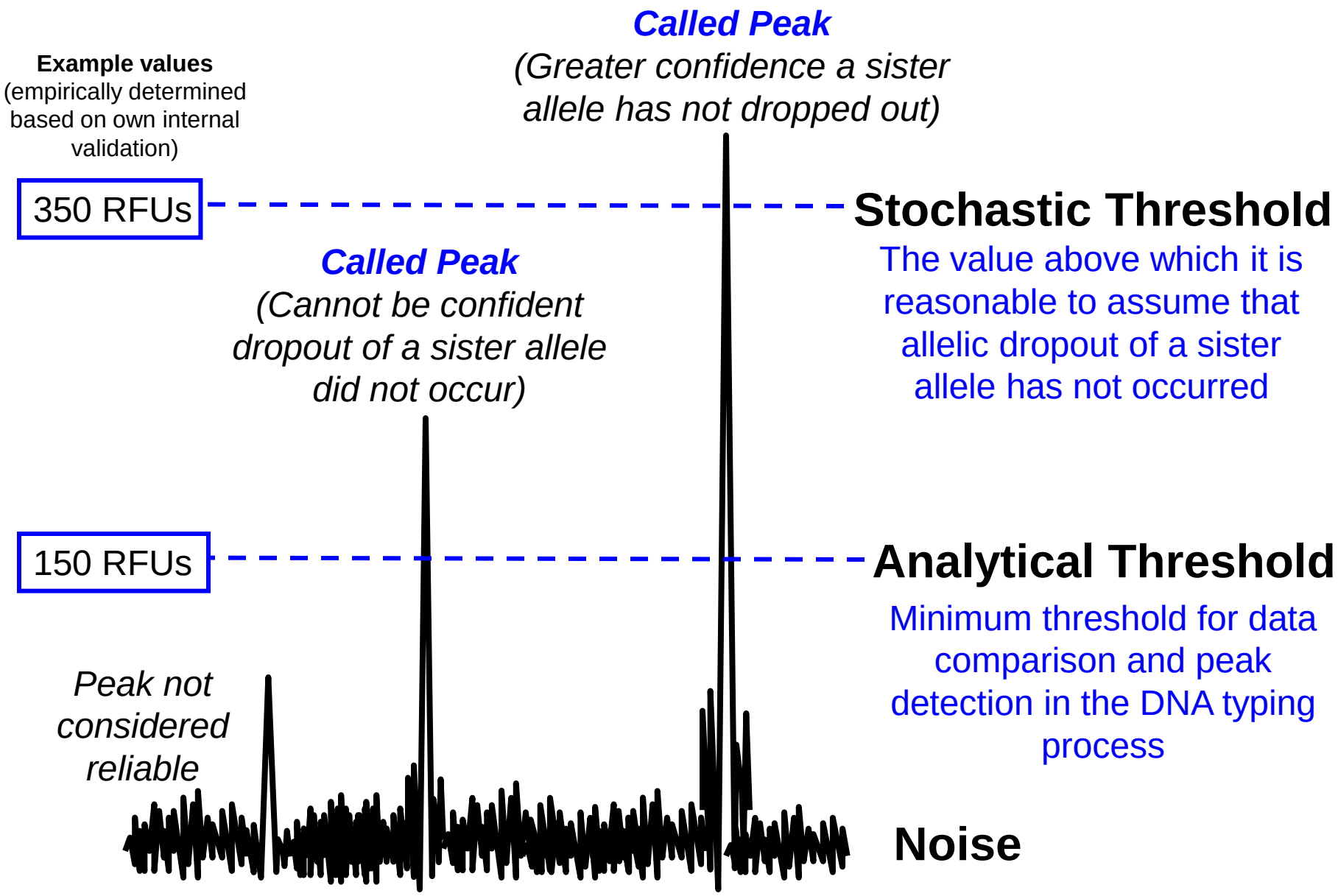
- Minor component identified correctly in a 1:10 mixture ratio

- Sensitivity data examined to set analytical and stochastic thresholds
  - Full (correct) profiles observed from 1.0 ng to 100 pg



Identifiler

Different Threshold Overview



Butler, J.M. (2009) *Fundamentals of Forensic DNA Typing*. Elsevier Academic Press: San Diego.

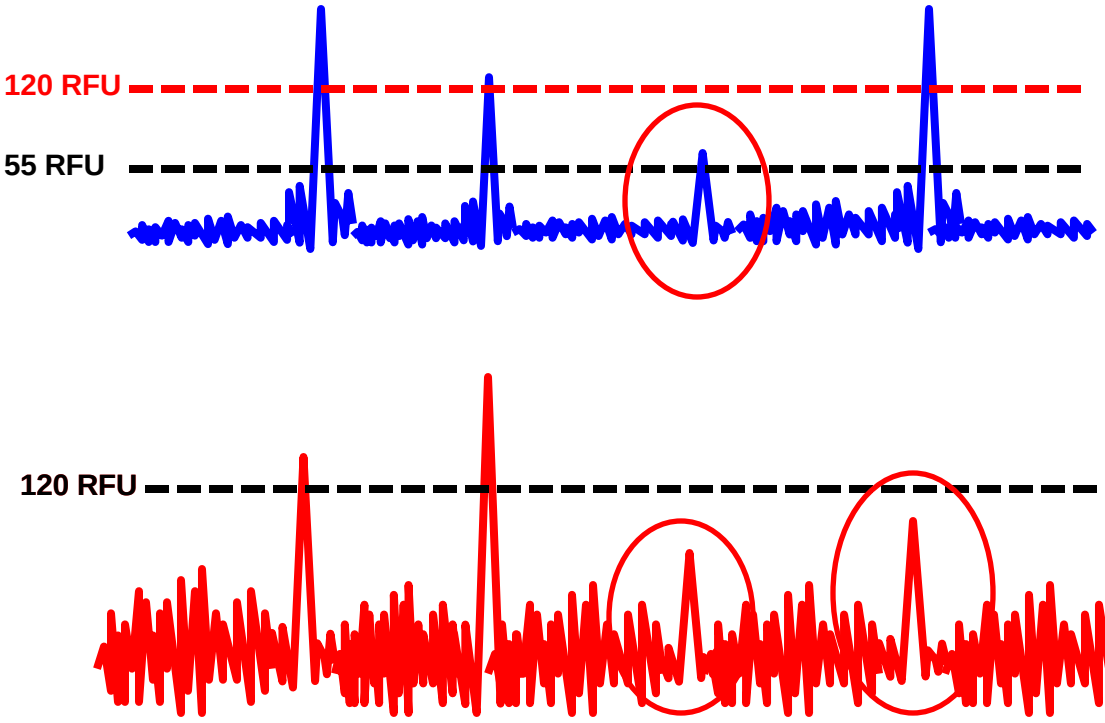
Analytical Threshold Methodology

- Baseline noise values calculated with data from the sensitivity study (DNA dilution series)
  - Threshold set at 1 RFU for all dye channels
  - Remove calls for all alleles and artifacts (stutter, n+4, pull-up, etc.)

- 4 methods to evaluate analytical thresholds calculated

- Analytical Threshold: Average RFU + (10 x Standard Deviation)

Different Thresholds



Single thresholds for all dye channels assumes all dye channels have the **same** amount of noise

Can cause data to fall below the analytical threshold and not be called

Dye-specific thresholds take into consideration that all dye channels do not have the same level of noise

Can increase the amount of data that is callable

n=84 samples

Analytical Threshold Calculation

Identifiler					
Dye Channel	**Average RFU**	**Stdev**	**Min RFU**	**Max RFU**	**Calculated Noise (RFU)**
Blue	9	8.4	1	66	93
Green	13	11.5	3	84	128
Yellow	22	11.6	4	88	138
Red	28	8.8	10	80	116

Single Threshold: 140 RFU

Dye-Specific: Rounded to nearest 5 RFU

Identifiler Plus					
Dye Channel	**Average RFU**	**Stdev**	**Min RFU**	**Max RFU**	**Calculated Noise (RFU)**
Blue	10	4.6	3	68	55
Green	16	5.6	3	78	72
Yellow	24	7.9	7	63	103
Red	31	8.9	7	81	120

Single Threshold: 120 RFU

Dye-Specific: Rounded to nearest 5 RFU

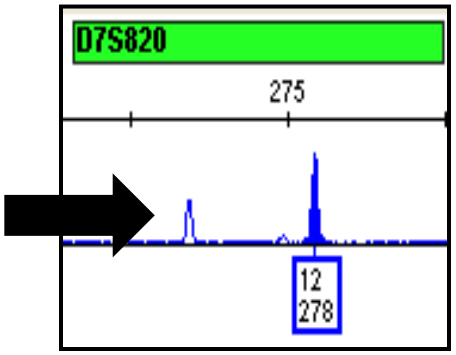
- Statistical difference was calculated between dye channels using a z-test
- Statistically each dye channel is <u>different</u> for both Identifiler and Identifiler Plus
  - Must be treated independently

n=560 alleles

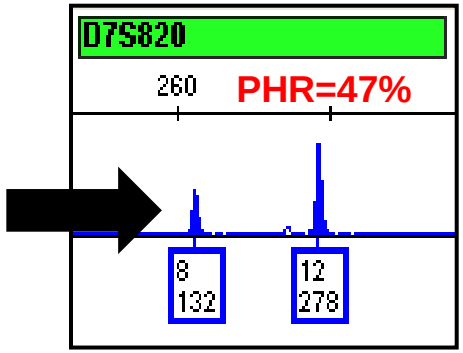
Threshold Comparison

Total of 560 alleles examined (50 pg, 30 pg, and 10 pg) where dropout was observed

Single Threshold (140 RFU)



Dye-Specific Thresholds



14.8% of the total possible allele calls were lost using a single threshold rather than using dye-specific thresholds with **Identifiler**

22.0% of the total possible allele calls were lost using a single threshold rather than using dye-specific thresholds with **Identifiler Plus**

Setting Stochastic Methodology

- Analyzed data from the sensitivity study (DNA dilution series) analyzed with dye specific analytical thresholds

- Examined sample amounts where dropout was observed (50 pg, 30 pg, 10 pg for Identifiler and Identifiler Plus)
  - Used to examine stochastic effects including severe imbalance of heterozygous alleles and allele dropout

- Stochastic Threshold: The RFU value of highest surviving false homozygous peak per dye channel

n=84 samples

Summary of Thresholds

Both AT and ST values rounded to the nearest 5 RFU value

Expected peak height ratio (PHR) is assuming the possibility of having one peak at the AT and one peak at the ST

Expected PHR = AT/ST

Identifiler: 7 sec @ 1.2 kV (28 cycles)				
	AT (RFU)	**Highest Surviving Peak (RFU)**	**ST (RFU)**	**Lowest Expected PHR**
Blue	95	344	345	28%
Green	130	435	435	30%
Yellow	140	409	410	34%
Red	120	309	310	39%

Identifiler Plus: 5 sec @ 1.2 kV (28 cycles)				
	AT (RFU)	**Highest Surviving Peak (RFU)**	**ST (RFU)**	**Lowest Expected PHR**
Blue	55	288	290	19%
Green	75	383	385	19%
Yellow	105	414	415	25%
Red	120	265	265	45%

Consumable RFID Tracking Limits

	RFID Hard Stops	Usage Comments From a Research Laboratory Standpoint
Array	None	1. Very easy to change between HID and sequencing 2. Array from validation was stored at least twice and reinstalled on 3500 during validation
Buffer	Expiration Date 7 Days on Instrument # Injections	1. Can no longer use in-house buffer 2. Very easy to change on the instrument (snap-and-go)
Polymer	Expiration Date # Samples # Injections	1. Hard stop with the expiration date has caused us to discard unused polymer we would have otherwise kept on the instrument 2. ~50% of total polymer remains in the pouch after "consumption" 3. Expiration dates have changed purchasing strategy (smaller batches, based on ongoing project needs)

Validation Conclusions

- The 3500 has proven to be reliable, reproducible and robust
  - Out of 498 samples between Identifiler and Identifiler Plus only 5 required reinjection
- Dye specific analytical thresholds resulted in less allelic and full locus dropout than applying one analytical threshold to all dyes
- Stochastic thresholds are linked to analytical thresholds
  - If the analytical threshold is adjusted, the stochastic threshold should be reevaluated along with expected peak height ratios
    - Requires consideration for overall interpretation workflow which we are still evaluating
- RFID tracking decreases flexibility in our research experience

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http://www.cstl.nist.gov/div831/strbase/pub_pres/ISFG_3500_Validation.pdf