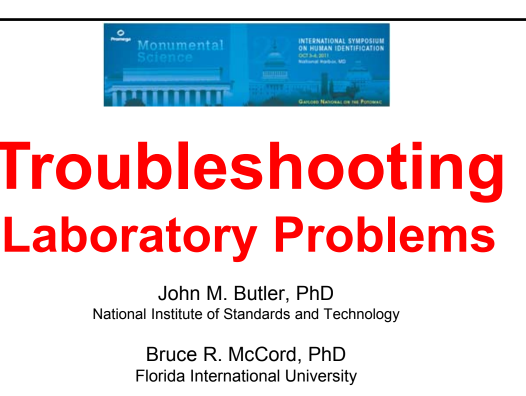


Purpose of This Workshop

- DNA labs often encounter challenges when working with the many variable aspects of STR analysis and capillary electrophoresis separation/detection.
- This workshop will explore common challenges experienced by forensic laboratories and suggest solutions for fixing various problems.
- Participants were invited to suggest problems that they would like to have reviewed in advance of the workshop.

Workshop Outline

- 15 min • Introductions
- 45 min (John) • Fundamentals of CE
  - sample prep, injection, separation, detection
- 30 min (Bruce) • Setting instrument parameters and thresholds
  - Applying validation data
  - Dealing with low-level DNA
- 15 min BREAK
- 45 min (Bruce) • Troubleshooting strategies and solutions
- 15 min (John) • ABI 3500 discussion
- 15 min (John/Bruce) • Questions

Our Backgrounds

John Butler
- NIST Fellow - **National Institute of Standards and Technology**
- PhD in Analytical Chemistry from University of Virginia (1995)
- Family: wife Terilynne and six children
- Hobbies: reading, writing, and making PowerPoint slides

Bruce McCord
- Professor of Analytical/Forensic Chemistry – **Florida International University**
- PhD in Analytical Chemistry from University of Wisconsin (1986)
- Family: wife Margie and three children
- Hobbies: dixieland jazz, windsurfing, sailing and editing John's slides

Background of Participants…

Your name
Your organization
Instrumentation in use (e.g., ABI 310, 3100, 3130xl, 3500)
What you hope to learn from this workshop

NIST and NIJ Disclaimer

Funding: Interagency Agreement between the National Institute of Justice and NIST Office of Law Enforcement Standards

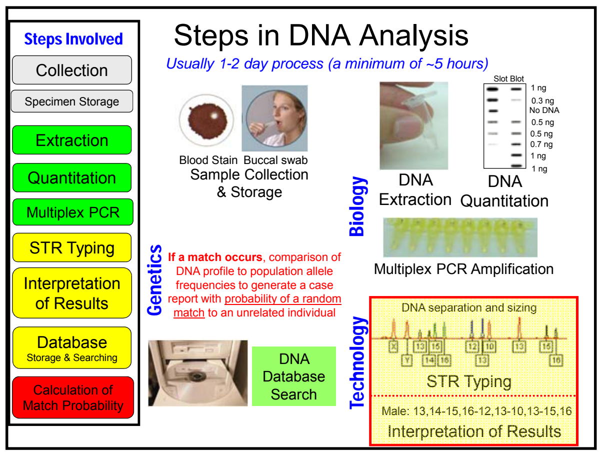
Points of view are the presenters and do not necessarily represent the official position or policies of the US Department of Justice or the National Institute of Standards and Technology.

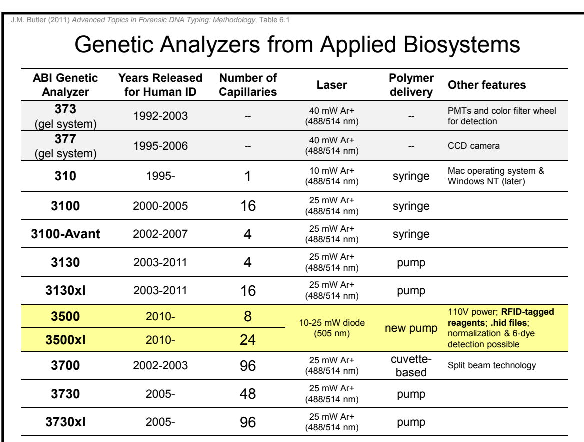
Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.

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

Fundamentals of Capillary Electrophoresis

John



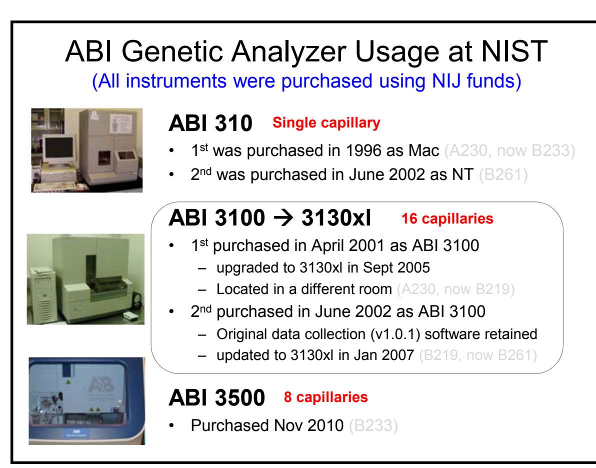


J.M. Butler (2011) *Advanced Topics in Forensic DNA Typing: Methodology*, Table 6.1

Genetic Analyzers from Applied Biosystems

ABI Genetic Analyzer	Years Released for Human ID	Number of Capillaries	Laser	Polymer delivery	Other features
373 (gel system)	1992-2003	--	40 mW Ar+ (488/514 nm)	--	PMTs and color filter wheel for detection
377 (gel system)	1995-2006	--	40 mW Ar+ (488/514 nm)	--	CCD camera
310	1995-	1	10 mW Ar+ (488/514 nm)	syringe	Mac operating system & Windows NT (later)
3100	2000-2005	16	25 mW Ar+ (488/514 nm)	syringe	
3100-Avant	2002-2007	4	25 mW Ar+ (488/514 nm)	syringe	
3130	2003-2011	4	25 mW Ar+ (488/514 nm)	pump	
3130xl	2003-2011	16	25 mW Ar+ (488/514 nm)	pump	
3500	2010-	8	10-25 mW diode (505 nm)	new pump	110V power; **RFID-tagged reagents**; **.hid files**; normalization & 6-dye detection possible
3500xl	2010-	24			
3700	2002-2003	96	25 mW Ar+ (488/514 nm)	cuvette-based	Split beam technology
3730	2005-	48	25 mW Ar+ (488/514 nm)	pump	
3730xl	2005-	96	25 mW Ar+ (488/514 nm)	pump	

Information courtesy of Michelle S. Shepherd, Applied Biosystems, LIFE Technologies.

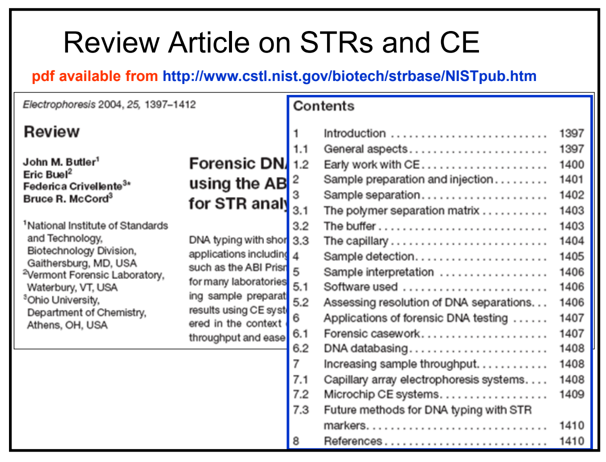


DNA Samples Run at NIST

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…



Review Article on STRs and CE

pdf available from http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

Electrophoresis 2004, *25*, 1397–1412

Review

John M. Butler[1]
Eric Buel[2]
Federica Crivellente[3]
Bruce R. McCord[3]

[1]National Institute of Standards and Technology, Biotechnology Division, Gaithersburg, MD, USA
[2]Vermont Forensic Laboratory, Waterbury, VT, USA
[3]Ohio University, Department of Chemistry, Athens, OH, USA

Forensic DN[A] using the AB[I] for STR anal[y]

DNA typing with shor[t] applications including such as the ABI Pris[m] for many laboratories ing sample preparat[ion] results using CE syst[em] ered in the context throughput and ease

Contents

1 Introduction 1397
1.1 General aspects 1397
1.2 Early work with CE 1400
2 Sample preparation and injection 1401
3 Sample separation 1402
3.1 The polymer separation matrix 1403
3.2 The buffer 1403
3.3 The capillary 1404
4 Sample detection 1405
5 Sample interpretation 1406
5.1 Software used 1406
5.2 Assessing resolution of DNA separations 1406
6 Applications of forensic DNA testing 1407
6.1 Forensic casework 1407
6.2 DNA databasing 1408
7 Increasing sample throughput 1408
7.1 Capillary array electrophoresis systems 1408
7.2 Microchip CE systems 1409
7.3 Future methods for DNA typing with STR markers 1410
8 References 1410

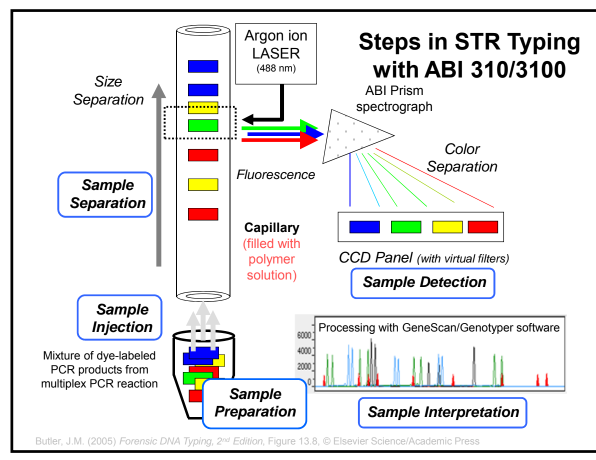
Analytical Requirements for STR Typing

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

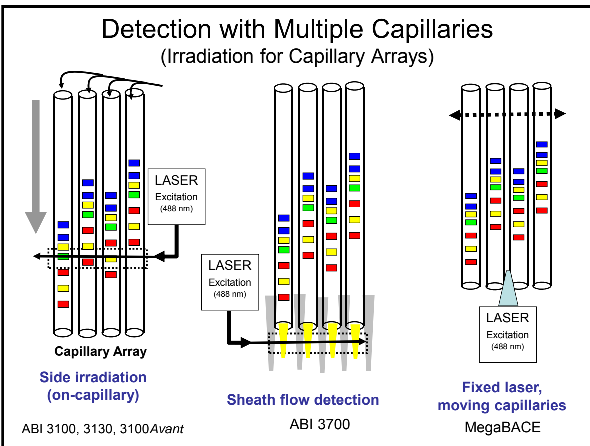
Raw data (w/ color overlap)

Spectrally resolved



Detection with Multiple Capillaries

(Irradiation for Capillary Arrays)



Process Involved in 310/3100 Analysis

- Separation
  - Capillary – 50um fused silica, 43 cm length (36 cm to detector)
  - POP-4 polymer – Polydimethyl acrylamide
  - Buffer - TAPS pH 8.0
  - Denaturants – urea, pyrolidinone
- Injection
  - electrokinetic injection process (formamide, water)
  - importance of sample stacking
- Detection
  - fluorescent dyes with excitation and emission traits
  - CCD with defined virtual filters produced by assigning certain pixels

Separation

Ohm's Law

- V = IR (where V is voltage, I is current, and R is resistance)
- Current, or the flow of ions, is what matters most in electrophoresis
- CE currents are much lower than gels because of a higher resistance in the narrow capillary
- CE can run a higher voltage because the capillary offers a higher surface area-to-volume ratio and can thus dissipate heat better from the ion flow (current)

DNA and Electrophoresis

"From a practical point of view it is disappointing that electrophoresis cannot be used to fractionate or analyze DNA's on the basis of size" Olivera, Biopolymers 1964, 2, 245

$\mu_{ep} = q/6\pi\eta r$

small ions with high charge move fastest

A T G C

PO- PO- PO-

As size increases so does charge!

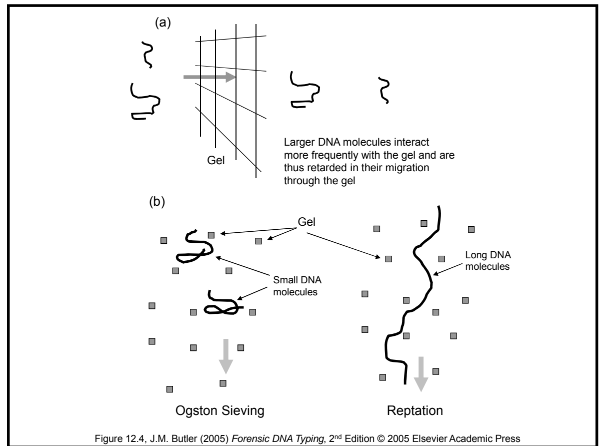


Figure 12.4, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

Separation Issues

- Electrophoresis buffer –
  - Urea for denaturing and viscosity
  - Buffer for consistent pH
  - Pyrolidinone for denaturing DNA
  - EDTA for stability and chelating metals
- Polymer solution -- POP-4 (but others work also)
- Capillary wall coating -- dynamic coating with polymer
  - Wall charges are masked by methyl acrylamide
- Run temperature -- 60 °C helps reduce secondary structure on DNA and improves precision. (Temperature control affects DNA sizing)

What is in POP-4 and Genetic Analyzer Buffer?

© 1997 Oxford University Press — Nucleic Acids Research, 1997, Vol. 25, No. 19 3925–3929

Improved single-strand DNA sizing accuracy in capillary electrophoresis

Barnett B. Rosenblum*, Frank Oaks, Steve Menchen and Ben Johnson

PE Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404, USA

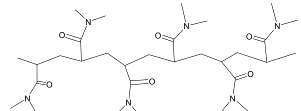
Received May 29, 1997; Revised and Accepted August 6, 1997

See also Wenz *et al.* (1998) *Genome Research* 8: 69-80

POP-4 (4% poly-dimethylacrylamide, 8 M urea, 5% 2-pyrrolidinone)

US Patent 5,552,028 covers POP-4 synthesis

Running buffer contains 100 mM TAPS and 1 mM EDTA (adjusted to pH 8.0 with NaOH) *TAPS = N-Tris-(hydroxymethyl)methyl-3-aminopropane-sulfonic acid*



Capillary Wall Coatings Impact DNA Separations

Electrophoretic flow

− EOF Bulk Flow DNA- DNA- DNA- +

Capillary Wall SiOH ⟷ SiO- + H+

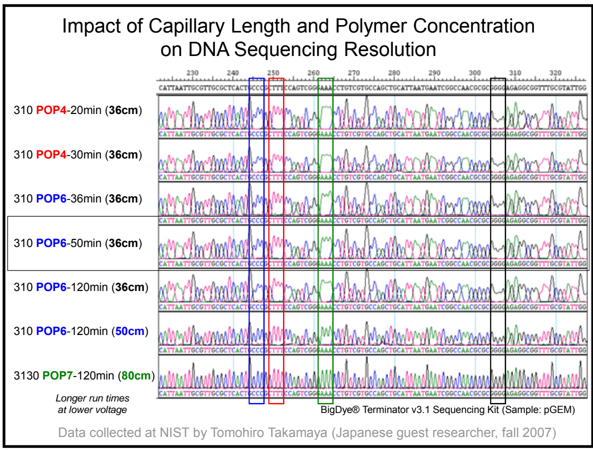
Electroosmotic flow (EOF)

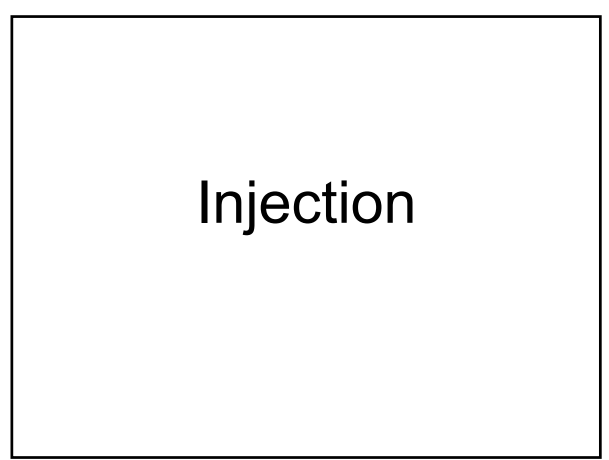
Solvated ions drag solution towards cathode in a flat flow profile

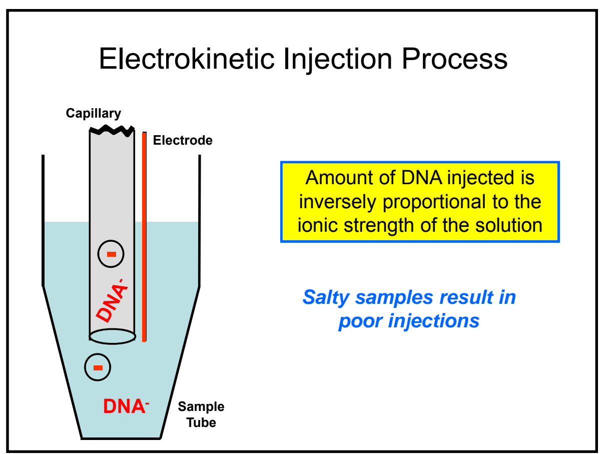
How to Improve Resolution?

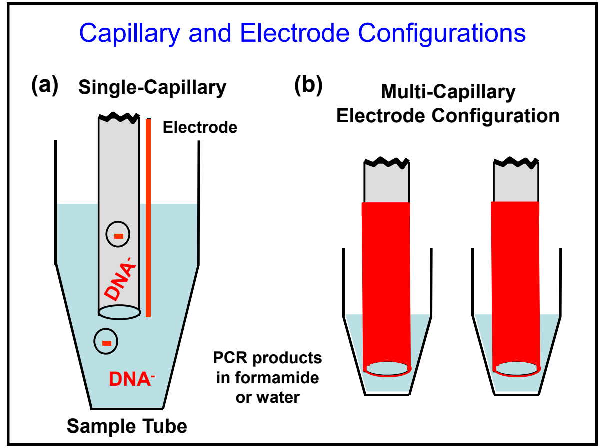
1. **Lower Field Strength**
2. **Increase Capillary Length**
3. **Increase Polymer Concentration**
4. **Increase Polymer Length**

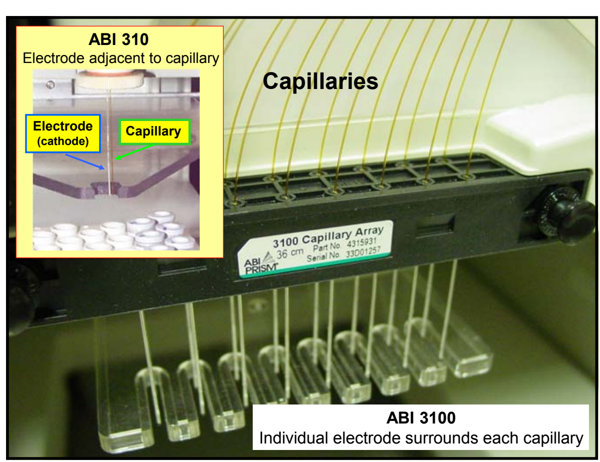
All of these come at a cost of longer separation run times

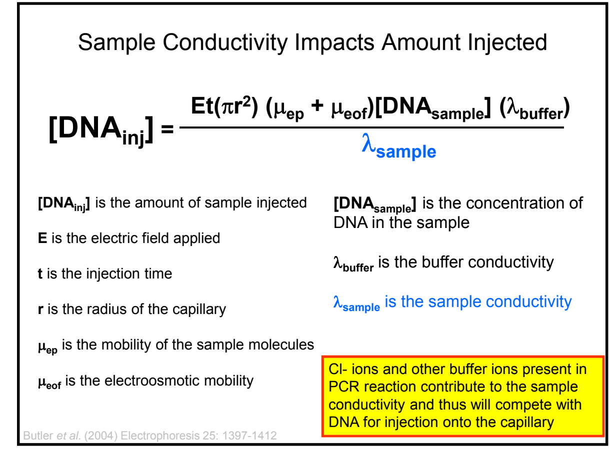

Impact of Capillary Length and Polymer Concentration on DNA Sequencing Resolution
310 POP4-20min (36cm)
310 POP4-30min (36cm)
310 POP6-36min (36cm)
310 POP6-50min (36cm)
310 POP6-120min (36cm)
310 POP6-120min (50cm)
3130 POP7-120min (80cm)
Longer run times at lower voltage
BigDye® Terminator v3.1 Sequencing Kit (Sample: pGEM)
Data collected at NIST by Tomohiro Takamaya (Japanese guest researcher, fall 2007)


Injection


Electrokinetic Injection Process
Capillary
Electrode
DNA-
Sample Tube
Amount of DNA injected is inversely proportional to the ionic strength of the solution
Salty samples result in poor injections


Capillary and Electrode Configurations
(a) Single-Capillary
Electrode
DNA-
Sample Tube
PCR products in formamide or water
(b) Multi-Capillary Electrode Configuration


ABI 310
Electrode adjacent to capillary
Electrode (cathode)
Capillary
Capillaries
3100 Capillary Array
ABI 3100
Individual electrode surrounds each capillary


Sample Conductivity Impacts Amount Injected
$$[DNA_{inj}] = \frac{Et(\pi r^2)(\mu_{ep} + \mu_{eof})[DNA_{sample}](\lambda_{buffer})}{\lambda_{sample}}$$
$[DNA_{inj}]$ is the amount of sample injected
E is the electric field applied
t is the injection time
r is the radius of the capillary
μ_{ep} is the mobility of the sample molecules
μ_{eof} is the electroosmotic mobility
$[DNA_{sample}]$ is the concentration of DNA in the sample
λ_{buffer} is the buffer conductivity
λ_{sample} is the sample conductivity
Cl- ions and other buffer ions present in PCR reaction contribute to the sample conductivity and thus will compete with DNA for injection onto the capillary
Butler et al. (2004) Electrophoresis 25: 1397-1412

Steps Performed in Standard Module

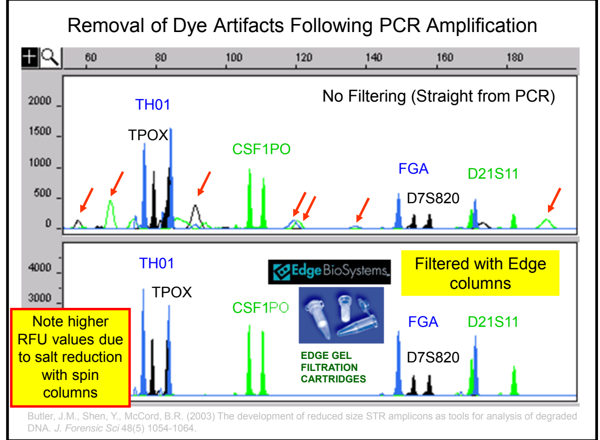
See J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition; Chapter 14

- **Capillary fill** – polymer solution is forced into the capillary by applying a force to the syringe
- **Pre-electrophoresis –** the separation voltage is raised to 10,000 volts and run for 5 minutes;
- **Water wash of capillary – capillary is dipped several times in deionized water to remove buffer salts that would interfere with the injection process**
- **Sample injection** – the autosampler moves to position A1 (or the next sample in the sample set) and is moved up onto the capillary to perform the injection; a voltage is applied to the sample and a few nanoliters of sample are pulled onto the end of the capillary; the default injection is 15 kV (kilovolts) for 5 seconds
- **Water wash of capillary** – capillary is dipped several times in waste water to remove any contaminating solution adhering to the outside of the capillary
- **Water dip** – capillary is dipped in clean water (position 2) several times
- **Electrophoresis** – autosampler moves to inlet buffer vial (position 1) and separation voltage is applied across the capillary; the injected DNA molecules begin separating through the POP-4 polymer solution
- **Detection** – data collection begins; raw data is collected with no spectral deconvolution of the different dye colors; the matrix is applied during Genescan analysis

Comments on Sample Preparation

- Use high quality formamide (<100 μS/cm)
- Denaturation with heating and snap cooling is not needed (although most labs still do it…)
- **Post-PCR purification reduces salt levels** and leads to more DNA injected onto the capillary

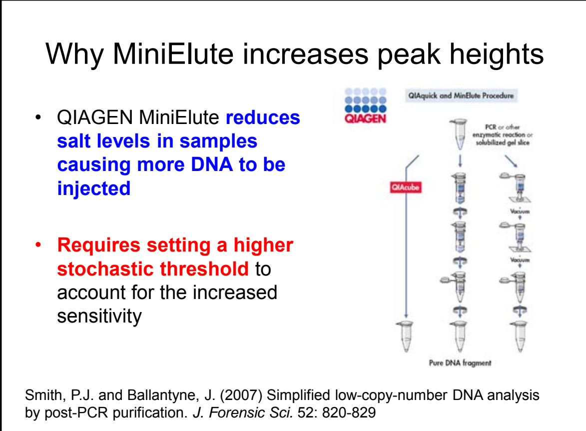
Removal of Dye Artifacts Following PCR Amplification



Butler, J.M., Shen, Y., McCord, B.R. (2003) The development of reduced size STR amplicons as tools for analysis of degraded DNA. *J. Forensic Sci* 48(5) 1054-1064.

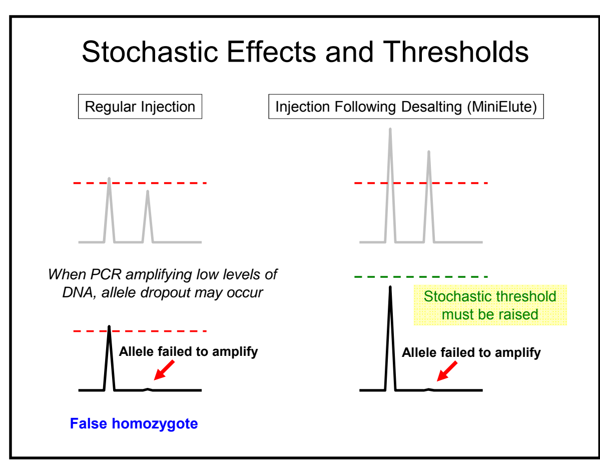
Why MiniElute increases peak heights

- QIAGEN MiniElute **reduces salt levels in samples causing more DNA to be injected**
- **Requires setting a higher stochastic threshold** to account for the increased sensitivity

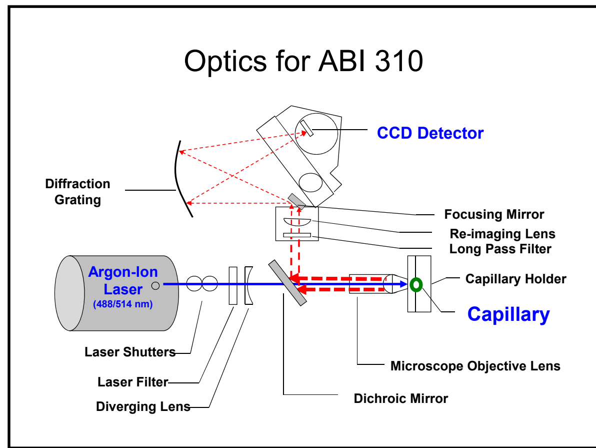


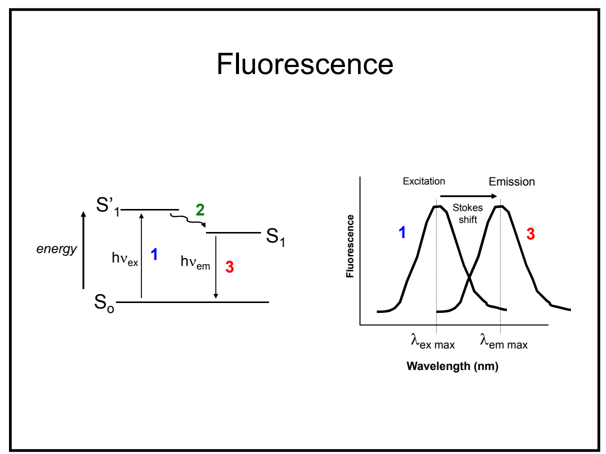
Smith, P.J. and Ballantyne, J. (2007) Simplified low-copy-number DNA analysis by post-PCR purification. *J. Forensic Sci.* 52: 820-829

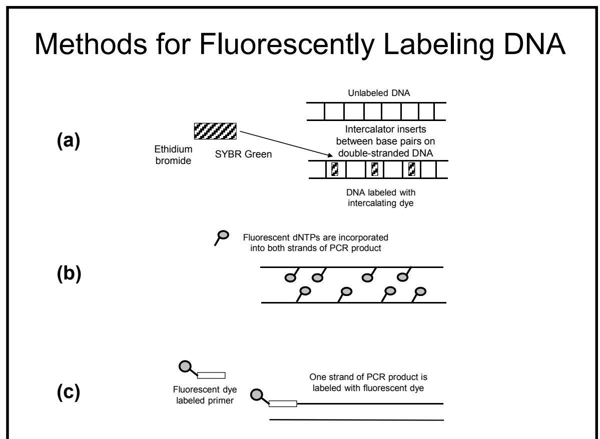
Stochastic Effects and Thresholds

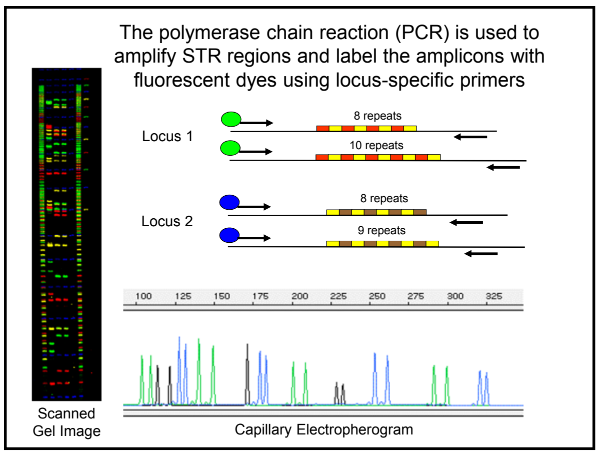


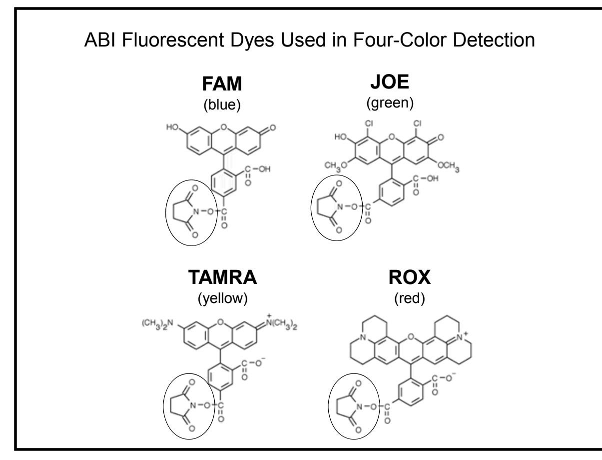
Detection

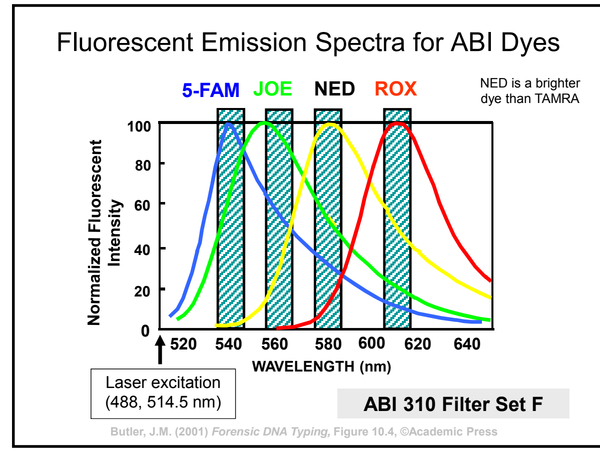

Optics for ABI 310
CCD Detector
Diffraction Grating
Focusing Mirror
Re-imaging Lens
Long Pass Filter
Argon-Ion Laser
(488/514 nm)
Capillary Holder
Capillary
Laser Shutters
Microscope Objective Lens
Laser Filter
Diverging Lens
Dichroic Mirror

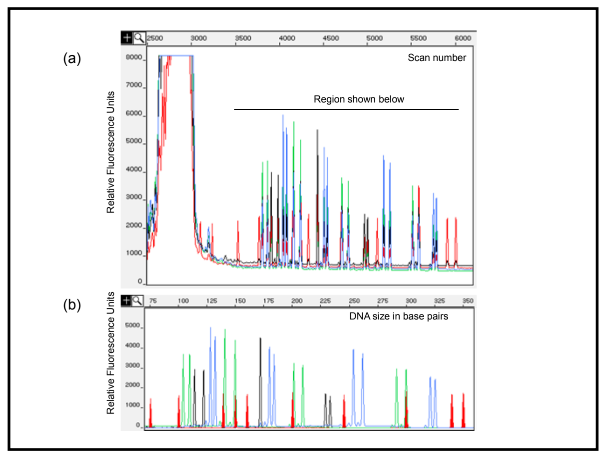

Fluorescence
energy
Excitation
Emission
Stokes shift
Fluorescence
Wavelength (nm)

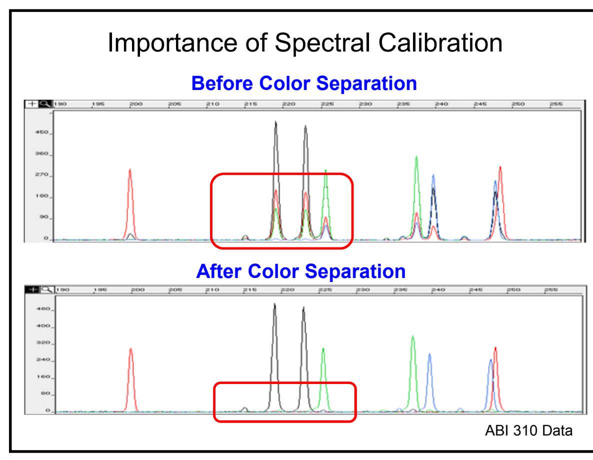

Methods for Fluorescently Labeling DNA
(a)
Unlabeled DNA
Ethidium bromide
SYBR Green
Intercalator inserts between base pairs on double-stranded DNA
DNA labeled with intercalating dye
(b)
Fluorescent dNTPs are incorporated into both strands of PCR product
(c)
Fluorescent dye labeled primer
One strand of PCR product is labeled with fluorescent dye

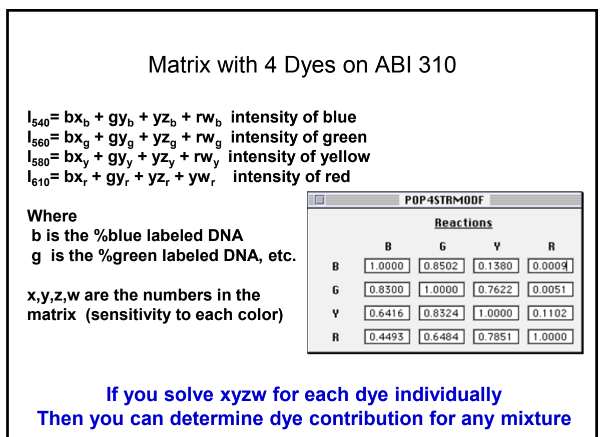

The polymerase chain reaction (PCR) is used to amplify STR regions and label the amplicons with fluorescent dyes using locus-specific primers
Locus 1
8 repeats
10 repeats
Locus 2
8 repeats
9 repeats
100 125 150 175 200 225 250 275 300 325
Scanned Gel Image
Capillary Electropherogram


ABI Fluorescent Dyes Used in Four-Color Detection
FAM
(blue)
JOE
(green)
TAMRA
(yellow)
ROX
(red)


Fluorescent Emission Spectra for ABI Dyes
5-FAM JOE NED ROX
NED is a brighter dye than TAMRA
Normalized Fluorescent Intensity
100
80
60
40
20
0
520 540 560 580 600 620 640
WAVELENGTH (nm)
Laser excitation
(488, 514.5 nm)
ABI 310 Filter Set F
Butler, J.M. (2001) Forensic DNA Typing, Figure 10.4, ©Academic Press



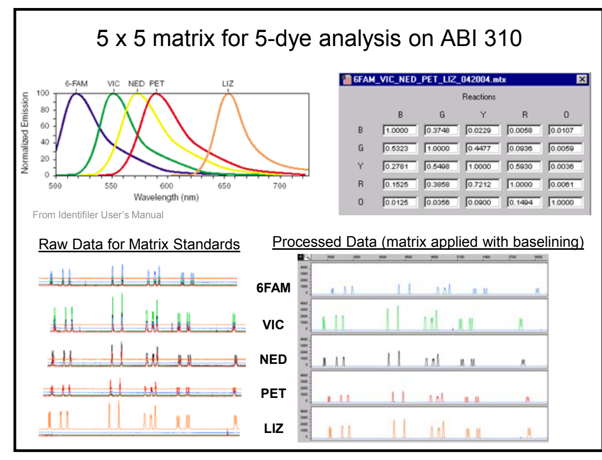




POP4STRMODF

Reactions

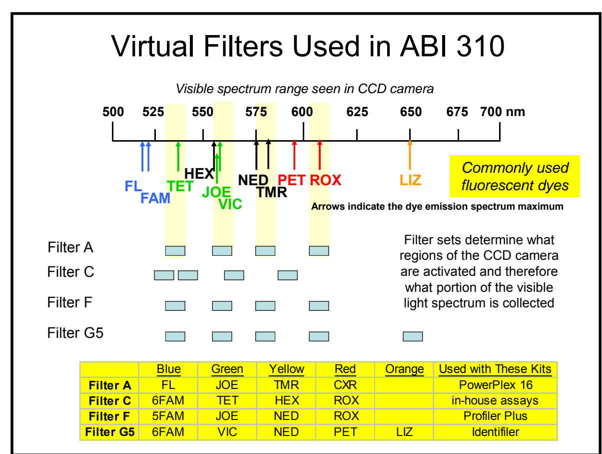
	B	G	Y	R
B	1.0000	0.8502	0.1380	0.0009
G	0.8300	1.0000	0.7622	0.0051
Y	0.6416	0.8324	1.0000	0.1102
R	0.4493	0.6484	0.7851	1.0000



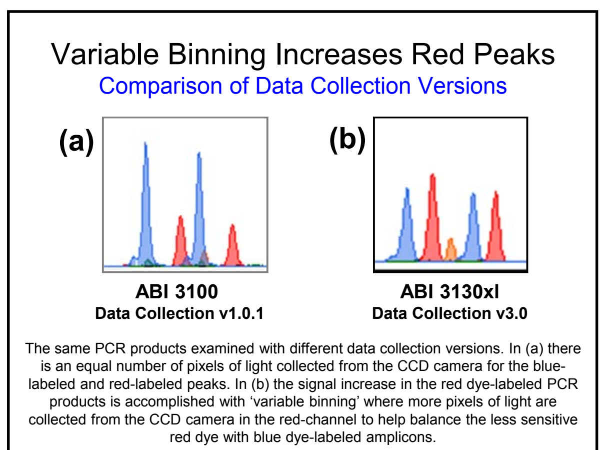
6FAM_VIC_NED_PET_LIZ_042004.mtx

Reactions

	B	G	Y	R	O
B	1.0000	0.3748	0.0229	0.0058	0.0107
G	0.5323	1.0000	0.4477	0.0936	0.0059
Y	0.2781	0.5498	1.0000	0.5930	0.0036
R	0.1525	0.3858	0.7212	1.0000	0.0061
O	0.0125	0.0356	0.0900	0.1494	1.0000



	Blue	Green	Yellow	Red	Orange	Used with These Kits
Filter A	FL	JOE	TMR	CXR		PowerPlex 16
Filter C	6FAM	TET	HEX	ROX		in-house assays
Filter F	5FAM	JOE	NED	ROX		Profiler Plus
Filter G5	6FAM	VIC	NED	PET	LIZ	Identifiler



Maintenance of ABI 310/3100/3130

- Syringe – leaks cause capillary to not fill properly
- Capillary storage & wash – **it dries, it dies!**
- Pump block – cleaning helps insure good fill
- Change the running buffer regularly

YOU MUST BE CLEAN AROUND A CE!

Protocols Used for STR Typing

- Most forensic DNA laboratories follow PCR amplification and CE instrument protocols provided by the manufacturer
- Comments
  - Lower volume reactions may work fine and reduce costs
  - No heat denaturation/snap cooling is required prior to loading samples into ABI 310 or ABI 3100
  - Capillaries do not have to be thrown away after 100 runs
  - POP-4 polymer lasts much longer than 5 days on an ABI 310
  - **Validation does not have to be an overwhelming task**

Setting Instrument Parameters and Thresholds

Bruce

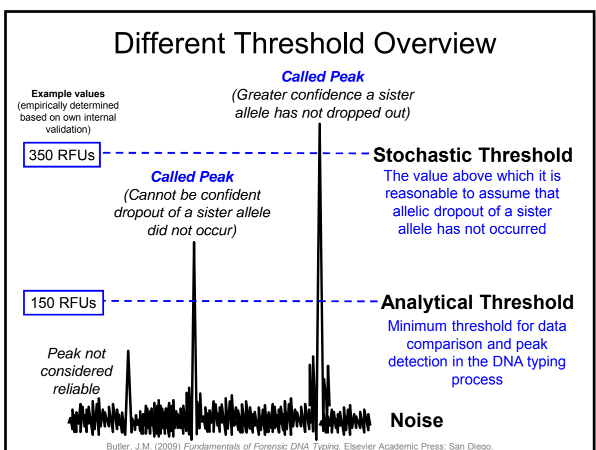
Setting thresholds for the ABI 310/3100

(Determining a true allele)

- Every laboratory needs to set an analytical threshold, a stochastic threshold, a limit of linearity and minimum peak height threshold.
- Can these values be set globally for the entire lab or are they instrument dependent?
- How do these values affect detection, stutter, pull-up, mixture interpretation, low copy DNA?

Signal (S)
Noise (N)
Allele 1
Allele 2
Heterozygote peak balance
Stutter product
True allele

Different Threshold Overview



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

Fundamental parameters for allele detection by CE

Detection Limit (analytical threshold): 3x the standard deviation of the noise. Estimated using 2x peak to peak noise. (approximately 35 - 50 RFUs) Peaks below this level may be random noise

Limit of Quantitation: 10x the standard deviation of the noise Estimated using **7x peak to peak noise** (~100 RFUs) Below this point estimates of peak area or height are unreliable and may not be reliable indicators of mixture ratios

Stochastic Threshold: Level of DNA below which a significant chance of allele dropout can occur. Set high enough that a heterozygous peak will produce its companion allele in the grey zone between stochastic and analytical threshold. (150-200 RFUs)

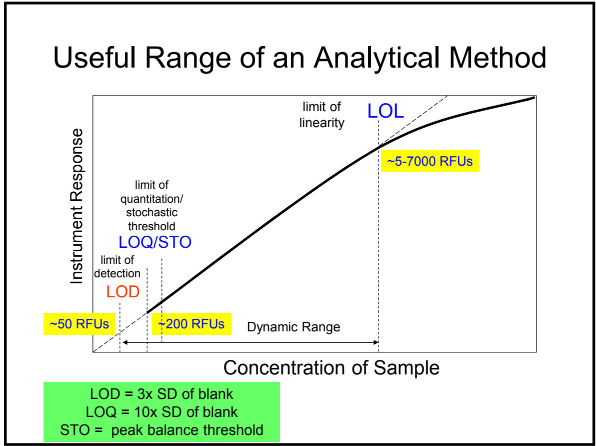
Limit of linearity: The level of DNA above which enhanced pull-up, flat top peaks and elevated stutter occurs. Determined by examining the relationship between input DNA and fluorescence signal varies with instrument- (~4500 RFUs for ABI 310)

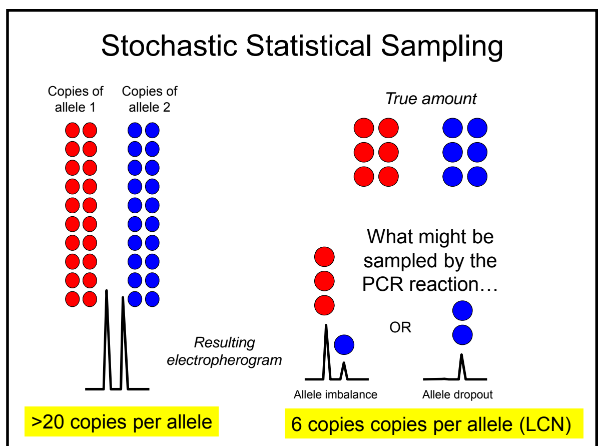
Heterozygous peak ratio: The minimum peak height ratio expected for a clean, single source DNA sample at a particular concentration (typically 60-70%)

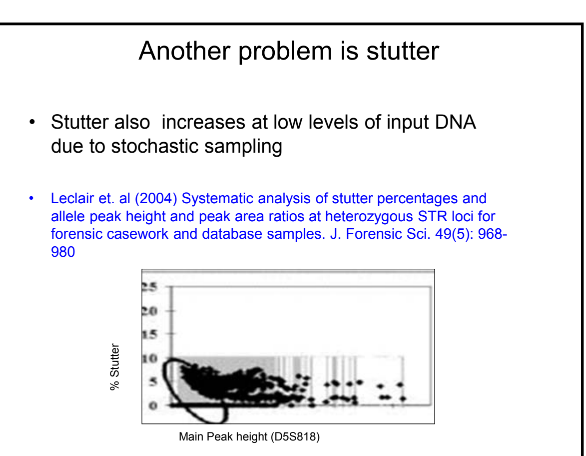
The Scientific Reasoning behind the Concept of an Analytical Threshold/limit of detection

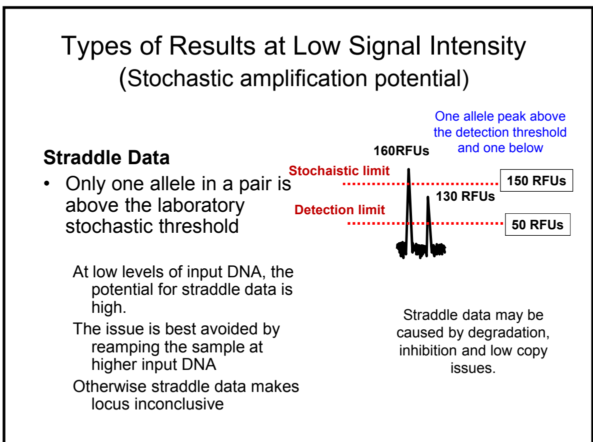
- This is fundamentally an issue of reliability
- **For a peak intensity below the LOD there is a very real chance that such a signal is the result of a random fluctuation**
- You want to be sure to avoid labeling noise!

Abracadabra! It's an allele









The Scientific Reasoning behind the LOQ/Stochastic threshold

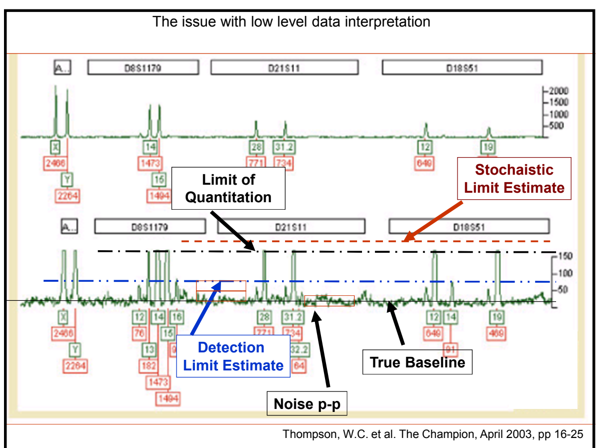
- With peak intensity below the LOQ, you have significant variation in height from one sample to the next.
- **Similarly due to stochaistic fluctuation in peak height ratios, interpreting data below the stochaistic threshold presents the real problem of allele dropout due to variation.**
- You rely on peak heights to detect major and minor profiles and you need to be certain when calculating statistics that you do not have a dropped heterozygous allele.

How low can you go?

Issues with Data below the Stochastic Threshold

- PCR artifacts and stutter become prevalent
- Low levels of bleed through are possible
- Instrument spikes are more numerous
- -A peaks may appear
- Dye blobs become more significant in overall e-gram
- Low level 2nd contributors may show peaks

The issue with low level data interpretation

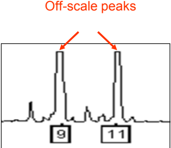


Thompson, W.C. et al. The Champion, April 2003, pp 16-25

Limit of Linearity (LOL)

- Point of saturation for an instrument detector so that higher amounts of analyte do not produce a linear response in signal
- In ABI 310 or ABI 3100 detectors, the CCD camera saturates leading to flat-topped peaks.

Off-scale peaks



Limit of Linearity/ Issues with off-scale peaks

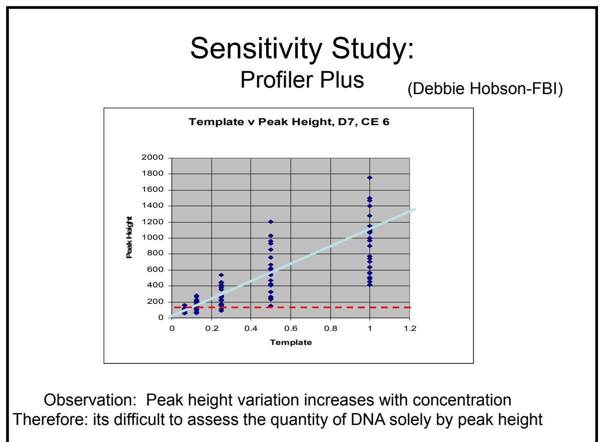
Artifacts produced by overloading: Non-specific amplification, poor adenylation, +4 stutter

Pull-up: odd peaks produced by non-linear matrix effects

Signal = x(fam)+y(vic) + z(ned) + a(pet) +b(liz)

Sensitivity Study: Profiler Plus

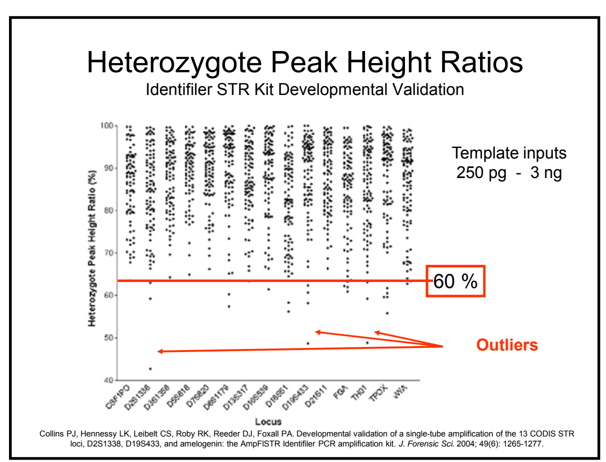
(Debbie Hobson-FBI)



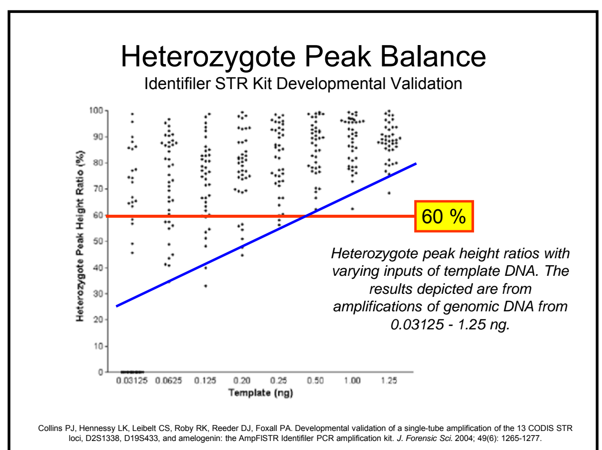
Observation: Peak height variation increases with concentration
Therefore: its difficult to assess the quantity of DNA solely by peak height

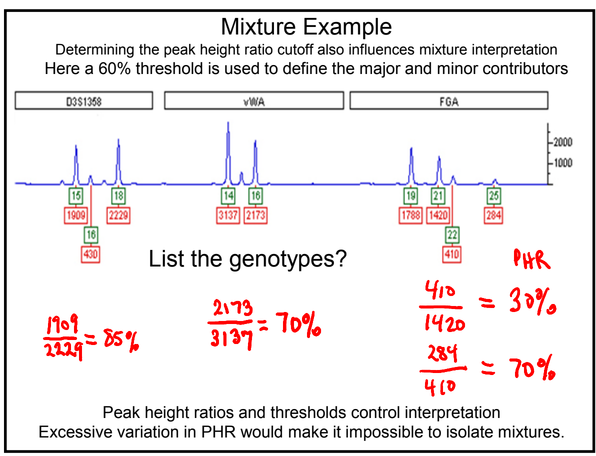
Heterozygote Peak Height Ratios

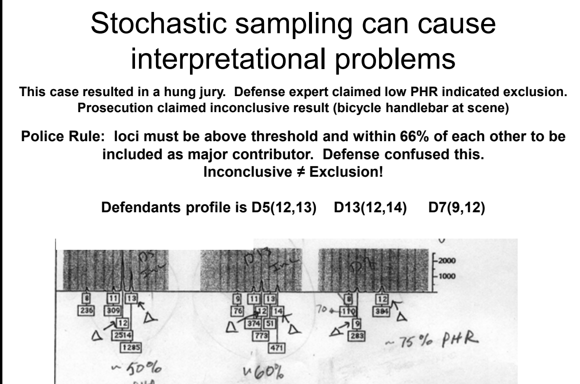
Identifiler STR Kit Developmental Validation

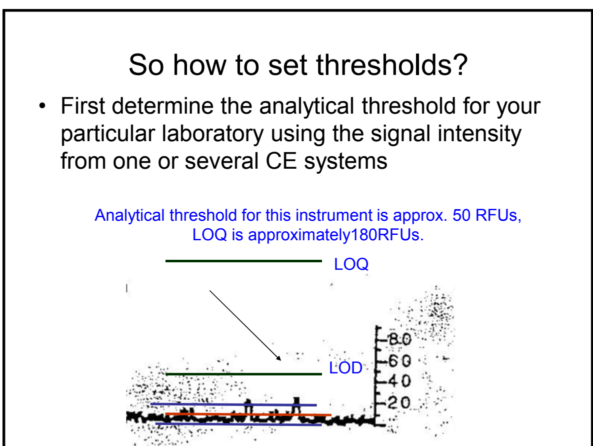


Collins PJ, Hennessy LK, Leibelt CS, Roby RK, Reeder DJ, Foxall PA. Developmental validation of a single-tube amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: the AmpFlSTR Identifiler PCR amplification kit. *J. Forensic Sci.* 2004; 49(6): 1265-1277.



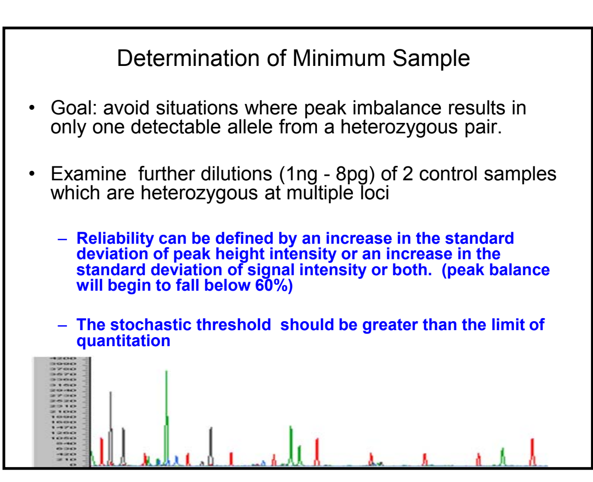






Next determine the dynamic range

1. Perform a series of amplifications of 5 different samples with 5.00, 2.00, 1.00, 0.50, 0.25, 0.13, 0.06, 0.03 ng DNA
2. Use your laboratory quantification system, your thermal cycler, and your 310.
3. Determine the average and standard deviation of each set of samples
4. Your dynamic range is the range of concentrations that are not overloaded. Overload point is where peaks flat top. (this can be checked by experiments)



Alternative Procedure for setting stochastic threshold

1. Since most estimates for LCN show up from 100-250pg DNA, select a low level- say 150pg as your stochastic limit.
2. Amplify 2 or more samples at a range of concentrations (1.0-0.005) ng multiple times and score the intensity
3. The stochastic limit is the intensity (RFUs) at which half the alleles have intensity above this value and half are below
4. In this way you define straddle data as at the point 50% of your alleles will be above this mark

Alternative Procedure (Mass State Police)

1. Since most estimates for LCN show up from 100-250pg DNA, select a low level- say 150pg as your stochastic limit.
2. Amplify 2 or more samples at a range of concentrations (1.0-0.005) ng multiple times and score the intensity
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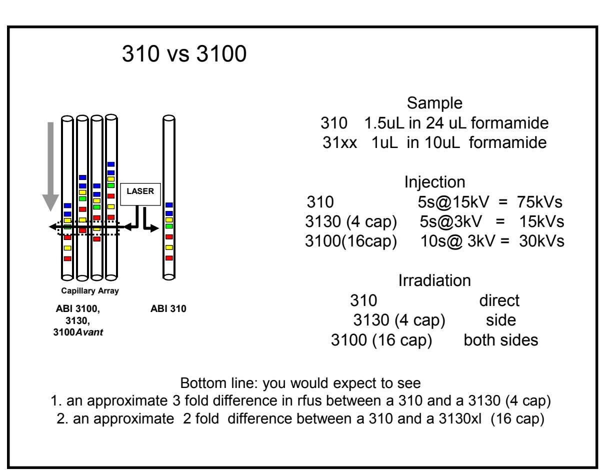
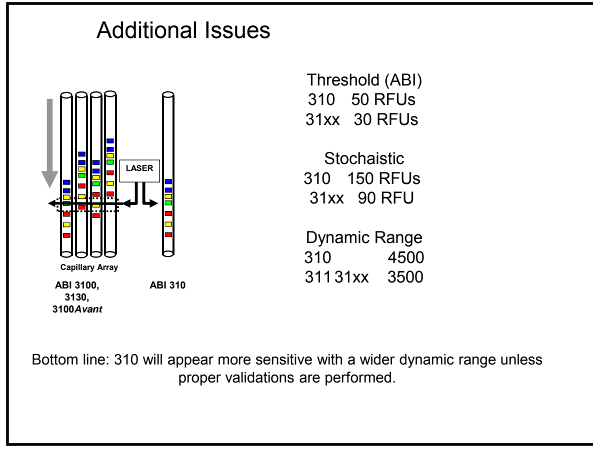
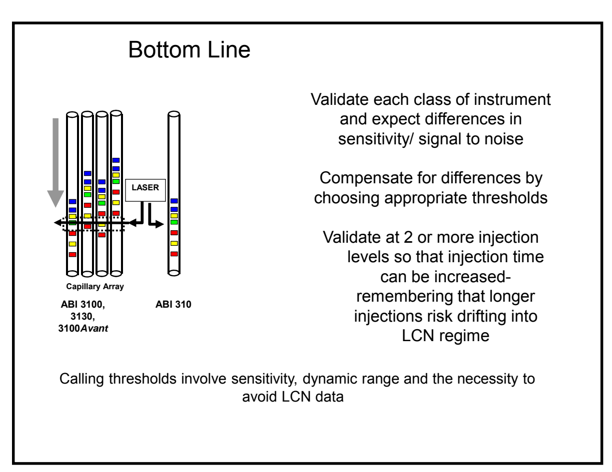
Instrument factors

1. Because only signal is measured (RFUs) in forensic DNA analysis, many labs find that one instrument or another is more sensitive
2. There are also differences in sensitivity based on injection parameters, capillary illumination (single vs multiple) and laser intensity
3. Lastly the variation in qPCR sensitivity affects the output of any system
4. These differences should be corrected by proper setting of threshold parameters and/or adjustment of qPCR results.
5. It is not realistic to constantly alter and adjust levels. Instead, thresholds should be set conservatively





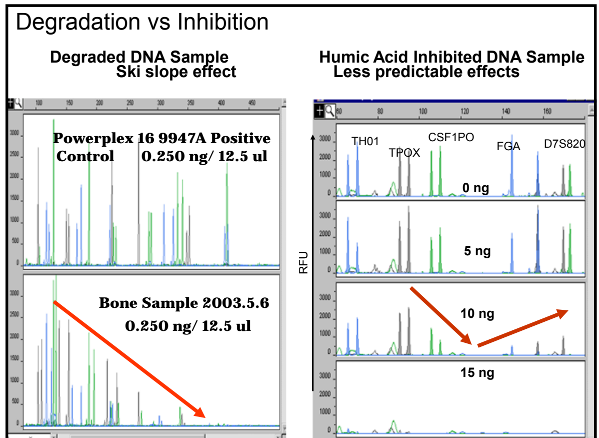


What else can go wrong?

Yarr,Take care mates!

- Most validation studies are performed on pristine samples derived from clean sources.
- DNA degradation will result in dropped alleles from larger sized amplicons
- DNA inhibition will result in dropped alleles from any location and the effects are difficult to predict
- Inhibition and degradation can produce stochastic effects – peak balance issues and allele dropout.

Degradation vs Inhibition



The bottom line:

1. Low signal levels are bad because:
   a. They may indicate low copy # DNA = inconsistent or misleading results
   b. They often coincide with peak imbalance
   c. PCR and instrumental artifacts appear at these levels
2. Relying on signal level to determine DNA quantity can be misleading
   a. There is wide variation in signal strength of amplified DNA
   b. Inhibitors and mixtures complicate interpretation
      1. peak imbalance can occur even in single source samples due to inhibition and degradation
      2. instruments can vary in sensitivity

Fuzzy Logic in Data Interpretation

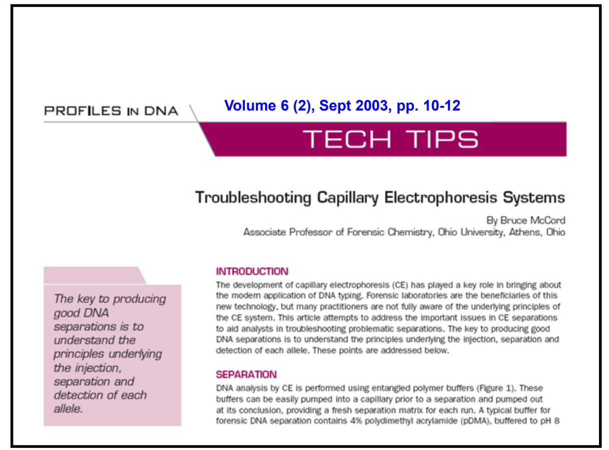
- Capillary Electrophoresis is a dynamic process
- Sensitivity varies with
  - Allele size
  - Injection solvent
  - Input DNA
  - Instrument factors
  - Presence of PCR inhibitors
  - Gel matrix

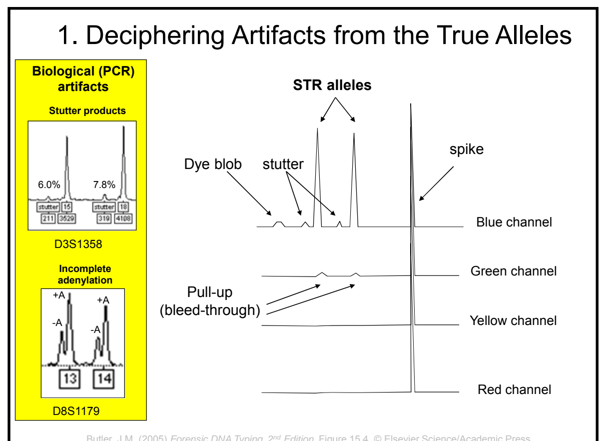
- Thus interpretation must be conservative and data from these studies yields guidelines, not rules.
- These guidelines must be based on in-house validation.
- In addition the interpretation and its significance cannot be dissociated from the overall facts of the case.

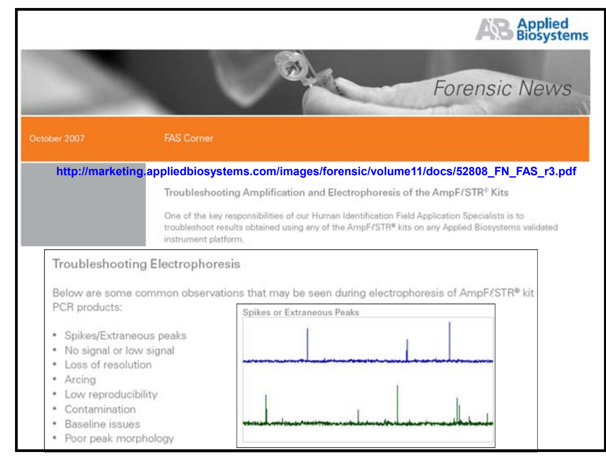
Conclusions

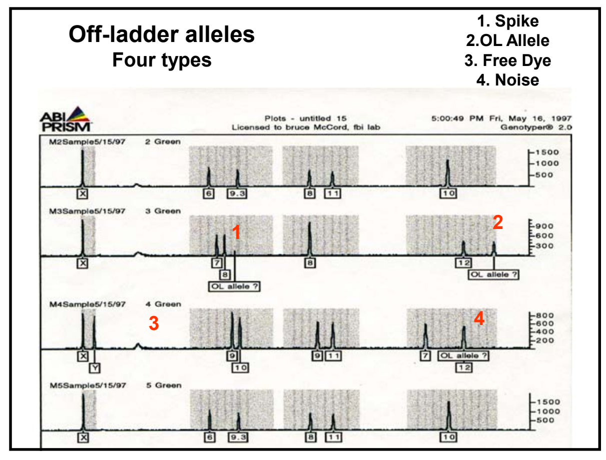
- Be conservative in interpretation
  - Set thresholds based on signal to noise and stochaistic amplification (2 thresholds). Base these numbers on controlled in-house experiments
  - Understand that different instruments may vary in sensitivity – set thresholds high enough to encompass this variation
  - Understand that even with such guidelines issues such as degradation and inhibition can skew results.
- Leave room for the facts of the sample in your interpretation

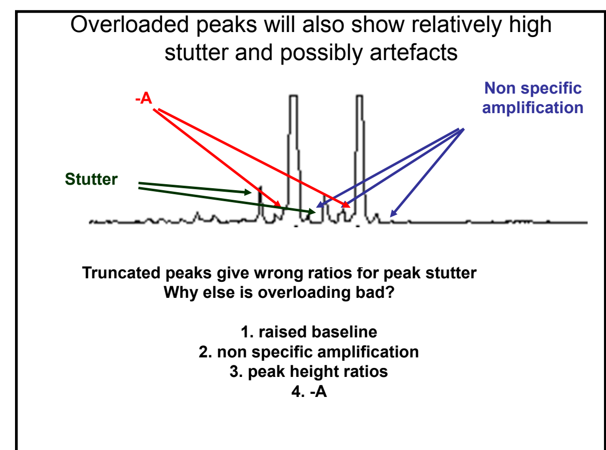
Troubleshooting:
Strategies and Solutions
Bruce
Hang in there……


PROFILES IN DNA
Volume 6 (2), Sept 2003, pp. 10-12
TECH TIPS
Troubleshooting Capillary Electrophoresis Systems
By Bruce McCord
Associate Professor of Forensic Chemistry, Ohio University, Athens, Ohio
The key to producing good DNA separations is to understand the principles underlying the injection, separation and detection of each allele.
INTRODUCTION
SEPARATION


1. Deciphering Artifacts from the True Alleles
Biological (PCR) artifacts
Stutter products
6.0%
7.8%
D3S1358
Incomplete adenylation
+A
-A
13
14
D8S1179
STR alleles
Dye blob
stutter
spike
Blue channel
Green channel
Pull-up (bleed-through)
Yellow channel
Red channel
Butler, J.M. (2005) Forensic DNA Typing, 2nd Edition, Figure 15.4, © Elsevier Science/Academic Press

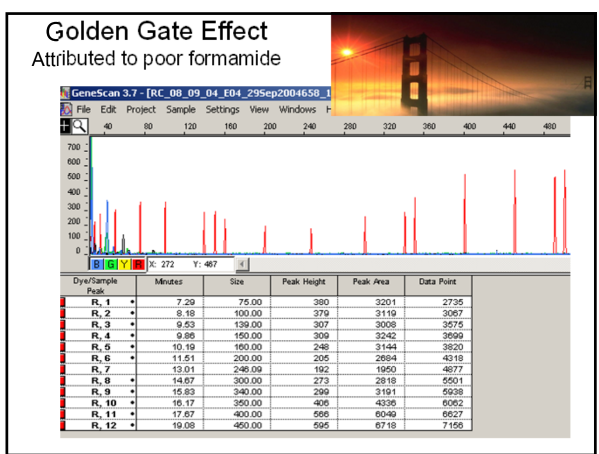

Applied Biosystems
Forensic News
October 2007
FAS Corner
http://marketing.appliedbiosystems.com/images/forensic/volume11/docs/52808_FN_FAS_r3.pdf
Troubleshooting Amplification and Electrophoresis of the AmpFℓSTR® Kits
Troubleshooting Electrophoresis
Below are some common observations that may be seen during electrophoresis of AmpFℓSTR® kit PCR products:
Spikes/Extraneous peaks
No signal or low signal
Loss of resolution
Arcing
Low reproducibility
Contamination
Baseline issues
Poor peak morphology
Spikes or Extraneous Peaks


Off-ladder alleles
Four types
1. Spike
2.OL Allele
3. Free Dye
4. Noise
ABI PRISM
OL allele ?

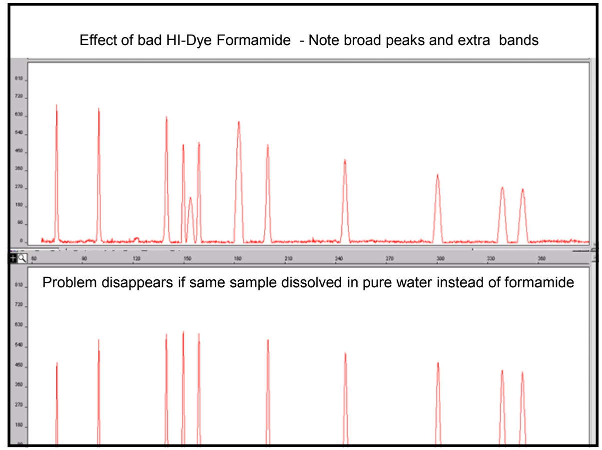

Overloaded peaks will also show relatively high stutter and possibly artefacts
-A
Non specific amplification
Stutter
Truncated peaks give wrong ratios for peak stutter
Why else is overloading bad?
1. raised baseline
2. non specific amplification
3. peak height ratios
4. -A

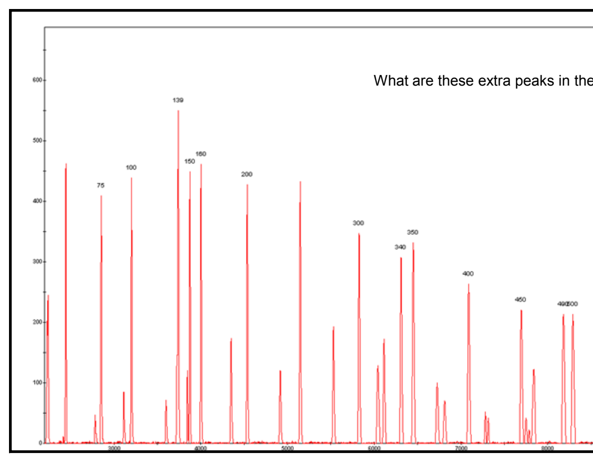
2. Sample Issues

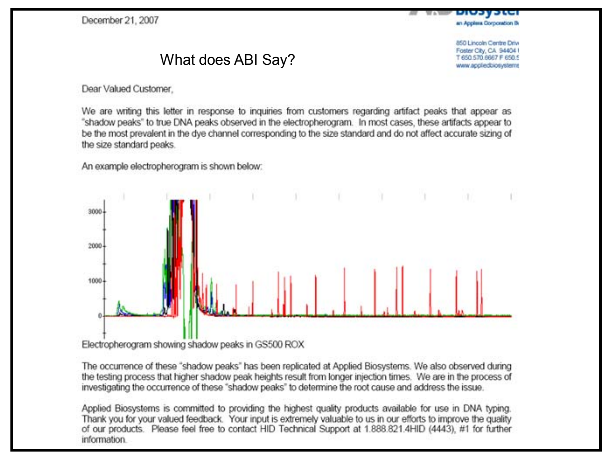
- Formamide Conductivity
- Excessive salt in sample due to evaporation
- Metal ion contamination
- Sensitivity issues with Microcon cleanup (salt removal)
- Dye “blobs” – artifacts from primer synthesis



Dye/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point
R, 1 •	7.29	75.00	380	3201	2735
R, 2 •	8.18	100.00	379	3119	3067
R, 3 •	9.53	139.00	307	3008	3575
R, 4 •	9.86	150.00	309	3242	3699
R, 5 •	10.19	160.00	248	3144	3820
R, 6 •	11.51	200.00	205	2684	4318
R, 7	13.01	246.09	192	1950	4877
R, 8 •	14.67	300.00	273	2818	5501
R, 9 •	15.83	340.00	299	3191	5938
R, 10 •	16.17	350.00	406	4336	6062
R, 11 •	17.67	400.00	566	6049	6627
R, 12 •	19.08	450.00	595	6718	7156







What does ABI Say?

December 21, 2007

850 Lincoln Centre Driv
Foster City, CA 94404
T 650.570.6667 F 650.5
www.appliedbiosystems

Dear Valued Customer,

We are writing this letter in response to inquiries from customers regarding artifact peaks that appear as “shadow peaks” to true DNA peaks observed in the electropherogram. In most cases, these artifacts appear to be the most prevalent in the dye channel corresponding to the size standard and do not affect accurate sizing of the size standard peaks.

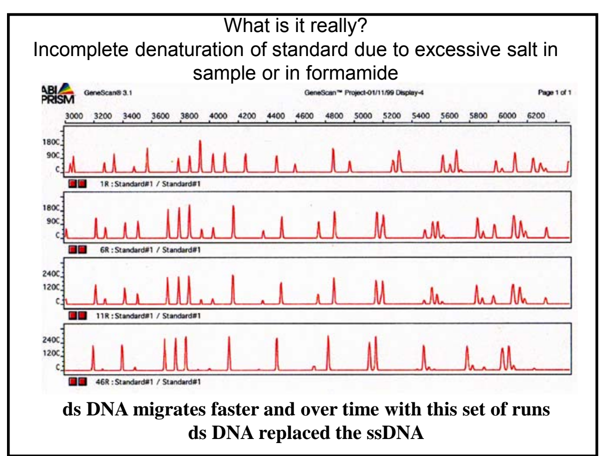
An example electropherogram is shown below:

Electropherogram showing shadow peaks in GS500 ROX

The occurrence of these “shadow peaks” has been replicated at Applied Biosystems. We also observed during the testing process that higher shadow peak heights result from longer injection times. We are in the process of investigating the occurrence of these “shadow peaks” to determine the root cause and address the issue.

Applied Biosystems is committed to providing the highest quality products available for use in DNA typing. Thank you for your valued feedback. Your input is extremely valuable to us in our efforts to improve the quality of our products. Please feel free to contact HID Technical Support at 1.888.821.4HID (4443), #1 for further information.

What is it really?
Incomplete denaturation of standard due to excessive salt in sample or in formamide



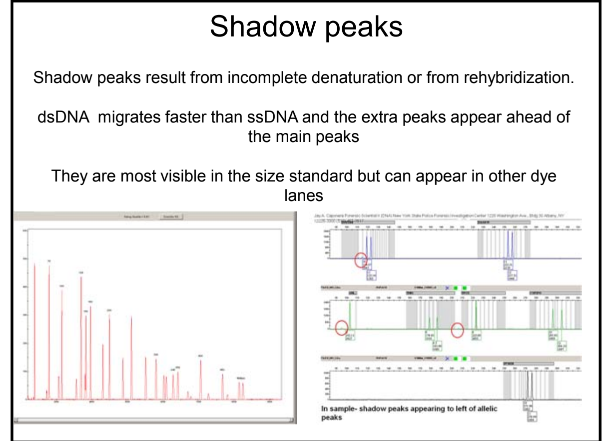
ds DNA migrates faster and over time with this set of runs ds DNA replaced the ssDNA

Shadow peaks

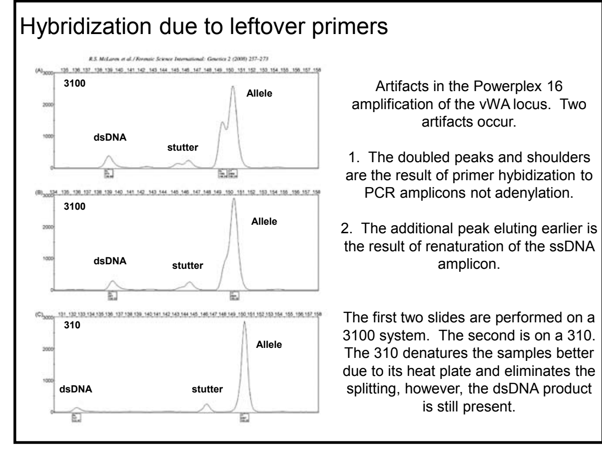
Shadow peaks result from incomplete denaturation or from rehybridization.

dsDNA migrates faster than ssDNA and the extra peaks appear ahead of the main peaks

They are most visible in the size standard but can appear in other dye lanes



Hybridization due to leftover primers



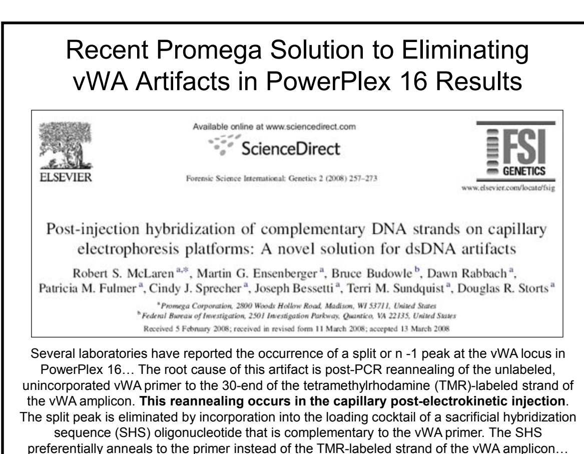
Artifacts in the Powerplex 16 amplification of the vWA locus. Two artifacts occur.

1. The doubled peaks and shoulders are the result of primer hybidization to PCR amplicons not adenylation.

2. The additional peak eluting earlier is the result of renaturation of the ssDNA amplicon.

The first two slides are performed on a 3100 system. The second is on a 310. The 310 denatures the samples better due to its heat plate and eliminates the splitting, however, the dsDNA product is still present.

Recent Promega Solution to Eliminating vWA Artifacts in PowerPlex 16 Results



Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

Forensic Science International: Genetics 2 (2008) 257–273

FSI GENETICS

www.elsevier.com/locate/fsig

Post-injection hybridization of complementary DNA strands on capillary electrophoresis platforms: A novel solution for dsDNA artifacts

Robert S. McLaren [a,*], Martin G. Ensenberger [a], Bruce Budowle [b], Dawn Rabbach [a], Patricia M. Fulmer [a], Cindy J. Sprecher [a], Joseph Bessetti [a], Terri M. Sundquist [a], Douglas R. Storts [a]

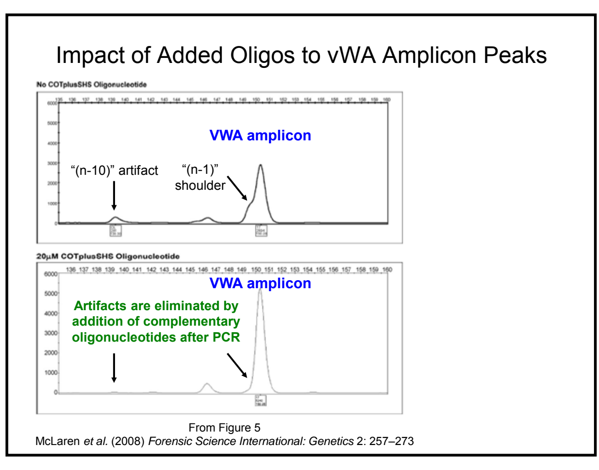
[a] Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711, United States

[b] Federal Bureau of Investigation, 2501 Investigation Parkway, Quantico, VA 22135, United States

Received 5 February 2008; received in revised form 11 March 2008; accepted 13 March 2008

Several laboratories have reported the occurrence of a split or n -1 peak at the vWA locus in PowerPlex 16… The root cause of this artifact is post-PCR reannealing of the unlabeled, unincorporated vWA primer to the 30-end of the tetramethylrhodamine (TMR)-labeled strand of the vWA amplicon. **This reannealing occurs in the capillary post-electrokinetic injection**. The split peak is eliminated by incorporation into the loading cocktail of a sacrificial hybridization sequence (SHS) oligonucleotide that is complementary to the vWA primer. The SHS preferentially anneals to the primer instead of the TMR-labeled strand of the vWA amplicon…

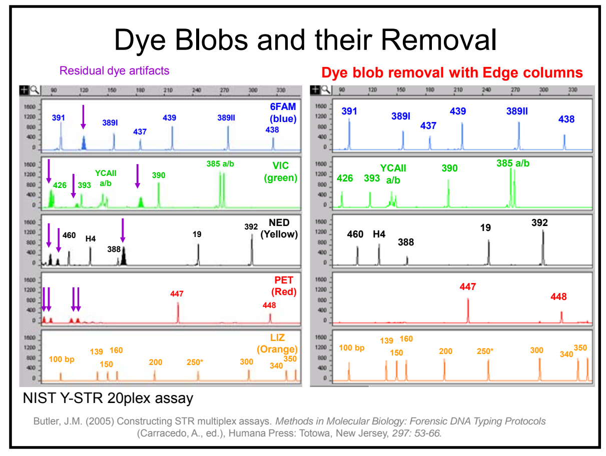
Impact of Added Oligos to vWA Amplicon Peaks



From Figure 5

McLaren *et al.* (2008) *Forensic Science International: Genetics* 2: 257–273

Dye Blobs and their Removal

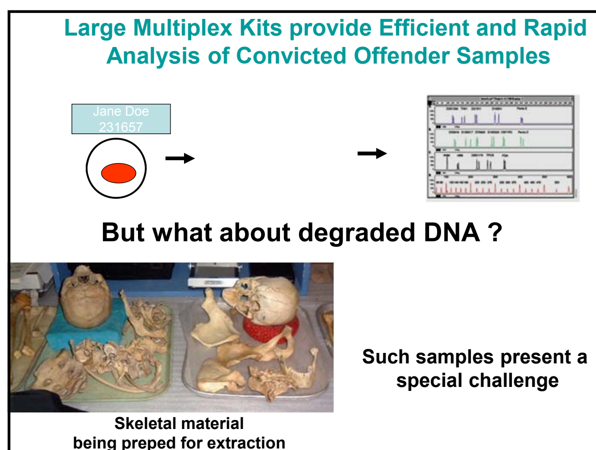


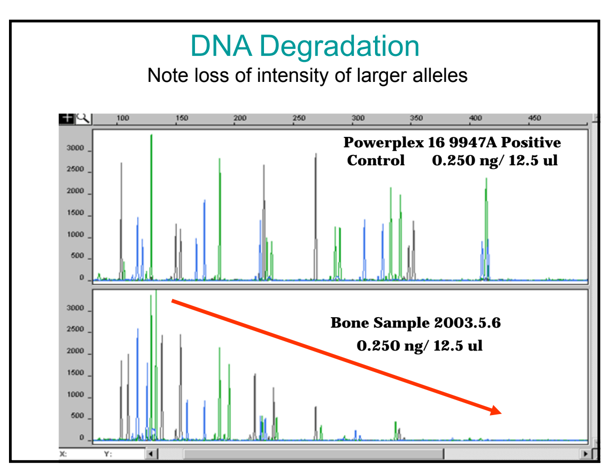
NIST Y-STR 20plex assay

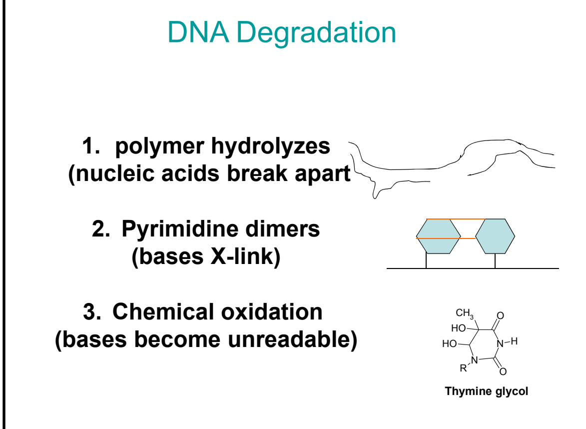
Butler, J.M. (2005) Constructing STR multiplex assays. *Methods in Molecular Biology: Forensic DNA Typing Protocols* (Carracedo, A., ed.), Humana Press: Totowa, New Jersey, *297: 53-66.*

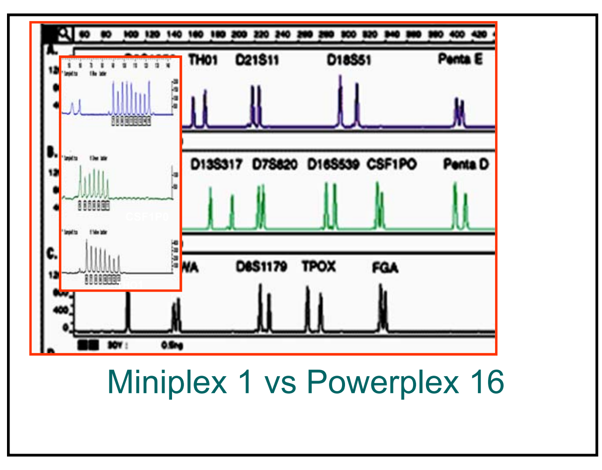
3. External Factors

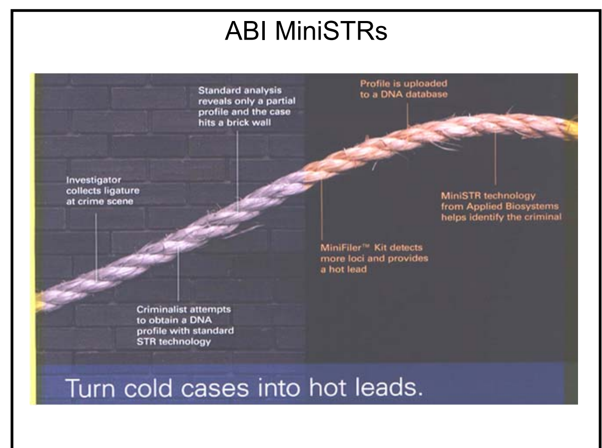
- Room temperature
  - Variations in room temperature can cause mobility shifts with band shifts and loss of calibration
  - Temperature is also important due to effects of high humidity on electrical conductance
- Cleanliness
  - Urea left in sample block can crystallize and catalyze further crystal formation causing spikes, clogs and other problems.
  - Best bet is to keep polymer in system and not remove or change block until polymer is used up.

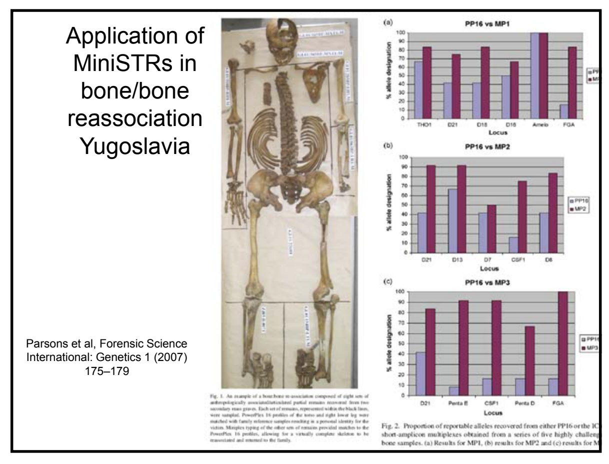

Large Multiplex Kits provide Efficient and Rapid Analysis of Convicted Offender Samples
Jane Doe 231657
But what about degraded DNA ?
Such samples present a special challenge
Skeletal material being preped for extraction

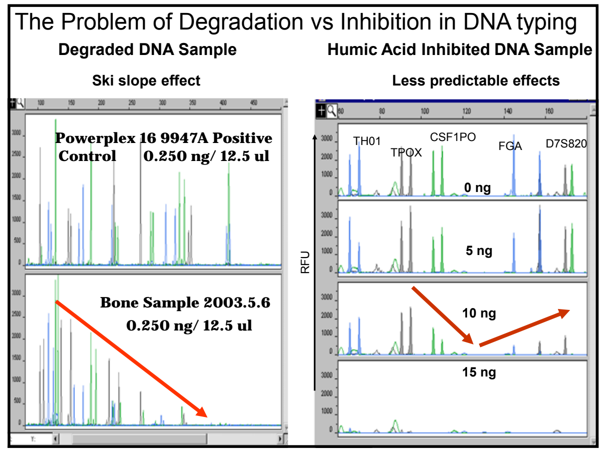

DNA Degradation
Note loss of intensity of larger alleles
Powerplex 16 9947A Positive Control 0.250 ng/ 12.5 ul
Bone Sample 2003.5.6 0.250 ng/ 12.5 ul

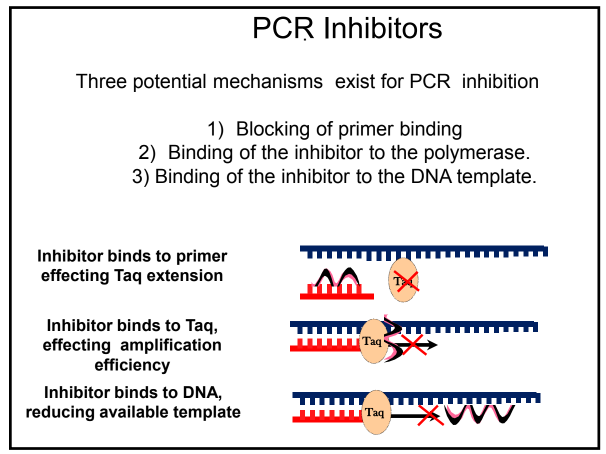

DNA Degradation
1. polymer hydrolyzes (nucleic acids break apart
2. Pyrimidine dimers (bases X-link)
3. Chemical oxidation (bases become unreadable)
Thymine glycol

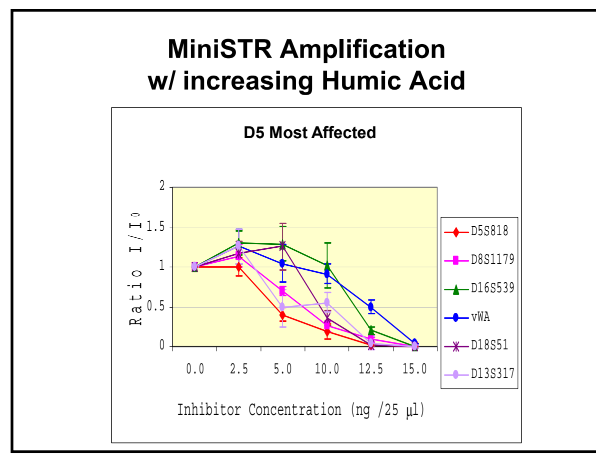

TH01
D21S11
D18S51
Penta E
D13S317
D7S820
D16S539
CSF1PO
Penta D
D8S1179
TPOX
FGA
Miniplex 1 vs Powerplex 16

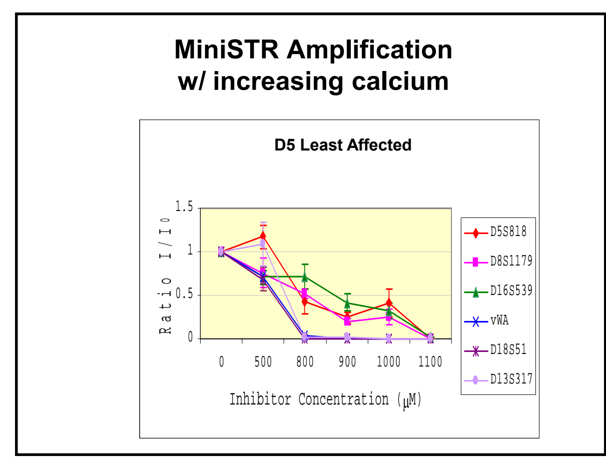

ABI MiniSTRs
Standard analysis reveals only a partial profile and the case hits a brick wall
Profile is uploaded to a DNA database
Investigator collects ligature at crime scene
MiniSTR technology from Applied Biosystems helps identify the criminal
MiniFiler™ Kit detects more loci and provides a hot lead
Criminalist attempts to obtain a DNA profile with standard STR technology
Turn cold cases into hot leads.

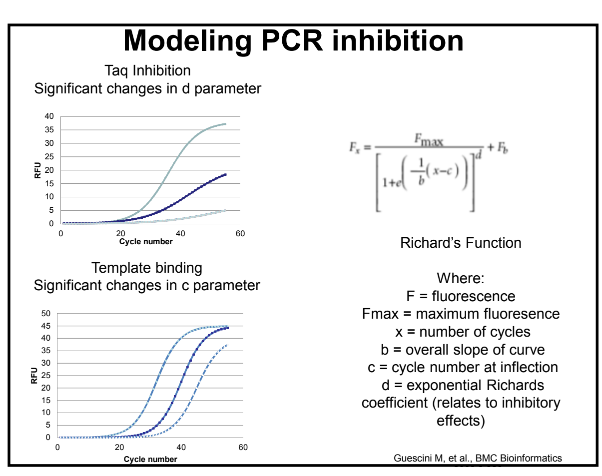

Application of MiniSTRs in bone/bone reassociation Yugoslavia
Parsons et al, Forensic Science International: Genetics 1 (2007) 175–179
PP16 vs MP1
PP16 vs MP2
PP16 vs MP3



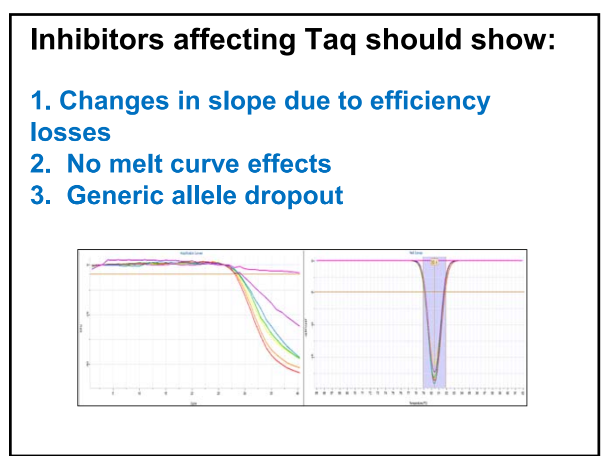


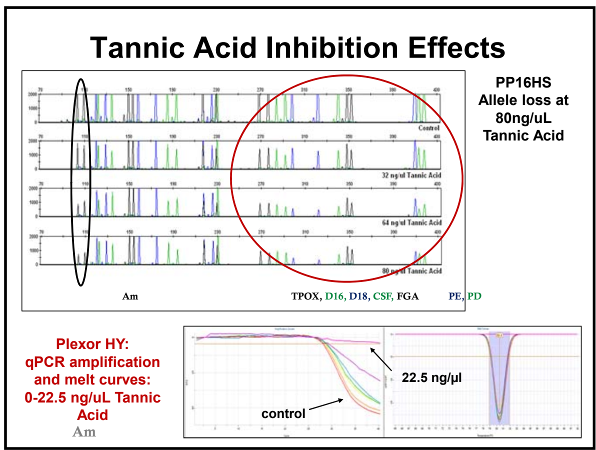


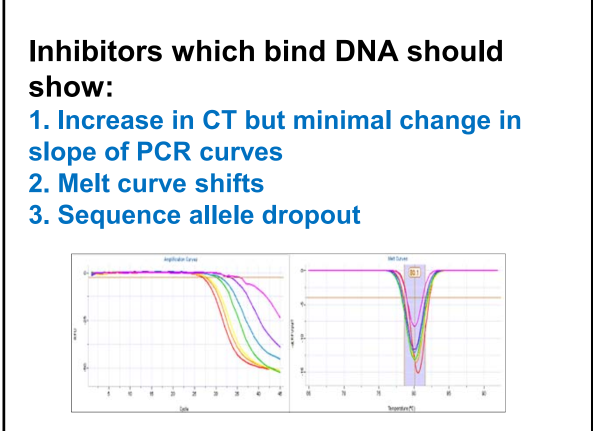


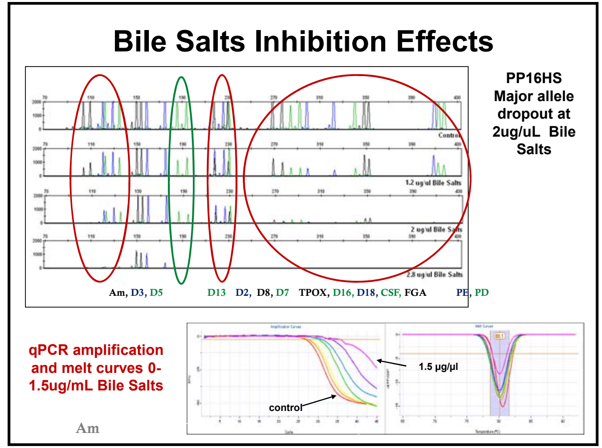


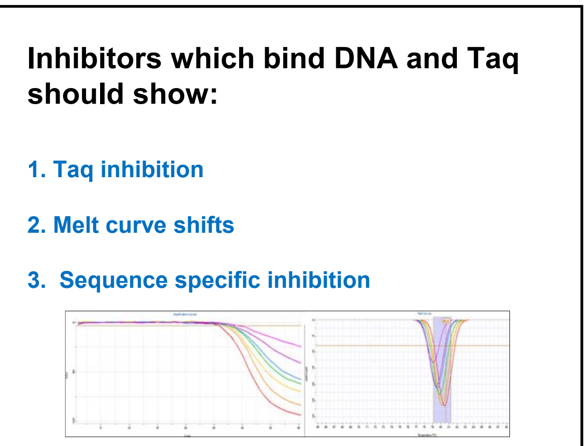
$$F_x = \frac{F_{max}}{\left[1+e^{\left(-\frac{1}{b}(x-c)\right)}\right]^d} + F_b$$

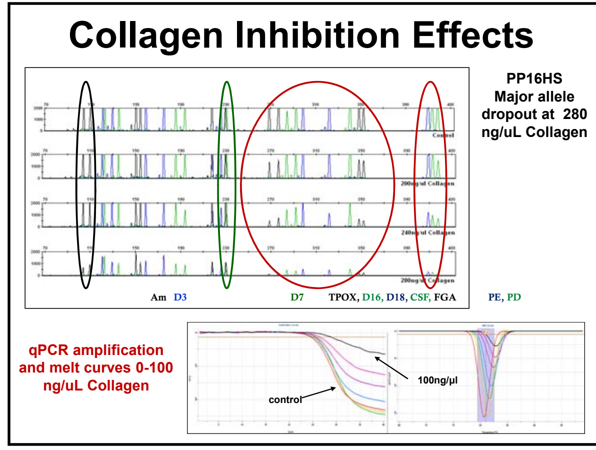






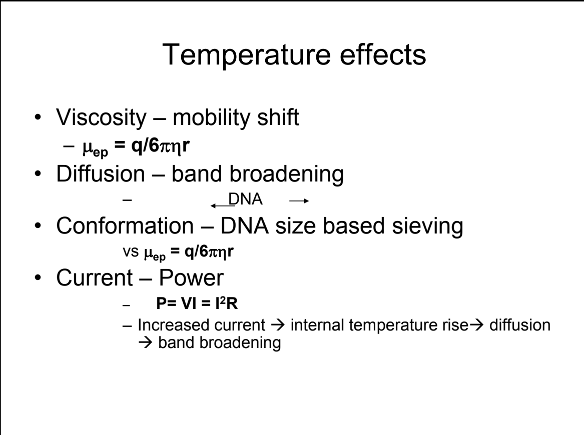


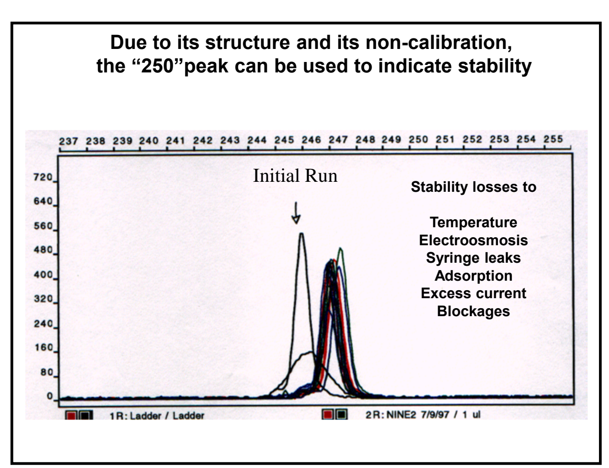


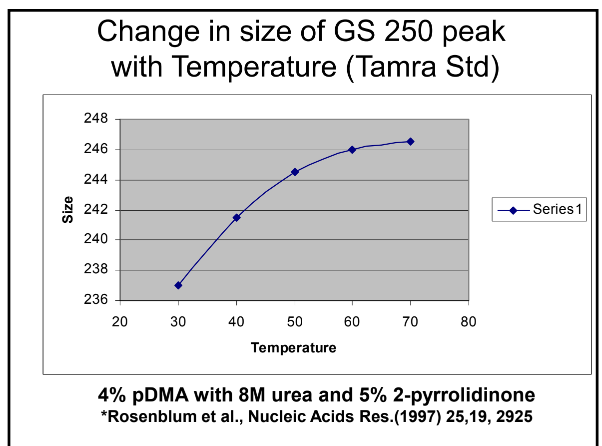


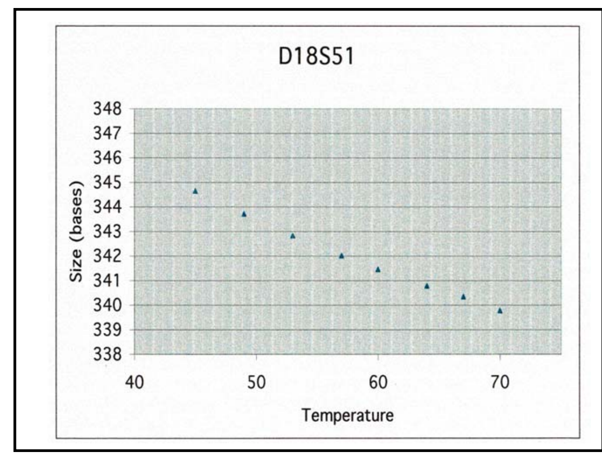
Overview

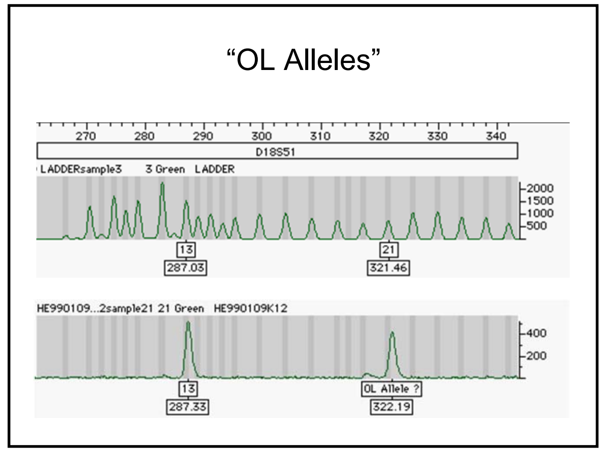
INHIBITOR	PLEXOR MODE OF INHIBITION	CONCENTRATION AT 50% qPCR INHIBITION	CONCENTRATION AT 50% DROP-OUT OF 1ST ALLELE	NOTED ALLELE DROP-OUT
Calcium	Taq	1.0 mM	0.8 mM	D2, D8, TPOX, D16, D18, CSF, FGA , PD, PE
Tannic Acid	Taq	15 ng/µL	32 ng/µL	Am, TPOX, D16, D18, CSF, FGA, PD, PE
Bile Salts	DNA	1.25 µg/µL	1.2 µg/µL	Am, D13, D8, TPOX, D16, D18, CSF, FGA, PE, PD
Humic Acid	DNA	20 ng/µL	8 ng/µL	Am, D8, TPOX, D16, D18, CSF, FGA, PD
Hematin	DNA	60 µM	48 µM	Am, D8, TPOX, D16, D18, CSF, PD
Melanin	DNA	40 µg/µL	32 µg/µL	D13, D8, D16, D18, CSF, PD
EDTA	DNA	1.2 mM	0.6 mM	Am, D3, D5, D13, D8, TPOX, D16, D18, CSF, FGA, PD, PE
Phenol	DNA/Taq	5 µg/µL	2.6 µg/µL	Am, D3, D5, D13, D8, D7, TPOX, D16, D18, CSF, FGA, PE, PD
Collagen	DNA/Taq	100 ng/µL	200 ng/µL	Am, D3, D5, D13, D8, D7, TPOX, D16, D18, CSF, FGA, PE, PD
Urea	DNA/Taq	400 mM	240 mM	Am, D13, D8, TPOX, D16, D18, CSF, FGA, PD, PE

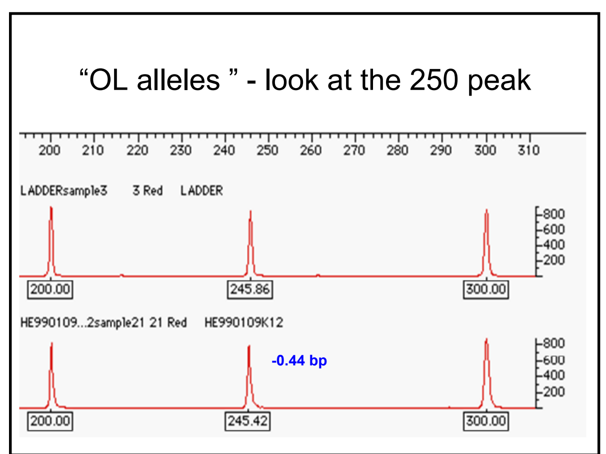

Temperature effects
• Viscosity – mobility shift
– $\mu_{ep} = q/6\pi\eta r$
• Diffusion – band broadening
– DNA
• Conformation – DNA size based sieving
vs $\mu_{ep} = q/6\pi\eta r$
• Current – Power
– P= VI = I²R
– Increased current → internal temperature rise→ diffusion → band broadening

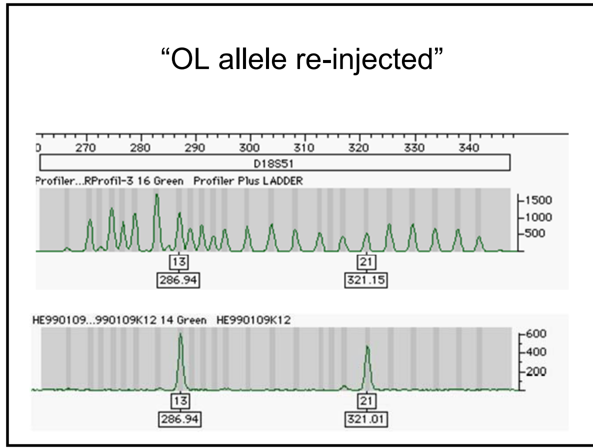

Due to its structure and its non-calibration, the "250"peak can be used to indicate stability
Initial Run
Stability losses to
Temperature
Electroosmosis
Syringe leaks
Adsorption
Excess current
Blockages
1R: Ladder / Ladder
2R: NINE2 7/9/97 / 1 ul

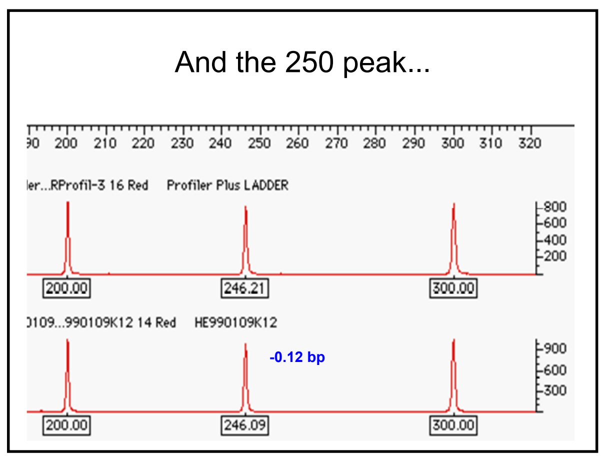

Change in size of GS 250 peak with Temperature (Tamra Std)
Size
Temperature
Series1
4% pDMA with 8M urea and 5% 2-pyrrolidinone
*Rosenblum et al., Nucleic Acids Res.(1997) 25,19, 2925

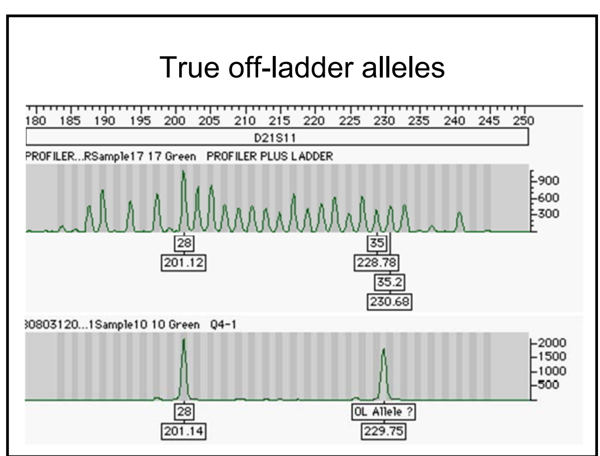

D18S51
Size (bases)
Temperature

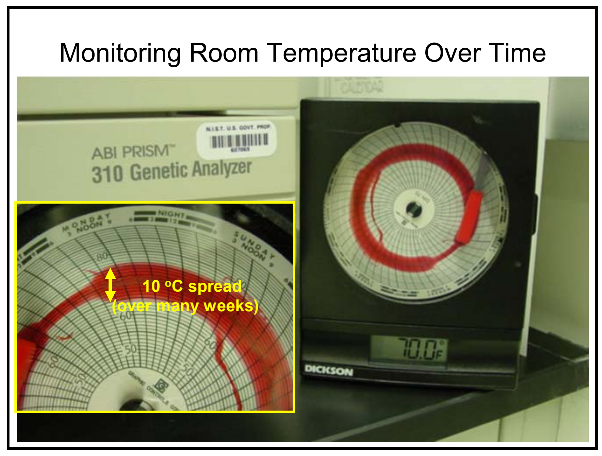

"OL Alleles"
D18S51
LADDERsample3 3 Green LADDER
13
287.03
21
321.46
HE990109...2sample21 21 Green HE990109K12
13
287.33
OL Allele ?
322.19

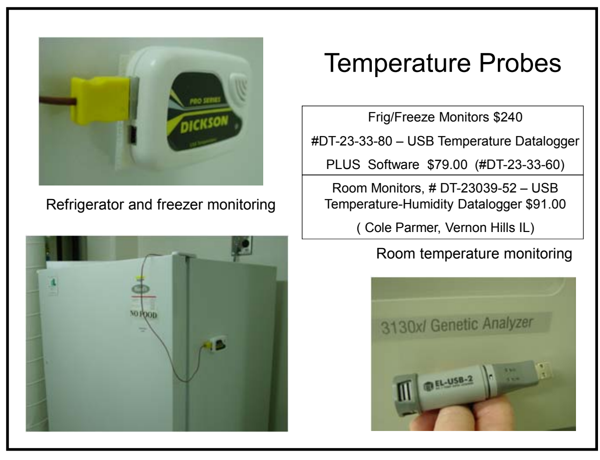

"OL alleles " - look at the 250 peak
LADDERsample3 3 Red LADDER
200.00
245.86
300.00
HE990109...2sample21 21 Red HE990109K12
-0.44 bp
200.00
245.42
300.00

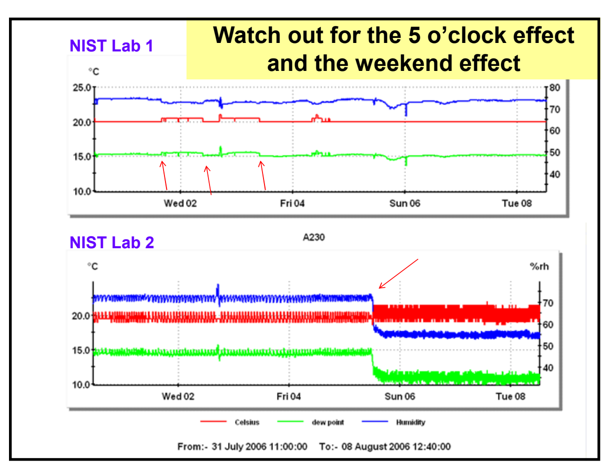

"OL allele re-injected"
D18S51
Profiler...RProfil-3 16 Green Profiler Plus LADDER
13
286.94
21
321.15
HE990109...990109K12 14 Green HE990109K12
13
286.94
21
321.01

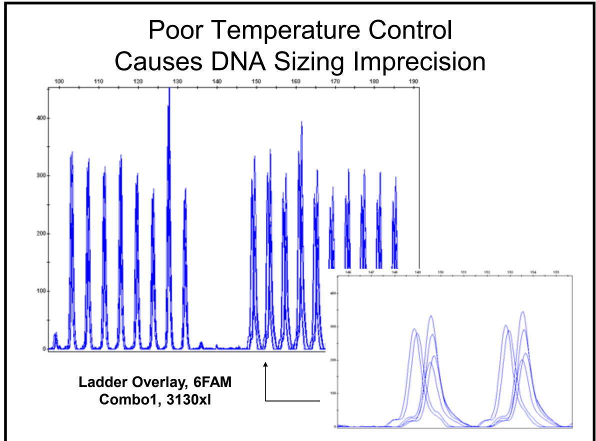

And the 250 peak...
ler...RProfil-3 16 Red Profiler Plus LADDER
200.00
246.21
300.00
109...990109K12 14 Red HE990109K12
-0.12 bp
200.00
246.09
300.00


True off-ladder alleles
D21S11
PROFILER...RSample17 17 Green PROFILER PLUS LADDER
28
201.12
35
228.78
35.2
230.68
30803120...1Sample10 10 Green Q4-1
28
201.14
OL Allele ?
229.75


Monitoring Room Temperature Over Time
ABI PRISM
310 Genetic Analyzer
10 °C spread
(over many weeks)
DICKSON


Temperature Probes
DICKSON
Refrigerator and freezer monitoring
Frig/Freeze Monitors $240
#DT-23-33-80 – USB Temperature Datalogger
PLUS Software $79.00 (#DT-23-33-60)
Room Monitors, # DT-23039-52 – USB
Temperature-Humidity Datalogger $91.00
(Cole Parmer, Vernon Hills IL)
Room temperature monitoring
3130xl Genetic Analyzer
EL-USB-2


NIST Lab 1
Watch out for the 5 o'clock effect
and the weekend effect
Wed 02
Fri 04
Sun 06
Tue 08
NIST Lab 2
A230
Wed 02
Fri 04
Sun 06
Tue 08
Celsius
dew point
Humidity
From:- 31 July 2006 11:00:00 To:- 08 August 2006 12:40:00

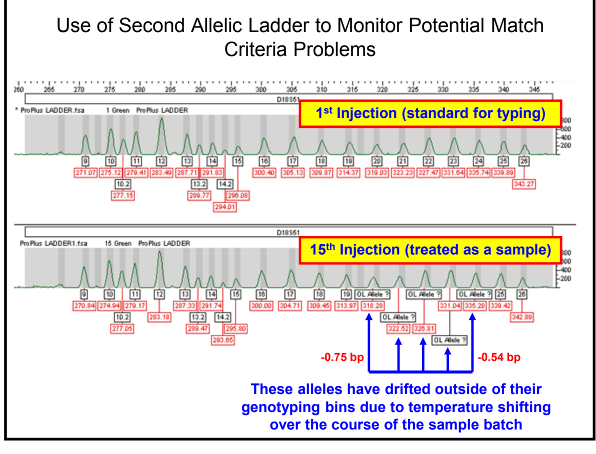


What to do if calibration is lost?

The 310 only calibrates to the first run ladder

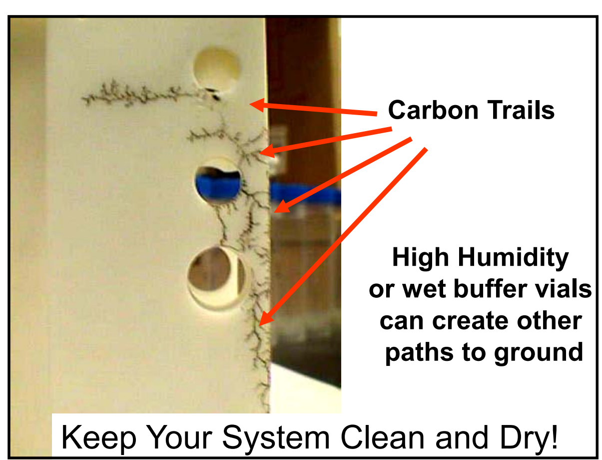
this ladder sample may have been run at a different temperature!

- If protocol permits
  - Go to the next ladder
  - Rerun sample
  - Check current
  - Check allelic ladder
- Always check the ROX size standard
  - Look for extra bands
  - Check peak height
  - Check parameters and alignment



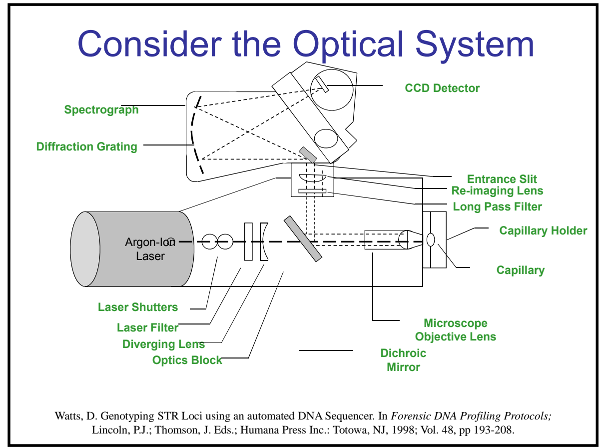
Cleanliness

- Urea sublimates and breaks down to ionic components - these find a path to ground
- Similarly wet buffer under a vial creates paths to ground
- Capillary windows must be clear or matrix effects will occur
- Laser will often assist in this process
- Vial caps will transfer low levels of DNA to capillary



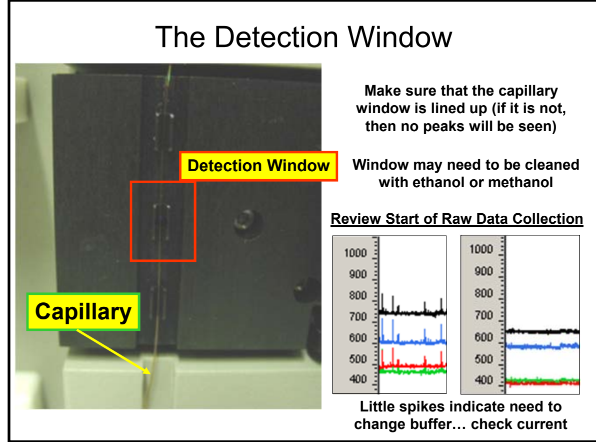
4. Instrumental Factors

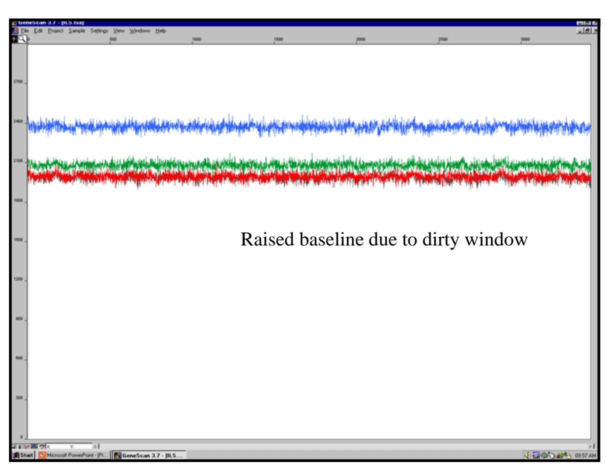
- Optical System
  - Sensitivity changes with age, capillary diameter, capillary cleanliness, instrument calibration
- Fluidic System
  - Effects of bubbles, dust, urea crystals, leaks in syringe and capillary ferrule
- Matrix Calculations
  - Changes in buffer, optics, sample dye can alter the software calibrations
- Capillary Problems
  - Chemisorbed materials on capillary surface can produce osmotic flow, DNA band broadening and inconsistent resolution (meltdowns)



Issues with the Optical System

- Argon Ion lasers outgas and eventually loose intensity; **take note of laser current and monitor it over time**
- Fluorescence expression:
  $I_f = I_0 k \varepsilon b C \varphi$ - changes in input intensity: I_0
  - changes in capillary diameter: b
  - cleanliness of capillary, optics: k
  - All these things directly affect peak RFUs, however, baseline noise is more affected by detector.
- **Thus by monitoring signal to noise, you can get a better picture of your optical system.**



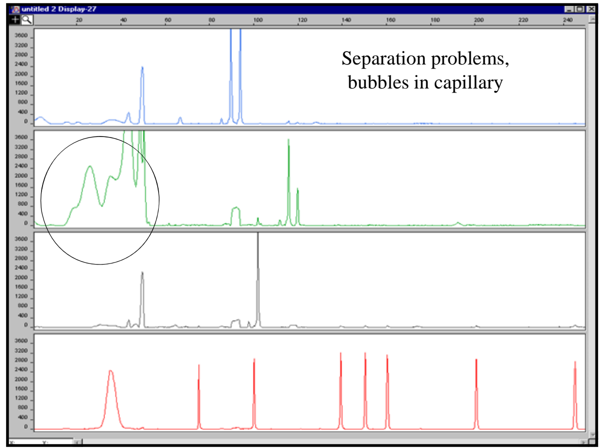


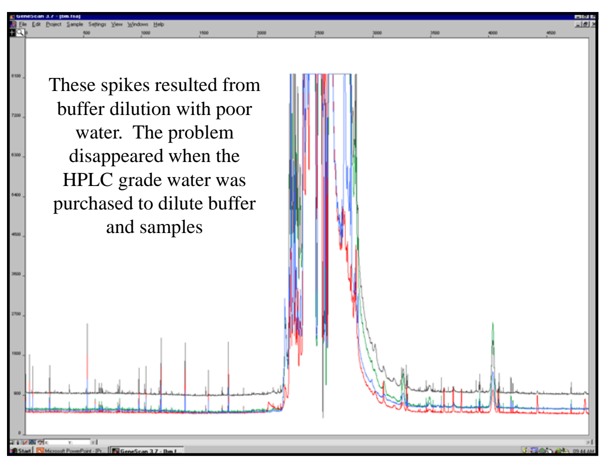
Buffer Issues

- The buffer and polymer affect the background fluorescence- affecting the matrix
- Urea crystals and dust may produce spikes
- High salt concentrations may produce reannealing of DNA
- High salt concentrations affect current
- Low polymer concentrations affect peak resolution

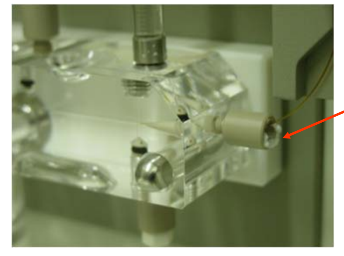
Remove all bubbles from the channels

Bubbles in the channels can prevent flow of ions and are usually exhibited by zero current when the voltage is applied





Beware of Urea Crystals



Urea crystals have formed due to a small leak where the capillary comes into the pump block

Urea sublimates and can evaporate to appear elsewhere

Use a small balloon to better grip the ferrule and keep it tight

Pump block should be well cleaned to avoid problems with urea crystal formation

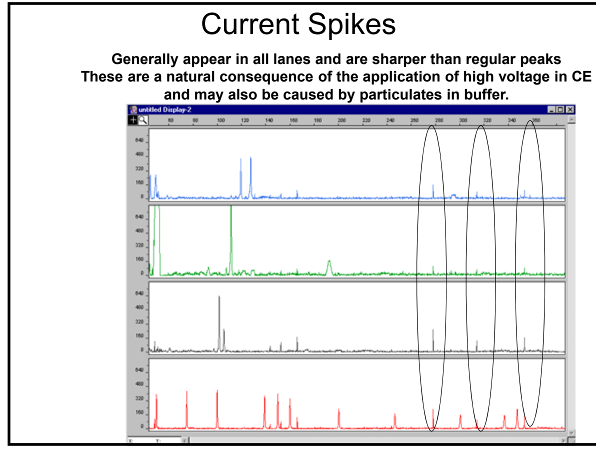
Maintenance of ABI 310/3100/3130

- Syringe – leaks cause capillary to not fill properly
- Capillary storage & wash – **it dries, it dies!**
- Pump block – cleaning helps insure good fill
- Change the running buffer regularly

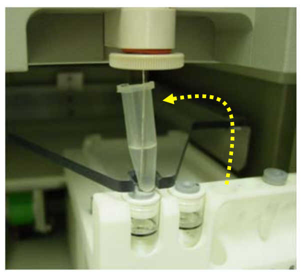
YOU MUST BE CLEAN AROUND A CE!

Current Spikes

Generally appear in all lanes and are sharper than regular peaks
These are a natural consequence of the application of high voltage in CE and may also be caused by particulates in buffer.

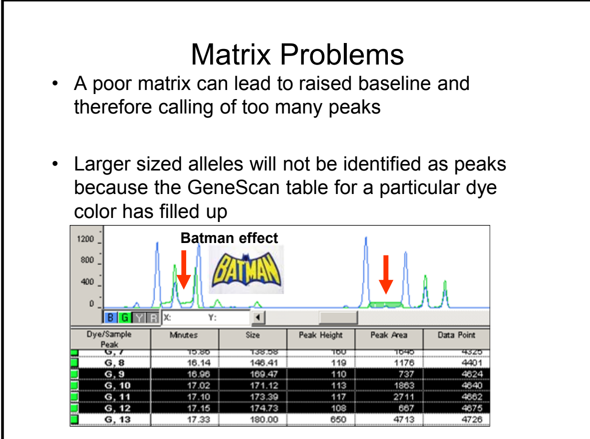


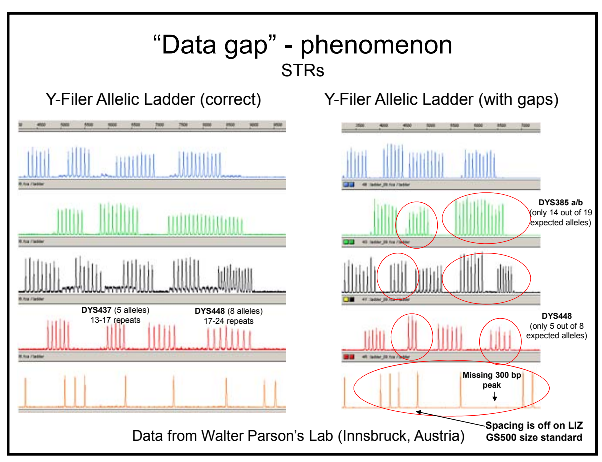
Storage when ABI 310 is not in use

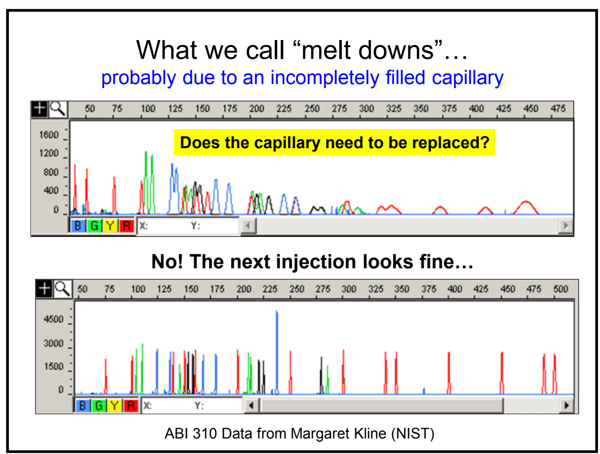


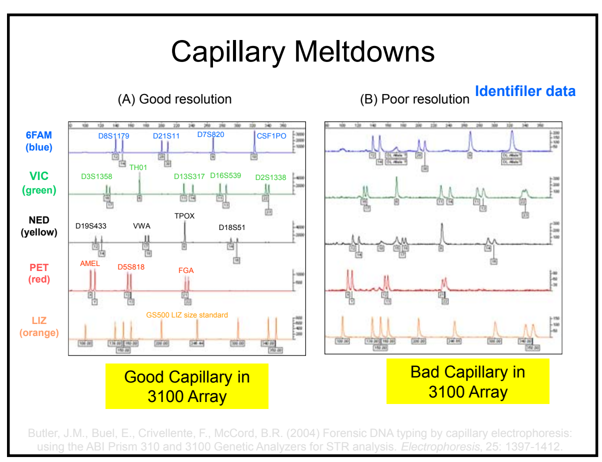
Remember that the water in the open tube will evaporate over time… Also this will destroy the electode if turned on without removing the tube

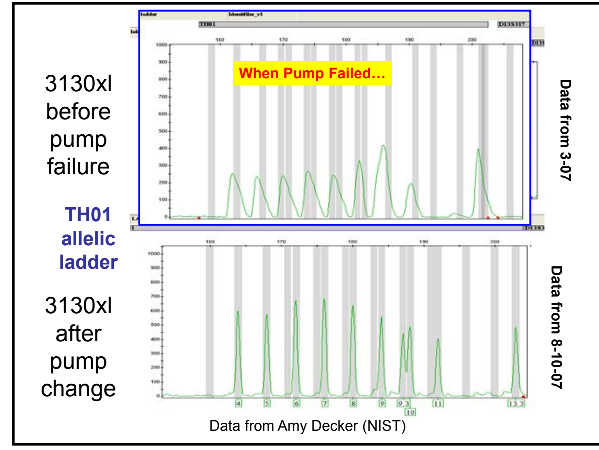
- Keep inlet of capillary in water…if it dries out then urea crystals from the polymer will clog the opening
- The waste vial (normally in position 3) can be moved into position
- A special device can be purchased from Suppelco to rinse the capillary off-line
- Store in distilled water
- Note that the laser is on when the instrument is on

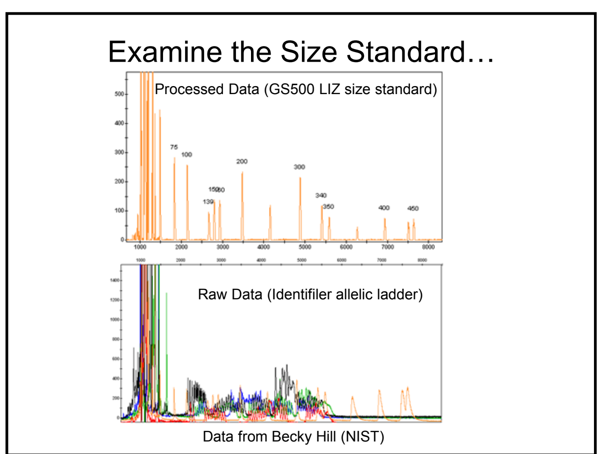

Matrix Problems
• A poor matrix can lead to raised baseline and therefore calling of too many peaks
• Larger sized alleles will not be identified as peaks because the GeneScan table for a particular dye color has filled up
Batman effect
BATMAN
Dye/Sample Peak | Minutes | Size | Peak Height | Peak Area | Data Point
G, 8 | 16.14 | 146.41 | 119 | 1176 | 4401
G, 9 | 16.96 | 169.47 | 110 | 737 | 4624
G, 10 | 17.02 | 171.12 | 113 | 1863 | 4640
G, 11 | 17.10 | 173.39 | 117 | 2711 | 4662
G, 12 | 17.15 | 174.73 | 108 | 667 | 4675
G, 13 | 17.33 | 180.00 | 650 | 4713 | 4726


"Data gap" - phenomenon
STRs
Y-Filer Allelic Ladder (correct)
Y-Filer Allelic Ladder (with gaps)
DYS385 a/b (only 14 out of 19 expected alleles)
DYS437 (5 alleles) 13-17 repeats
DYS448 (8 alleles) 17-24 repeats
DYS448 (only 5 out of 8 expected alleles)
Missing 300 bp peak
Spacing is off on LIZ GS500 size standard
Data from Walter Parson's Lab (Innsbruck, Austria)


What we call "melt downs"…
probably due to an incompletely filled capillary
Does the capillary need to be replaced?
No! The next injection looks fine…
ABI 310 Data from Margaret Kline (NIST)


Capillary Meltdowns
(A) Good resolution
(B) Poor resolution
Identifiler data
6FAM (blue)
VIC (green)
NED (yellow)
PET (red)
LIZ (orange)
D8S1179
D21S11
D7S820
CSF1PO
D3S1358
TH01
D13S317
D16S539
D2S1338
D19S433
VWA
TPOX
D18S51
AMEL
D5S818
FGA
GS500 LIZ size standard
Good Capillary in 3100 Array
Bad Capillary in 3100 Array
Butler, J.M., Buel, E., Crivellente, F., McCord, B.R. (2004) Forensic DNA typing by capillary electrophoresis: using the ABI Prism 310 and 3100 Genetic Analyzers for STR analysis. Electrophoresis, 25: 1397-1412.


3130xl before pump failure
When Pump Failed…
Data from 3-07
TH01 allelic ladder
3130xl after pump change
Data from 8-10-07
Data from Amy Decker (NIST)

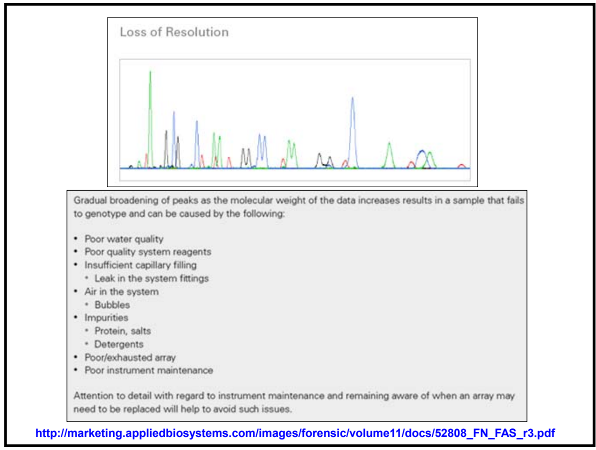

Examine the Size Standard…
Processed Data (GS500 LIZ size standard)
Raw Data (Identifiler allelic ladder)
Data from Becky Hill (NIST)

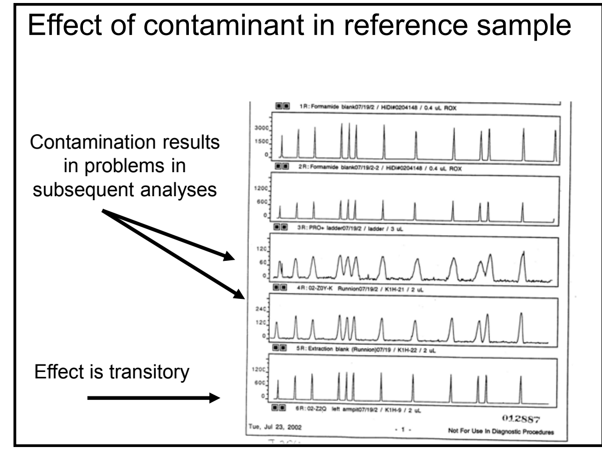
Meltdowns can be the result of

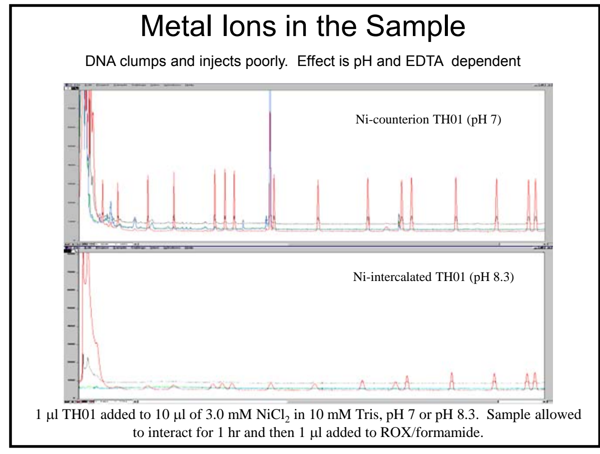
- Bad formamide
- Bubbles in the sample vial
- Water in the polymer buffer
- Syringe leak or bottom out
- Poisoned capillary
- Conductive polymer buffer due to urea degradation
- Crack/shift in capillary window
- Detergents and metal ions in sample

A permanent loss of resolution may mean

- Adsorptive sites on a capillary
- Initiation of electroosmotic flow
- Conductivity changes in buffer/polymer
- Wrong buffer formulation
- Bad formamide or internal lane standard
- Contaminated syringe







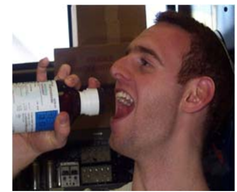
Troubleshooting benchmarks

- **Monitor run current**
- Observe syringe position and movement during a batch
- Examine ILS (ROX) peak height with no sample
- Observe "250 bp" peak in GS500 size standard
- Monitor resolution of TH01 9.3/10 in allelic ladder and size standard peak shapes
- **Keep an eye on the baseline signal/noise**
- Measure formamide conductivity
- Reagent blank – are any dye blobs present?
- See if positive control DNA is producing typical peak heights (along with the correct genotype)

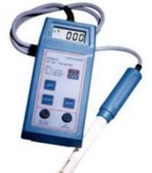
Measurement of Current

- V/I = R where R is a function of capillary diameter, [buffer], and buffer viscosity
- In a CE system the voltage is fixed, thus changes in resistance in the capillary will be reflected in the current observed
- Air bubbles, syringe leaks, alternate paths to ground, changes in temperature, changes in zeta potential, and contamination, will be reflected in the current
- A typical current for a CE system with POP4 buffer is **8-12 µA** (microamps)

Measuring Formamide Conductivity



(not this way)



The key is to measure the bottle when it comes in or buy the good stuff and immediately pipette it out into small tubes with or without ROX already added. Then freeze the tubes.

Do not ever open a cold bottle of formamide. Water will condense inside and aid in the formation of conductive formic acid.

Conclusion:
Troubleshooting is more than following the protocols

It means keeping watch on all aspects of the operation

1. Monitoring conductivity of sample and formamide
2. Keeping track of current and syringe position in log.
3. Watching the laser current
4. Watching and listening for voltage spikes
5. Monitoring room temperature and humidity

ABI 3500 Genetic Analyzer

John

ABI 3500 Genetic Analyzer

- **3500 (8 capillary)**
- **3500xl (24 capillary)**

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**

Details of the new ABI 3500

No lower pump block
(fewer air bubbles)

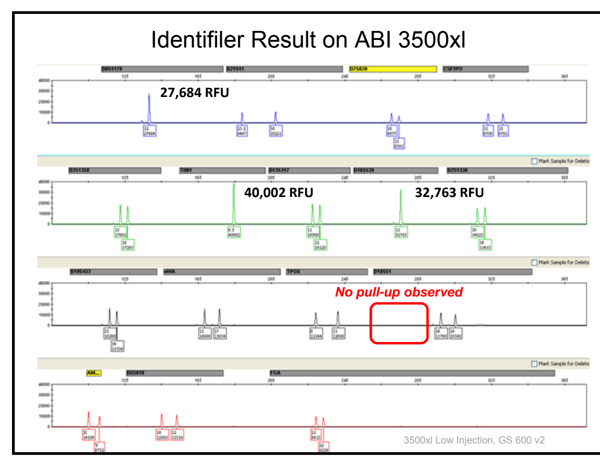
Improved sealing for better temperature control
(improved precision?)

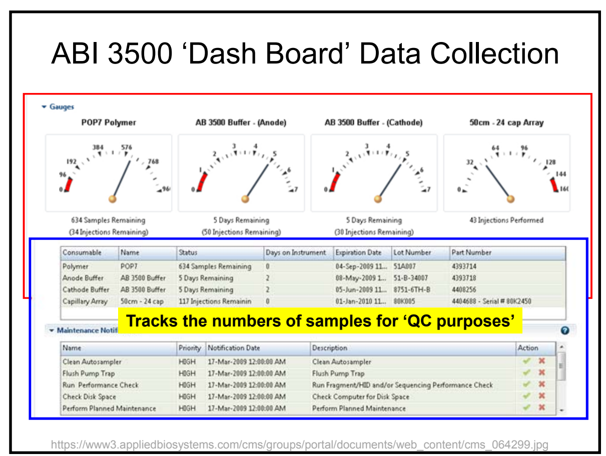
Better seal around the detector

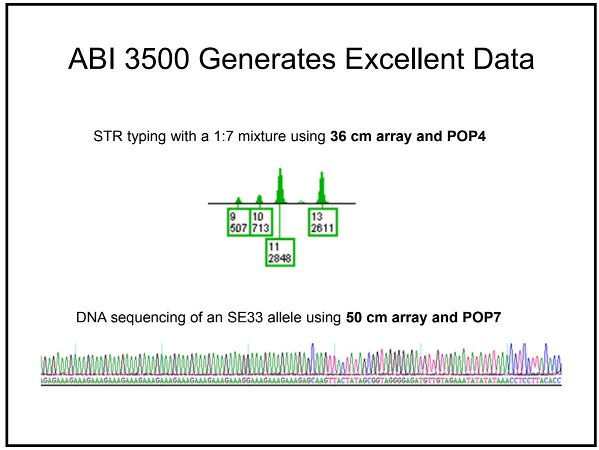
Reagents prepackaged with RFID tags

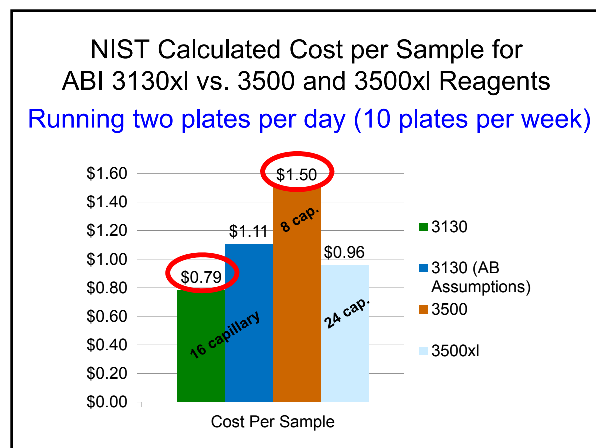
Primary Differences

	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









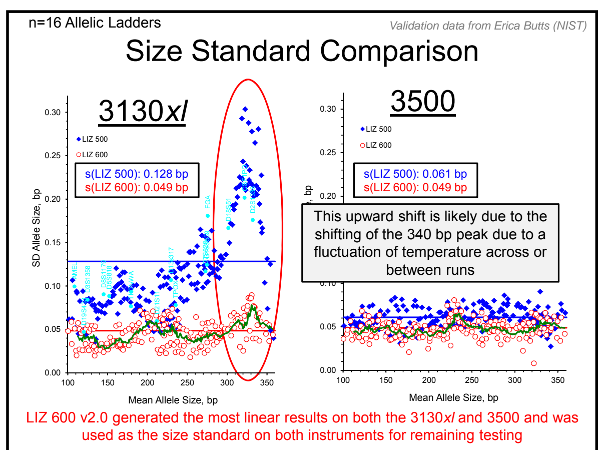
Consumable RFID Tracking Limits

	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)

n=16 Allelic Ladders

Validation data from Erica Butts (NIST)

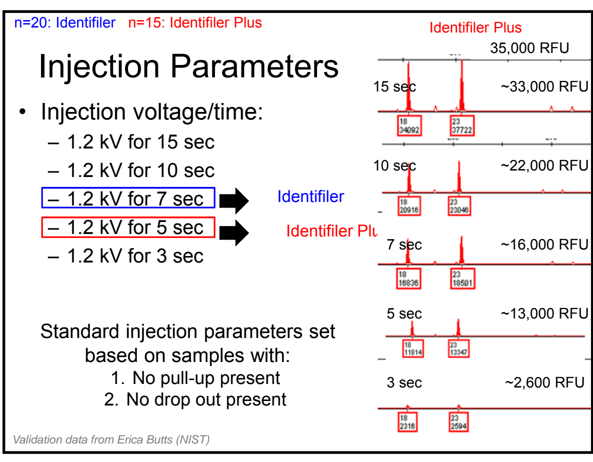
Size Standard Comparison



LIZ 600 v2.0 generated the most linear results on both the 3130*xl* and 3500 and was used as the size standard on both instruments for remaining testing

n=20: Identifiler n=15: Identifiler Plus

Injection Parameters

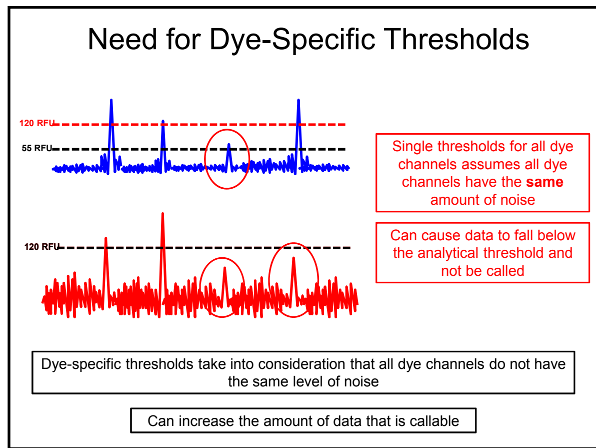


- Injection voltage/time:
  - 1.2 kV for 15 sec
  - 1.2 kV for 10 sec
  - 1.2 kV for 7 sec → Identifiler
  - 1.2 kV for 5 sec → Identifiler Plus
  - 1.2 kV for 3 sec

Standard injection parameters set based on samples with:
1. No pull-up present
2. No drop out present

Validation data from Erica Butts (NIST)

Need for Dye-Specific Thresholds



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

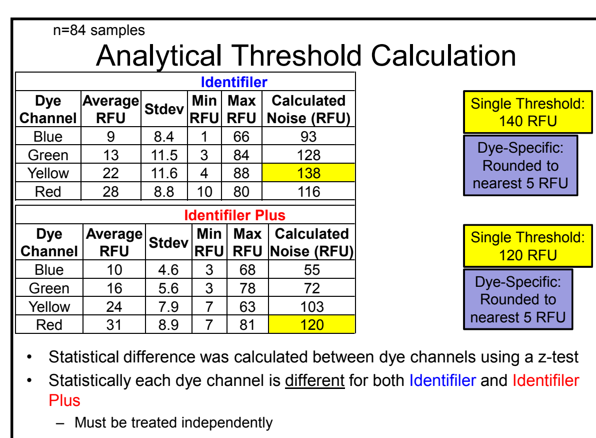
Can cause data to fall below the analytical threshold and not be called

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 different for both Identifiler and Identifiler Plus
  - Must be treated independently

Validation data from Erica Butts (NIST)

n=560 alleles

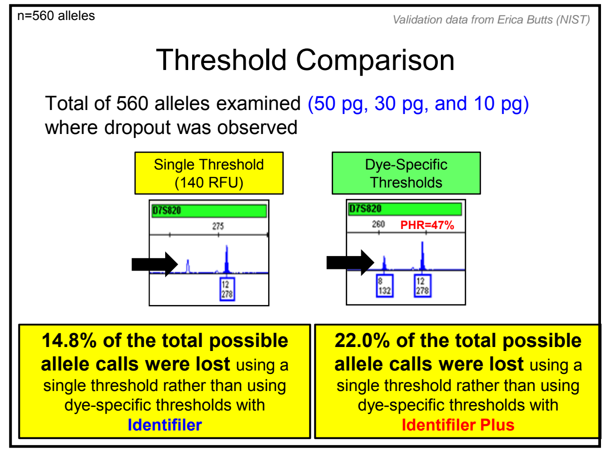
Validation data from Erica Butts (NIST)

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**

Validation data from Erica Butts (NIST)

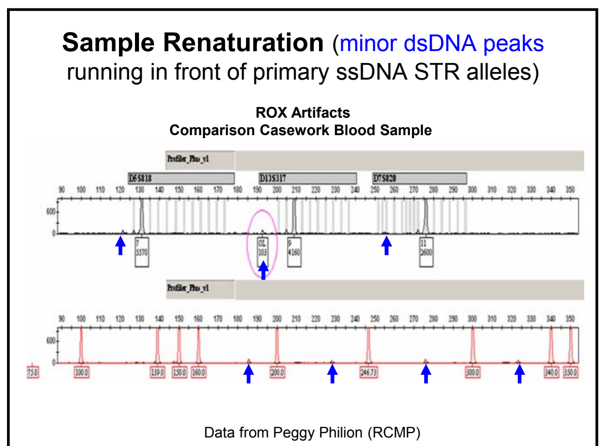
ABI 3500 Validation Considerations

- 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

Questions ?

Example Problems Seen
and Provided by Others in the Past

Sample Renaturation (minor dsDNA peaks running in front of primary ssDNA STR alleles)



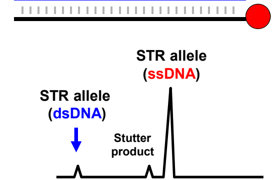
Data from Peggy Philion (RCMP)

Why dsDNA migrates through CE capillary faster than ssDNA…

- DNA molecule separation depends on interactions with the polymer
  - Higher polymer concentration (or longer polymer molecules) permits more polymer interactions and provides better resolution (i.e., POP-6 vs POP-4)
- **Single-stranded DNA (ssDNA) is more flexible than double-stranded DNA (dsDNA)** and therefore moves more slowly through the capillary because it is interacting with polymer strands more

dsDNA vs ssDNA CE Migration

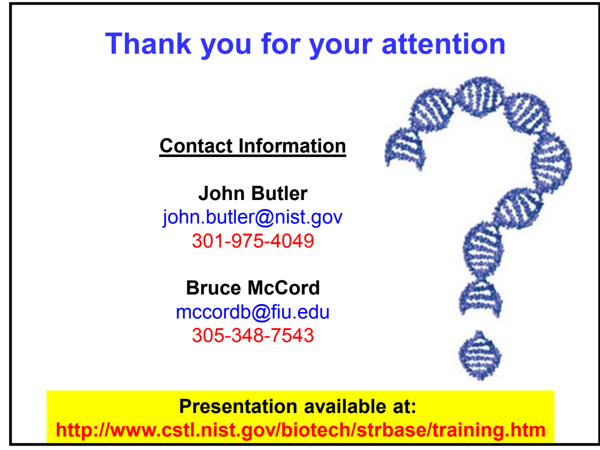
- If a small amount of the complementary strand re-hybridizes to the labeled STR allele strand, then a little peak will be seen in-front of each internal lane standard peak and



•Height of dsDNA peak will depend on amount of re-hybridization between the two strands (**some loci will re-hybridize more readily giving rise to larger dsDNA peaks**)

•Local temperature environment of capillary impacts amount of re-hybridization (may change over time)

Thank you for your attention



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http://www.cstl.nist.gov/biotech/strbase/training.htm