CIB Forensic Science Center
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Capillary Electrophoresis & Troubleshooting

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Presentation Outline

- History and background on CE
- Fundamentals of CE
  - sample prep, injection, separation, detection
- ABI 3500
- Troubleshooting strategies and solutions
- Questions

My Goal:
To help you understand the basic chemistry behind DNA separations and to help make CE instruments less of a "black box"

NIST and NIJ Disclaimer

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

Points of view are mine 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

Steps in DNA Analysis

Usually 1-2 day process (a minimum of ~5 hours)

Steps Involved

- Collection
- Specimen Storage
- Extraction
- Quantitation
- Multiplex PCR
- STR Typing
- Interpretation of Results
- Database Storage & Searching
- Calculation of Match Probability

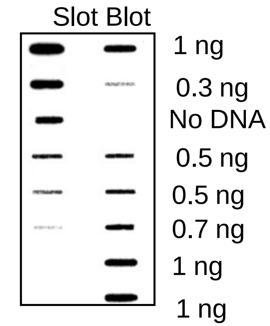
Blood Stain

Buccal swab

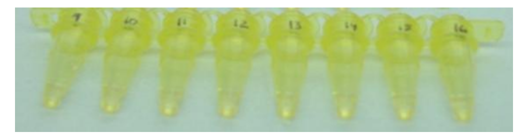
Sample Collection & Storage

Biology

DNA Extraction



DNA Quantitation



Multiplex PCR Amplification

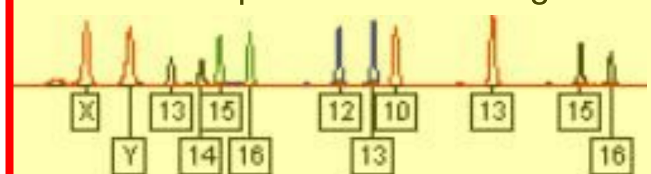
Genetics

If a match occurs, comparison of DNA profile to population allele frequencies to generate a case report with probability of a random match to an unrelated individual

DNA Database Search

Technology

DNA separation and sizing

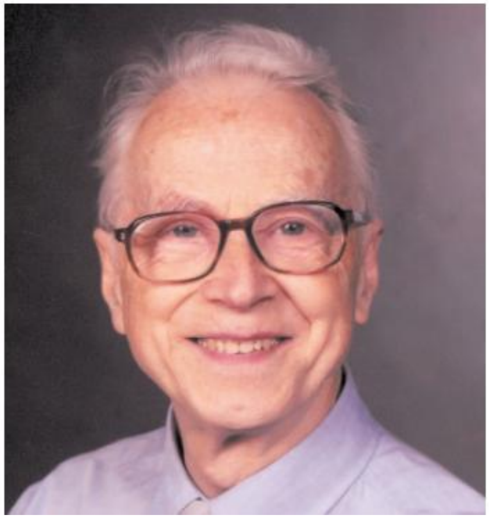


STR Typing

Male: 13,14-15,16-12,13-10,13-15,16

Interpretation of Results

Pioneers of Capillary Electrophoresis



Stellan Hjertén
Uppsala University

1967
First high voltage CE system (with rotating 3 mm i.d. capillaries)

James Jorgenson
University of North Carolina

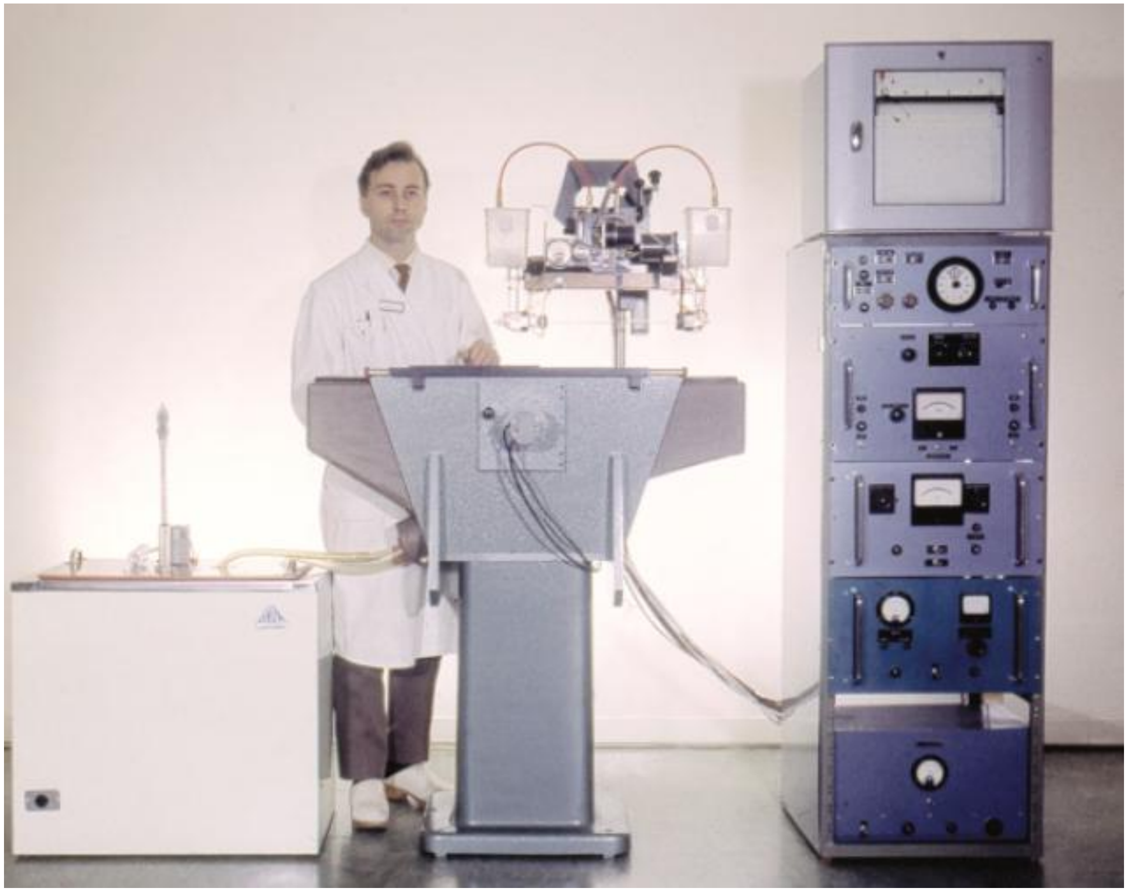
1981
First “modern” CE experiments (with 75 µm i.d. capillaries)

Barry Karger
Northeastern University

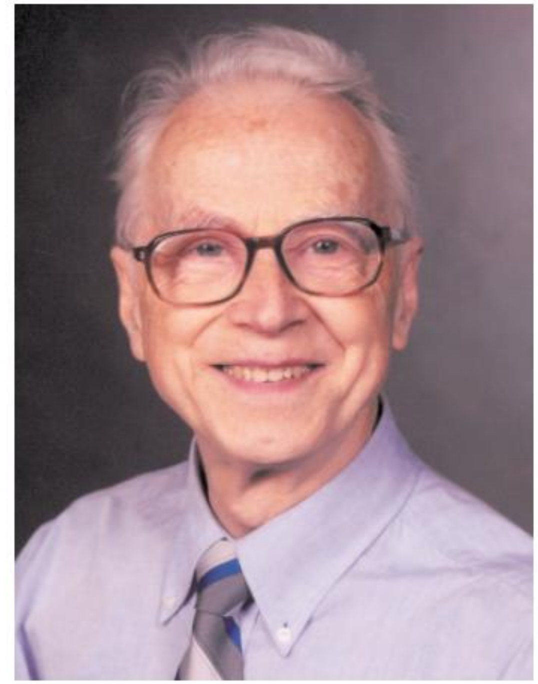
1988/90
First DNA separations in a capillary (gel-filled/ sieving polymer)

Stellan Hjertén

Uppsala University (Sweden)



With first fully automated capillary free zone electrophoresis apparatus in 1967



In 2003 at age 75

Received his PhD (1967) under Professor **Arne Tiselius** who had developed moving boundary zone electrophoresis in 1937 **(Noble Prize in 1948)**

A Brief History of Capillary Electrophoresis

- 1937 – Tiselius develops moving boundary electrophoresis
- 1967 – Hjertén uses rotating 3 mm i.d. tubes for CE
- **1981 – Jorgenson and Lukacs demonstrate first high performance CE separations with 75 µm i.d. capillary**
- 1988 – Karger's group shows DNA separations of single stranded oligonucleotides with gel-filled capillaries
- 1990 – Karger's group shows DNA separations with sieving polymers on DNA restriction fragments
- 1991 – Grossman expands work with sieving polymers
- 1992 – Bruce McCord starts working on PCR product separations with STR allelic ladders

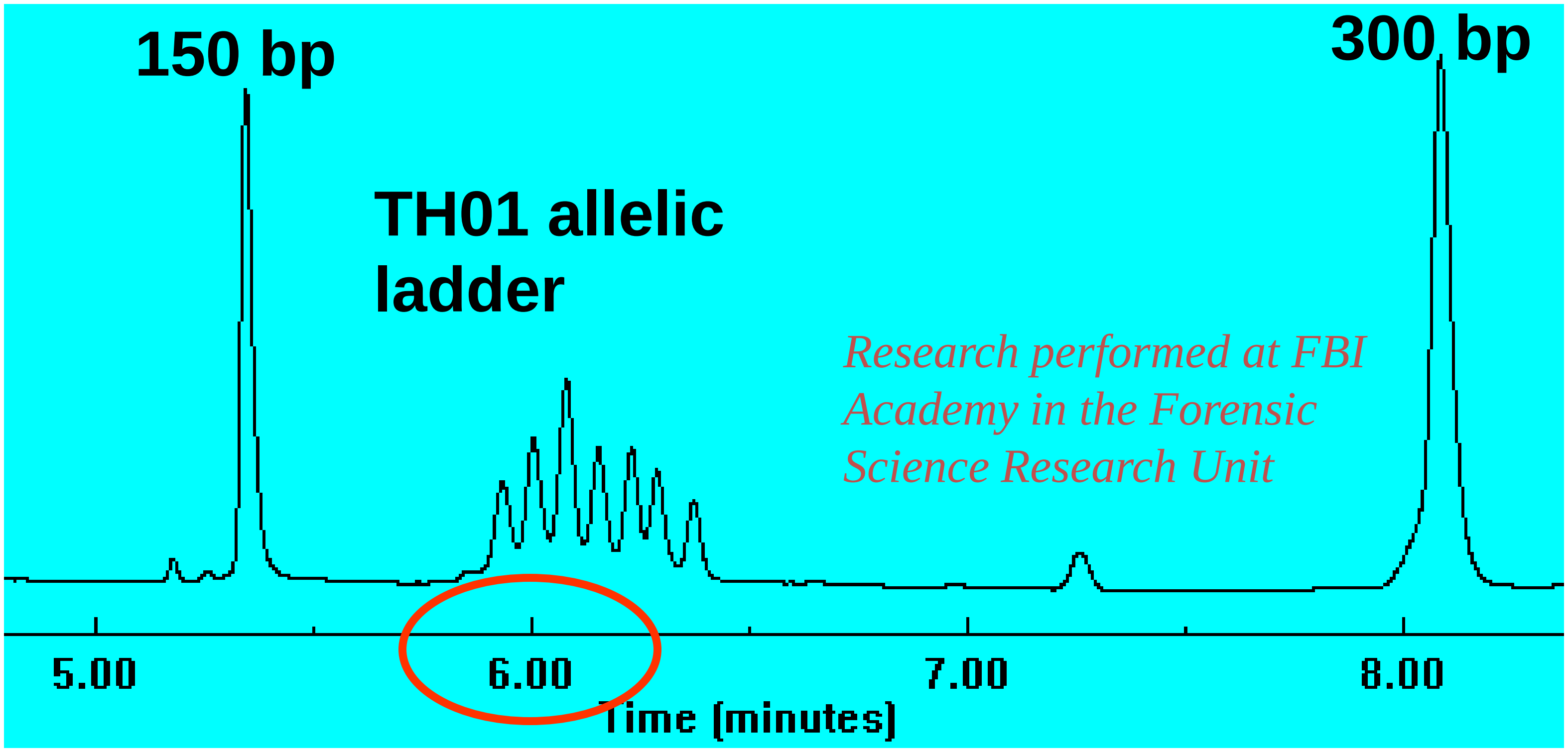
My Experience with CE, STRs, etc.

- May 1993 – began working in Bruce McCord's lab at Quantico
- Sept 1993 – developed mtDNA amplicon quantitation method (used in FBI casework from 1996 to present)
- Nov 1993 – **first demonstration of STR typing by CE** (using dual internal standards and TH01 ladder)
- July 1995 – defended Ph.D. dissertation entitled "Sizing and Quantitation of Polymerase Chain Reaction Products by Capillary Electrophoresis for Use in DNA Typing"
- July 1995 – ABI 310 Genetic Analyzer was released

First Rapid STR Typing with Capillary Electrophoresis

Single color detection with dual internal size standards

Butler *et al.* (1994) *BioTechniques* 17: 1062-1070



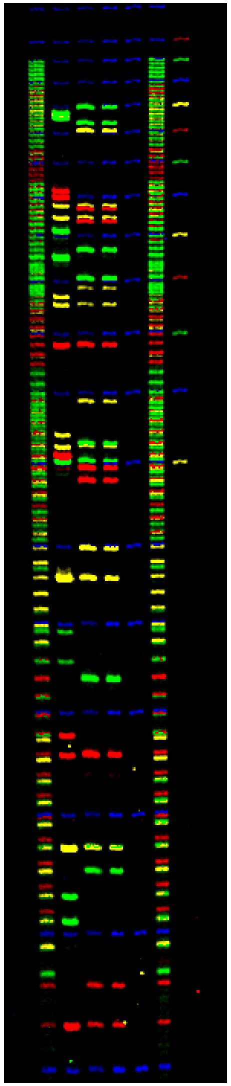
Performed in December 1993

Technology Implementation Takes Time – the FBI did not start running casework samples using STRs and CE until January 1999

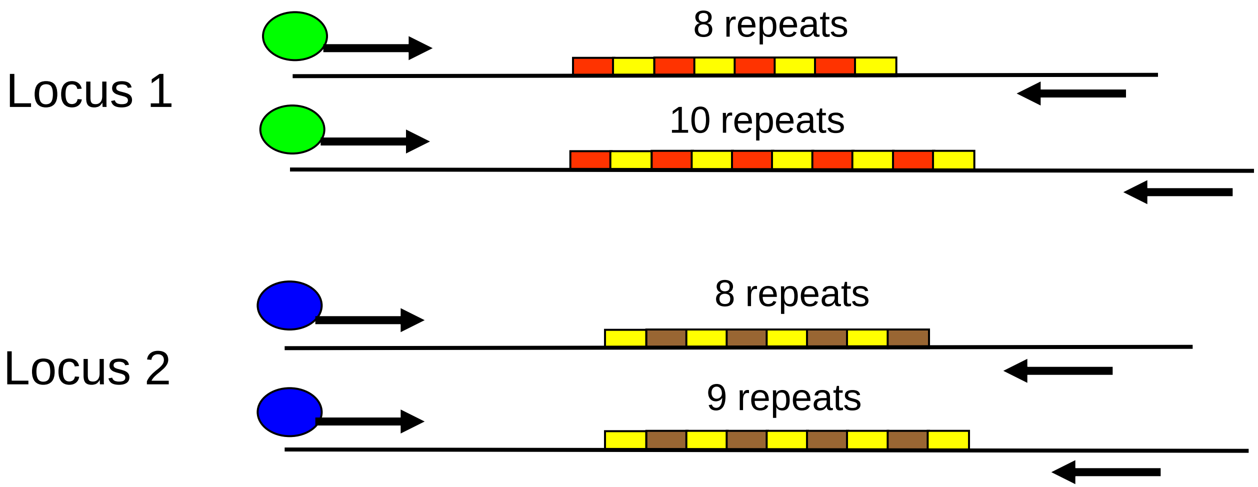
My Experience with CE, STRs, etc. (cont.)

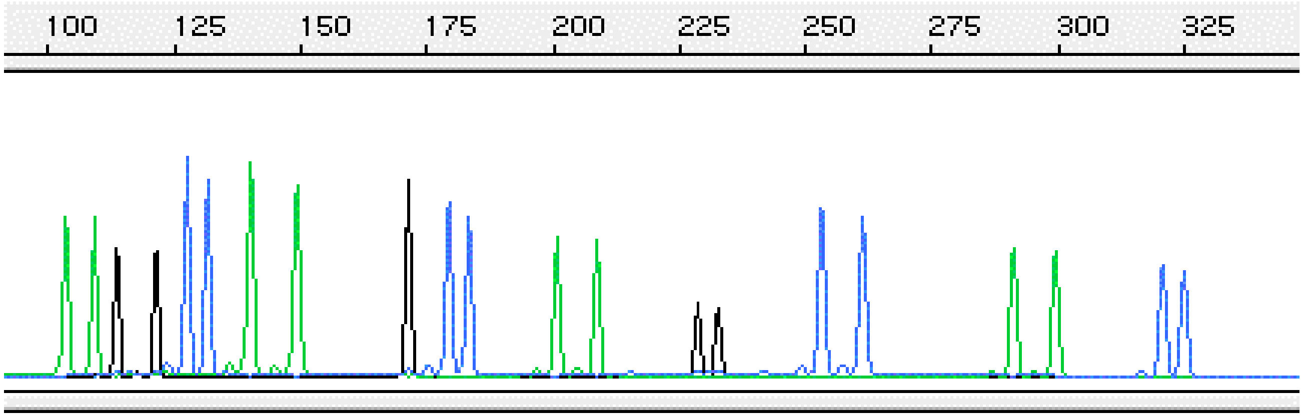
- 1996-1997 Developed STRBase while a postdoc at NIST
- Nov 1998 – GeneTrace Systems purchased a 310; typed several hundred samples with Profiler Plus and Cofiler kits and compared results to mass spec STR analysis
- **1999-present – Run thousands of samples with all STR kits available (except PP 1.2) and developed a number of new STR multiplex systems**
- Jan 2001 – Published "*Forensic DNA Typing: Biology and Technology behind STR Markers*" (2nd Edition in Feb 2005)
- April 2001-present – Use of ABI 3100 16-capillary array system

STR Allele Separation Can Be Performed by Gel or Capillary Electrophoresis with Detection of Fluorescent Dyes Labeling Each PCR Product



Scanned **Gel Image**





Capillary Electropherogram

Why Use CE for DNA Analysis?

1. Injection, separation, and detection are automated.
2. Rapid separations are possible
3. Excellent sensitivity and resolution
4. The time at which any band elutes is precisely determined
5. Peak information is automatically stored for easy retrieval

Symbol first used in Oct 1994 at the Promega meeting when I had a poster introducing the use of CE for STR typing

Important Differences Between CE and Gels

- **Room temperature control** is essential for run-to-run precision
  - CE uses sequential rather than simultaneous separations
  - Usually need < 2.0 ^{o}C (must inject allelic ladder regularly)

- **Lower amount of DNA loaded** (injection = nL vs µL) and thus detection sensitivity must be better

- Electrokinetic injection enables **dye artifacts** (blobs) to enter the capillary or microchip CE channel and thus possibly interfere with STR allele interpretation

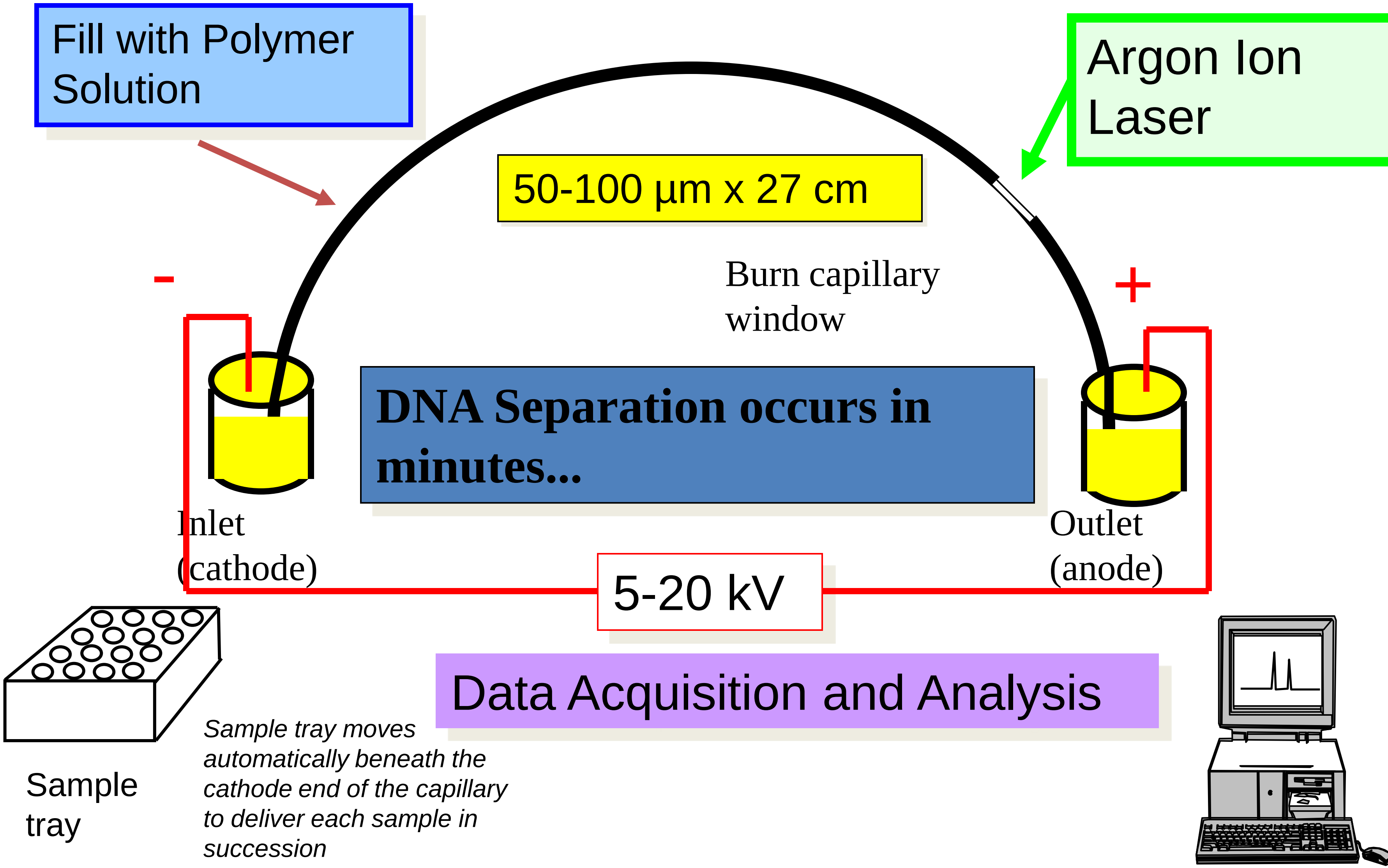
More Differences between CE and Gels…

- Filling the capillary (or microchip CE channel) is analogous to pouring a gel into a tiny tube…

- Must be more clean around a CE system
  - Because the capillaries (μCE channels) are small, particles of dust or urea crystals can easily plug them
  - Tips of capillary cannot dry out (once buffer solutions have been run through them) for the same reasons

- Bubbles are a BIG problem in CE as they can easily block current flow in the capillary…

Capillary Electrophoresis (CE)



Typical Instruments Used for STR Typing

Thermal Cycler for PCR Amplification

GeneAmp 9700

Capillary electrophoresis instruments for separating and sizing PCR products

single capillary

ABI 310

16-capillary array

ABI 3100

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.

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…

Review Article on STRs and CE

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

Electrophoresis 2004, *25*, 1397–1412

Review

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Forensic DN
using the AB
for STR analy

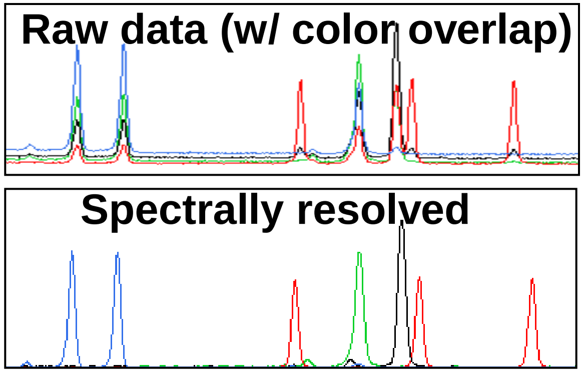
DNA typing with shor
applications includin
such as the ABI Prism
for many laboratories
ing sample preparati
results using CE syste
ered in the context
throughput and ease

Contents

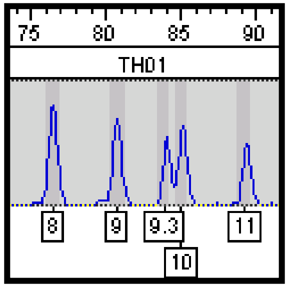
Analytical Requirements for STR Typing

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

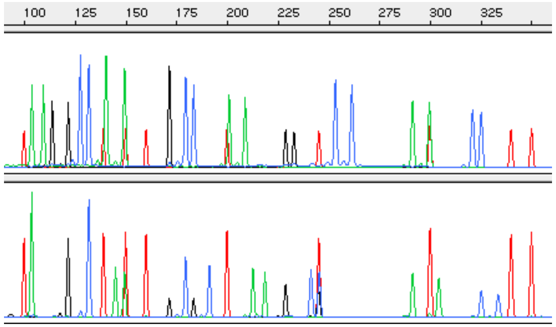
- Fluorescent dyes must be **spectrally resolved** in order to distinguish different dye labels on PCR products



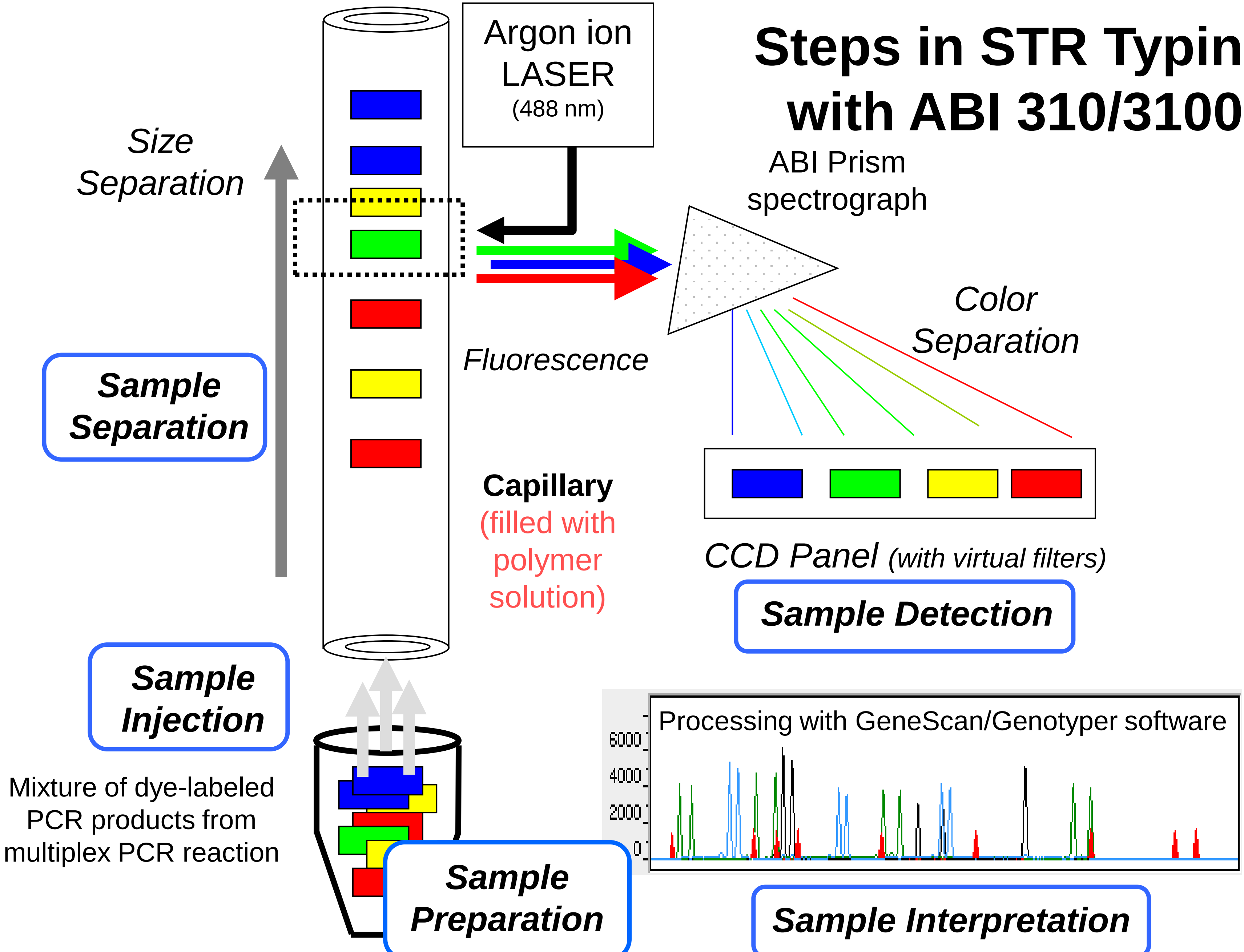
- PCR products must be **spatially resolved** – desirable to have single base resolution out to >350 bp in order to distinguish variant alleles



- High **run-to-run precision** – an internal sizing standard is used to calibrate each run in order to compare data over time

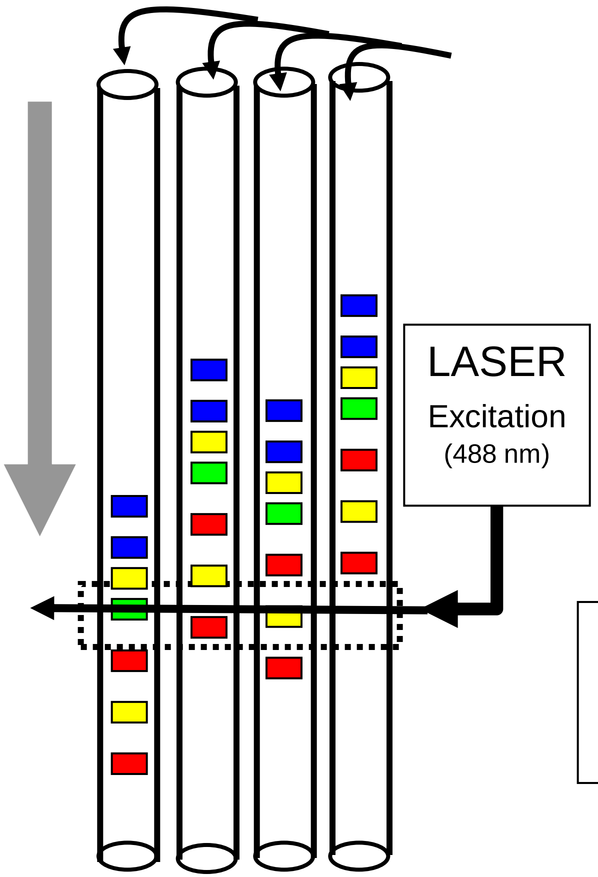


Steps in STR Typing with ABI 310/3100



Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 13.8, © Elsevier Science/Academic Press

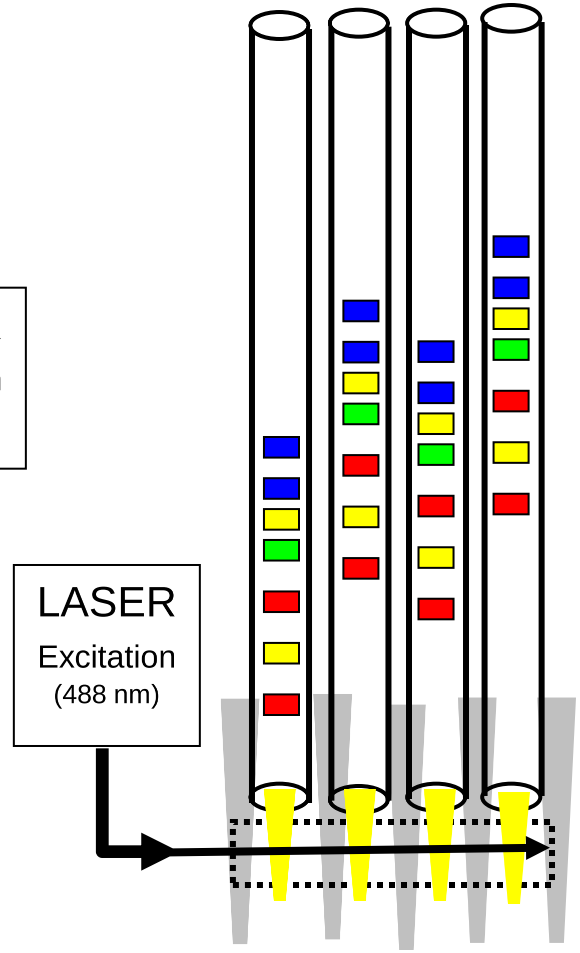
Detection with Multiple Capillaries (Irradiation for Capillary Arrays)



Capillary Array

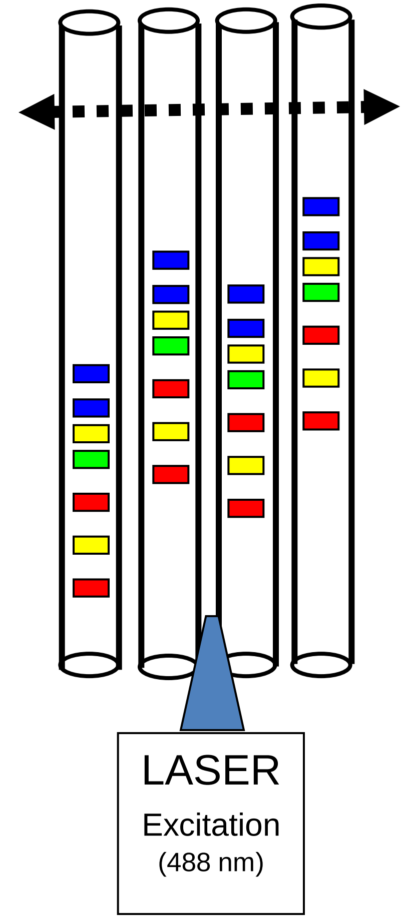
Side irradiation (on-capillary)

ABI 3100, 3130, 3100*Avant*



Sheath flow detection

ABI 3700



Fixed laser, moving capillaries

MegaBACE

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

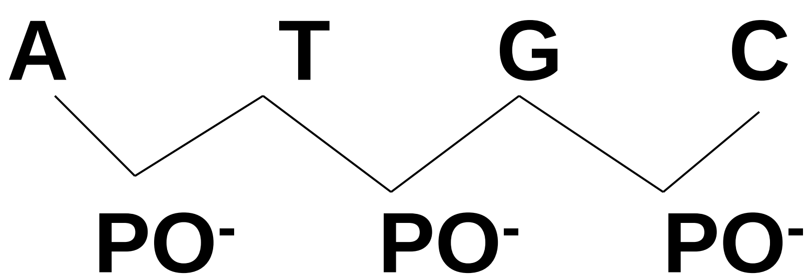
- V = IR (where V is voltage, I is current, and R is resistance)
- Current, or the flow of ions, is what matters most in electrophoresis
- CE currents are much lower than gels because of a higher resistance in the narrow capillary
- CE can run a higher voltage because the capillary offers a higher surface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



As size increases so does charge!

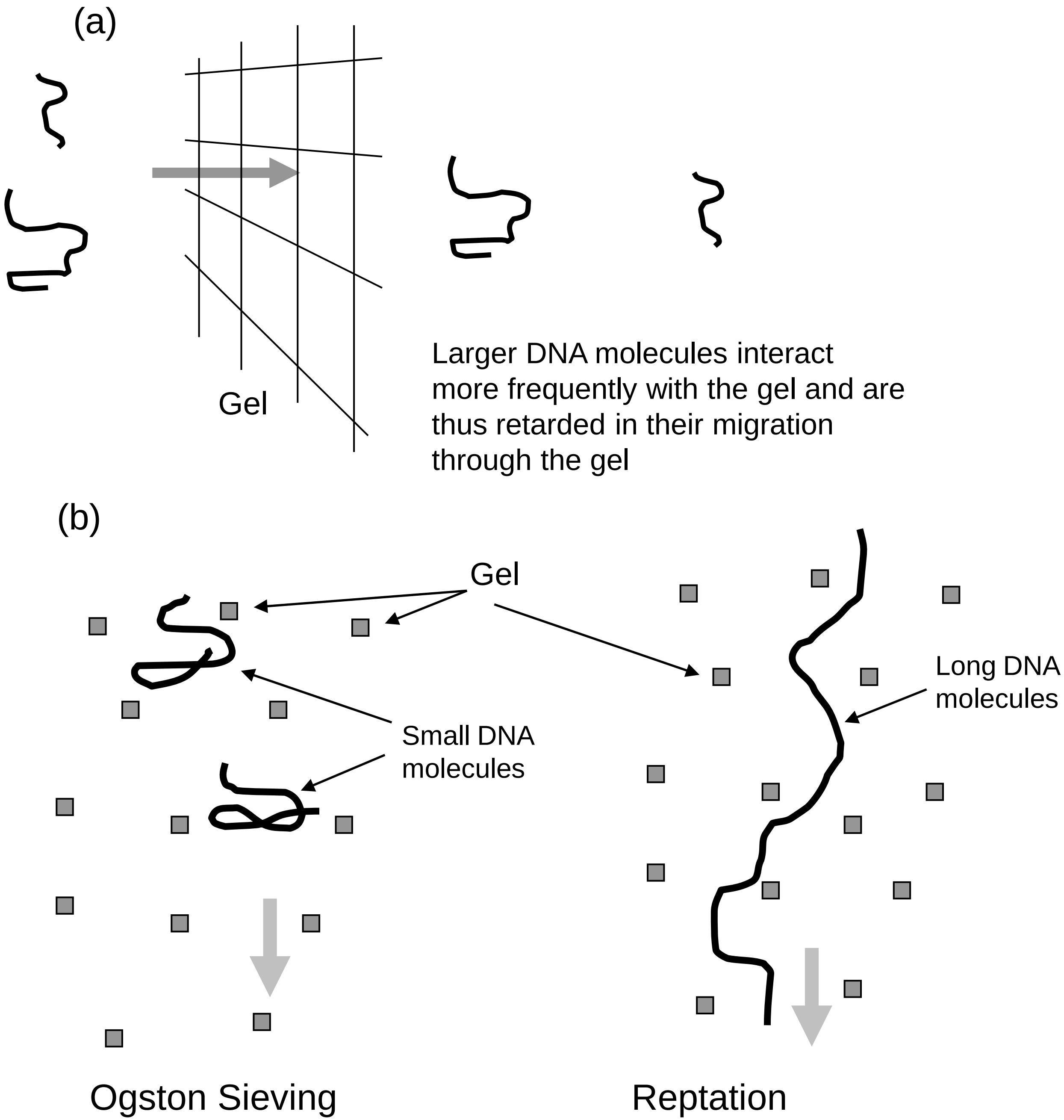


Figure 12.4, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

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 ^{o}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

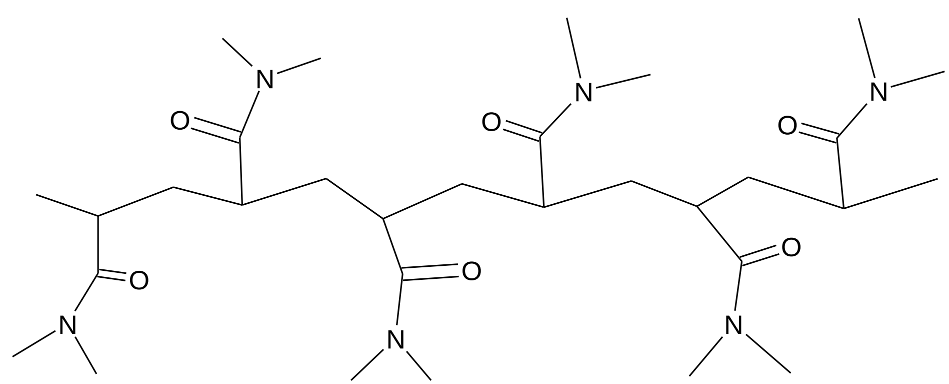
Received May 29, 1997; Revised and Accepted August 6, 1997

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

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

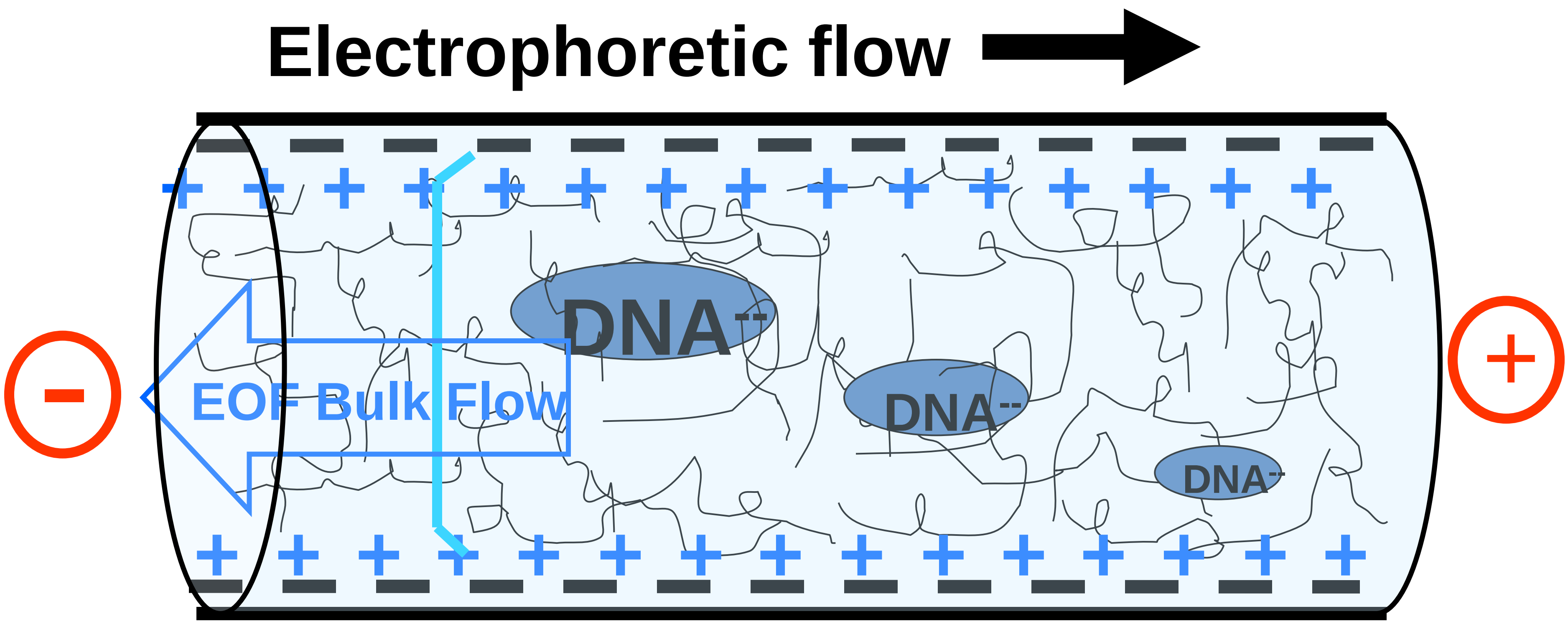
US Patent 5,552,028 covers POP-4 synthesis

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



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**)

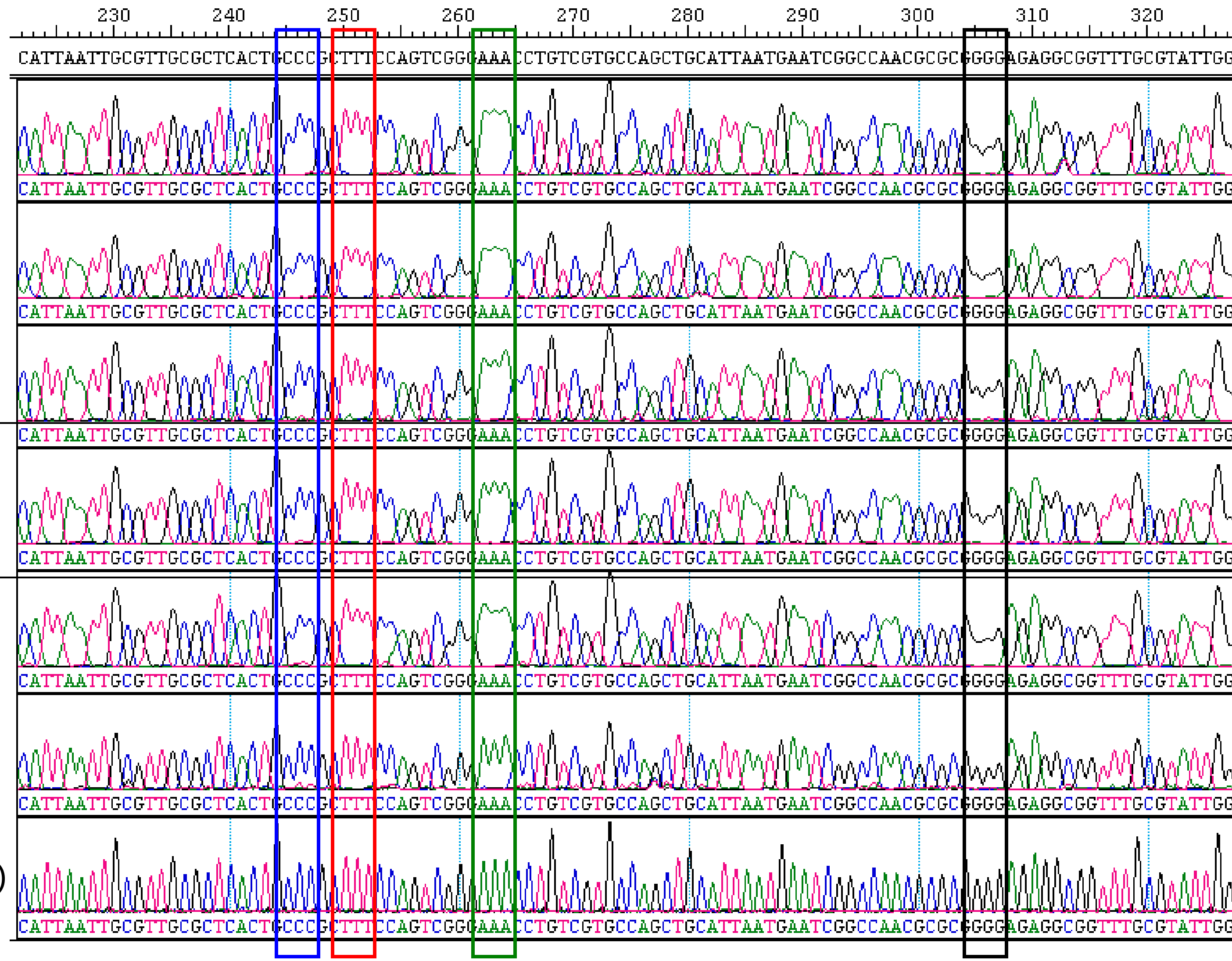
310 **POP6**-36min (**36cm**)

310 **POP6**-50min (**36cm**)

310 **POP6**-120min (**36cm**)

310 **POP6**-120min (**50cm**)

3130 **POP7**-120min (**80cm**)



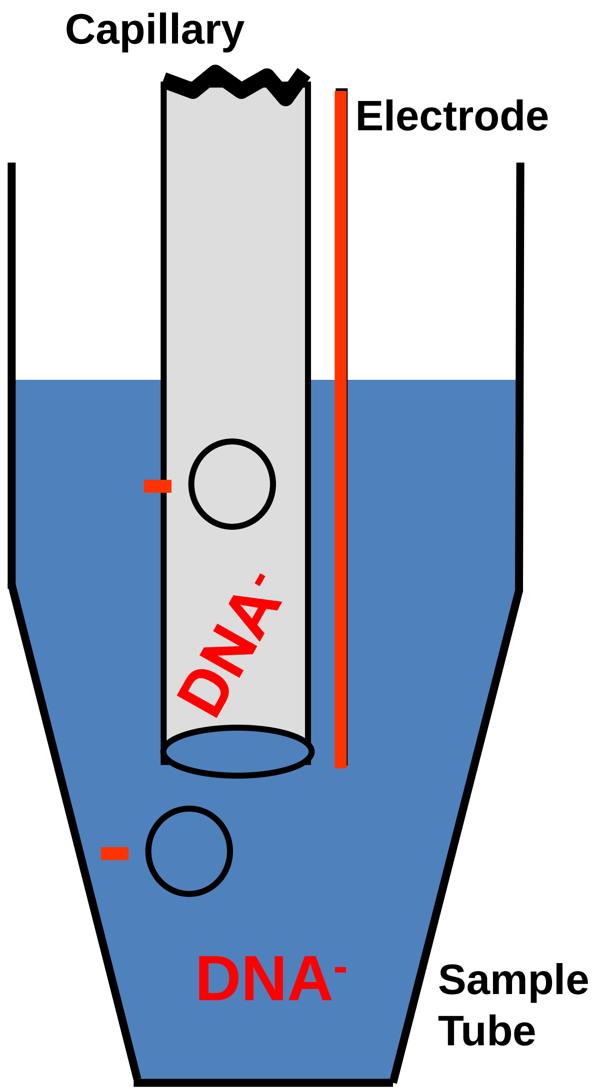
Longer run times at lower voltage

BigDye® Terminator v3.1 Sequencing Kit (Sample: pGEM)

Data collected at NIST by Tomohiro Takamaya (Japanese guest researcher, fall 2007)

Injection

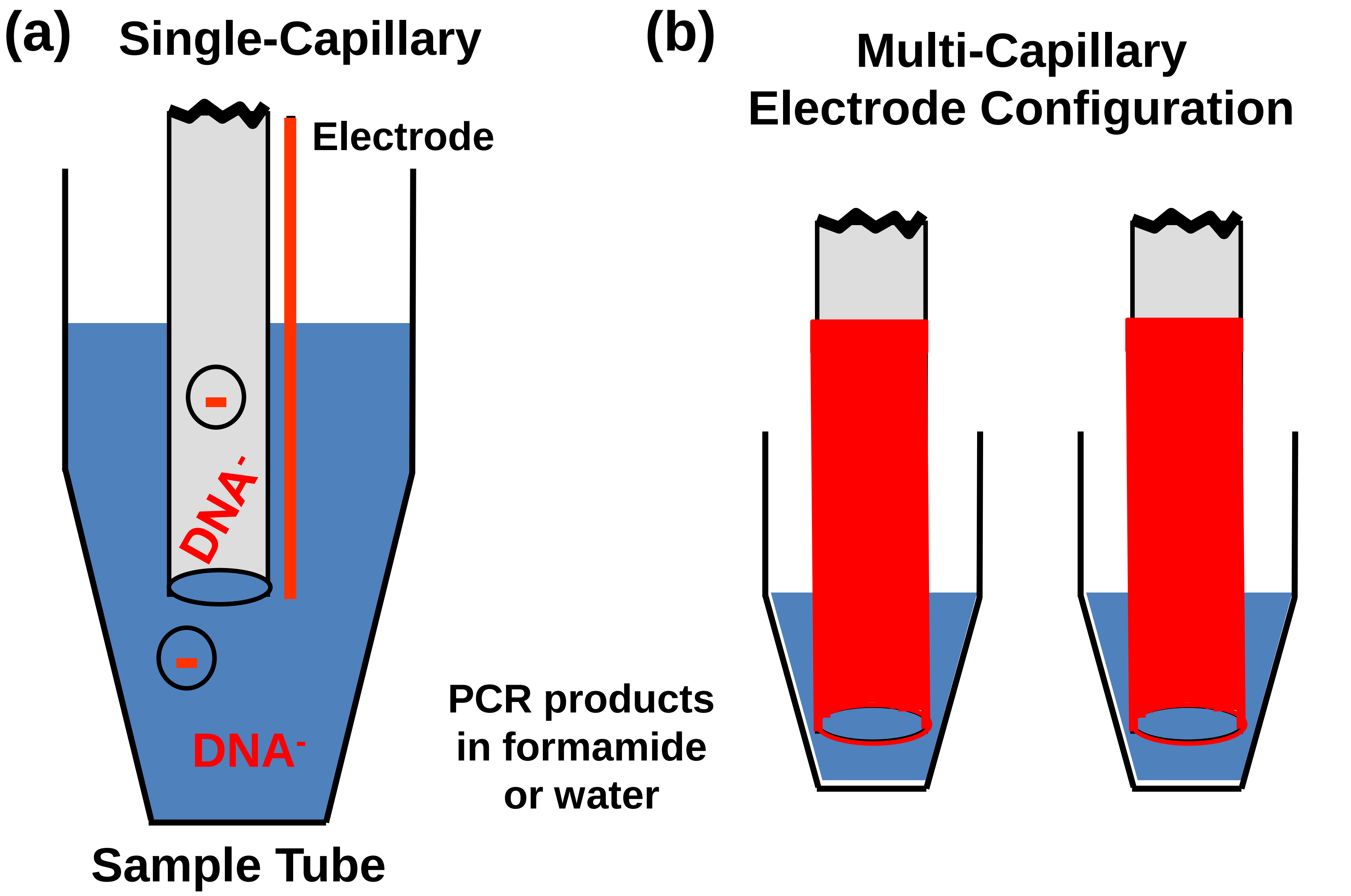
Electrokinetic Injection Process

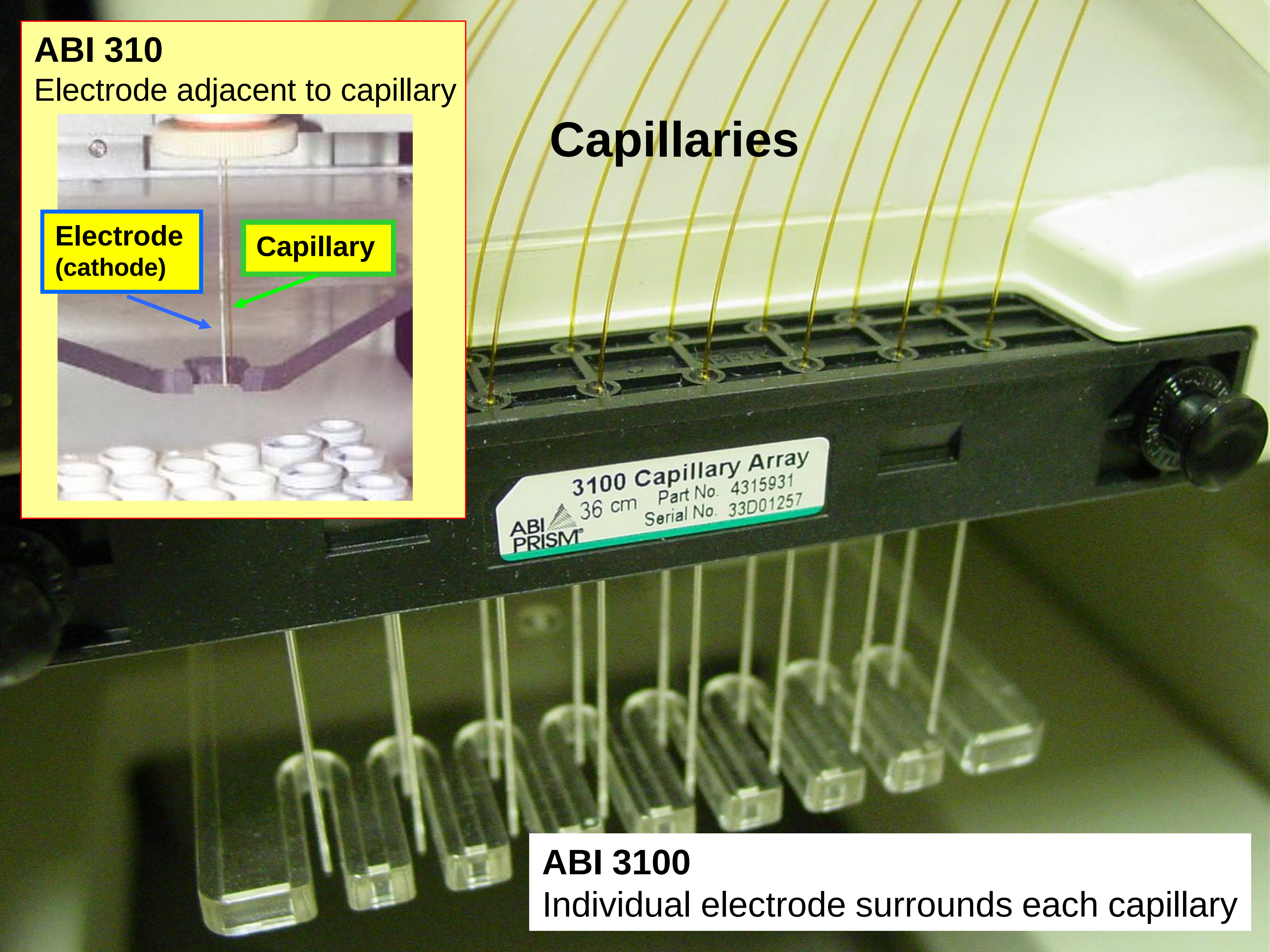


Amount of DNA injected is inversely proportional to the ionic strength of the solution

Salty samples result in poor injections

Capillary and Electrode Configurations




ABI 310
Electrode adjacent to capillary
Electrode (cathode)
Capillary
Capillaries
3100 Capillary Array
36 cm
Part No. 4315931
Serial No. 33D01257
ABI PRISM
ABI 3100
Individual electrode surrounds each capillary

Sample Conductivity Impacts Amount Injected

$$[\mathrm{DNA_{inj}}] = \frac{Et(\pi r^2)\,(\mu_{ep} + \mu_{eof})[\mathrm{DNA_{sample}}]\,(\lambda_{buffer})}{\lambda_{sample}}$$

$[\mathrm{DNA_{inj}}]$ 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

$[\mathrm{DNA_{sample}}]$ 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

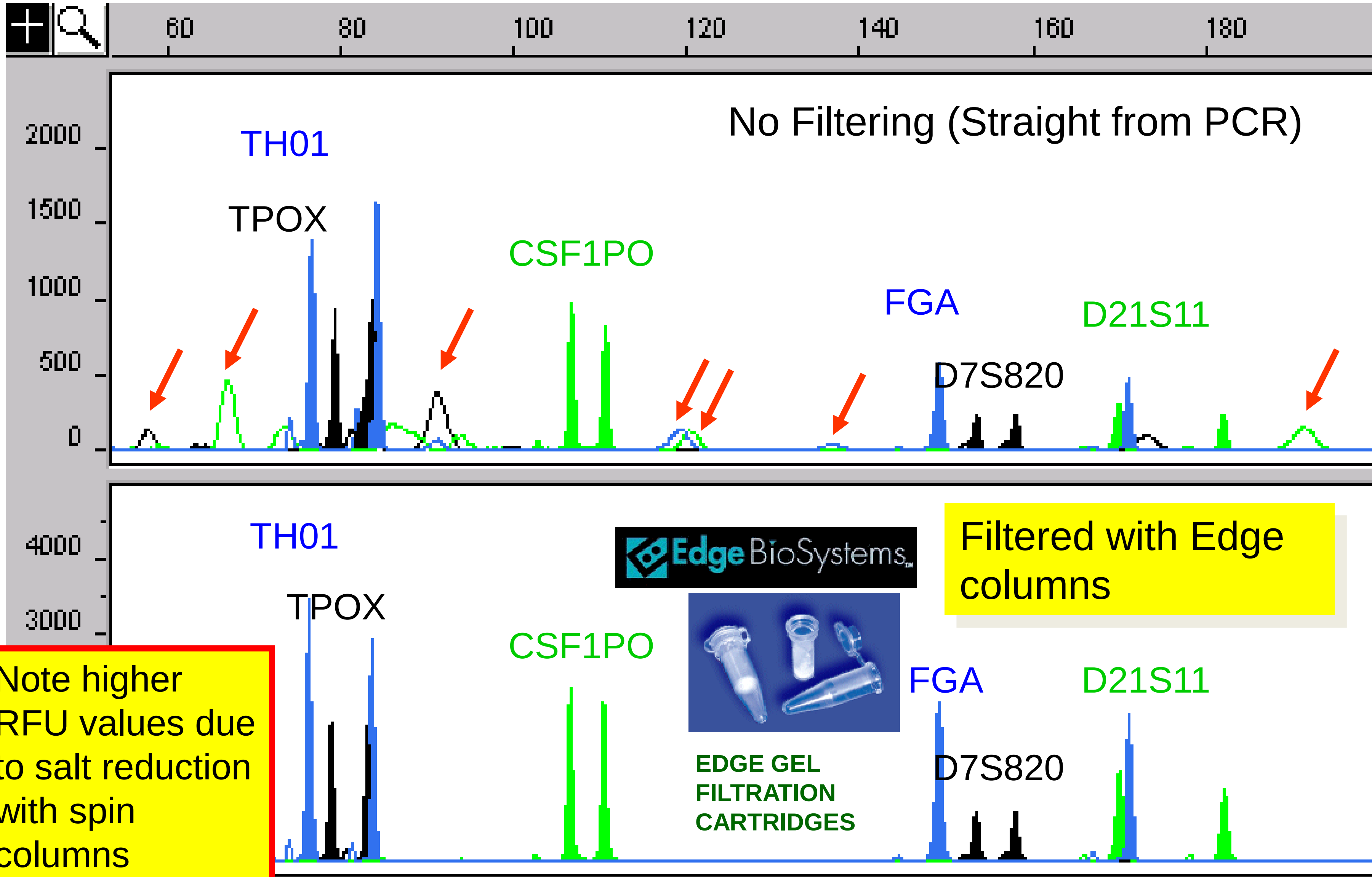
See J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition; Chapter 14

- **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



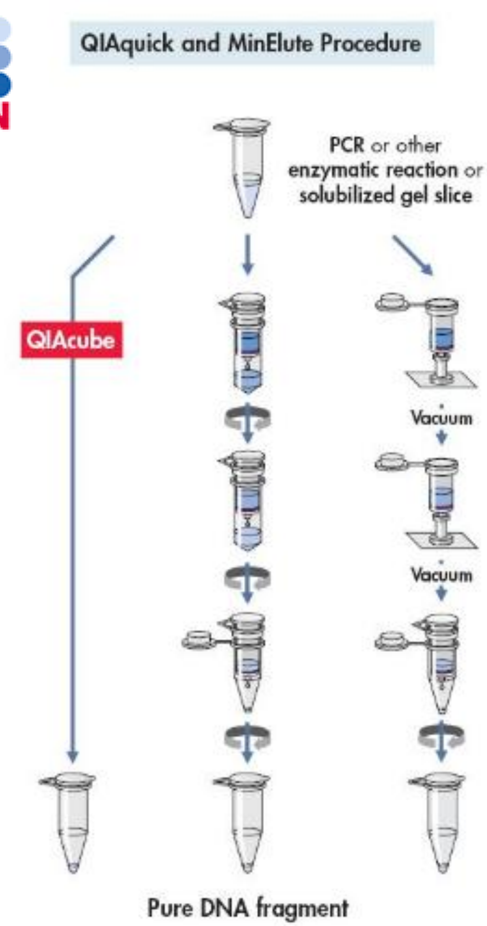
Note higher RFU values due to salt reduction with spin columns

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

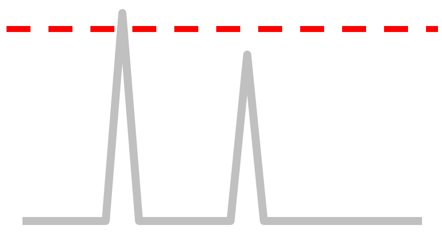


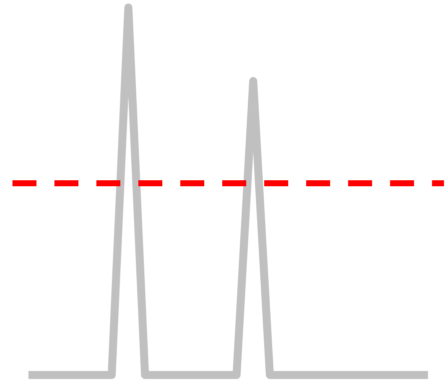
Smith, P.J. and Ballantyne, J. (2007) Simplified low-copy-number DNA analysis by post-PCR purification. *J. Forensic Sci.* 52: 820-829

Stochastic Effects and Thresholds

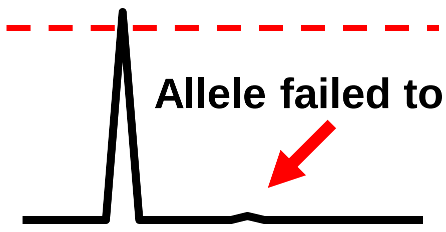
Regular Injection

Injection Following Desalting (MiniElute)



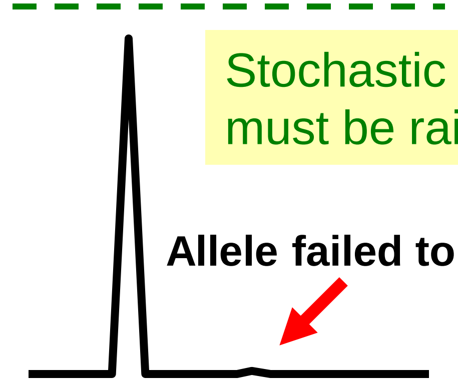


When PCR amplifying low levels of DNA, allele dropout may occur



Allele failed to amplify

False homozygote

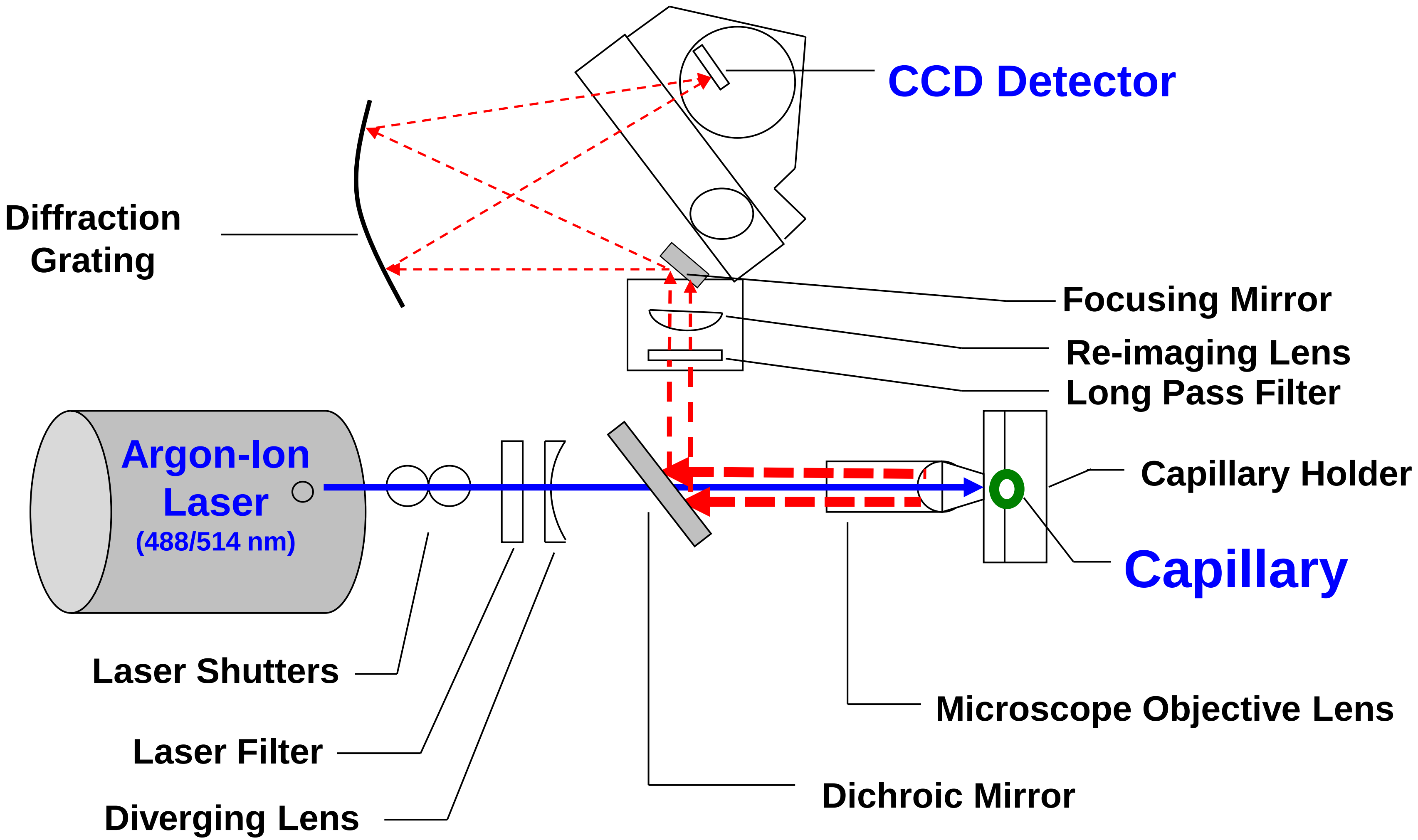


Stochastic threshold must be raised

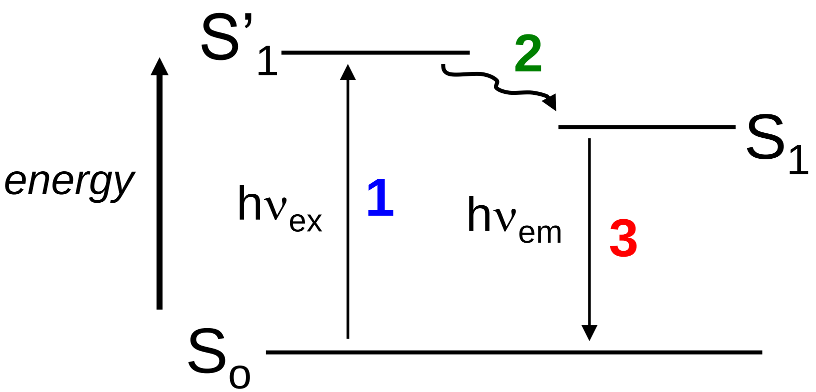
Allele failed to amplify

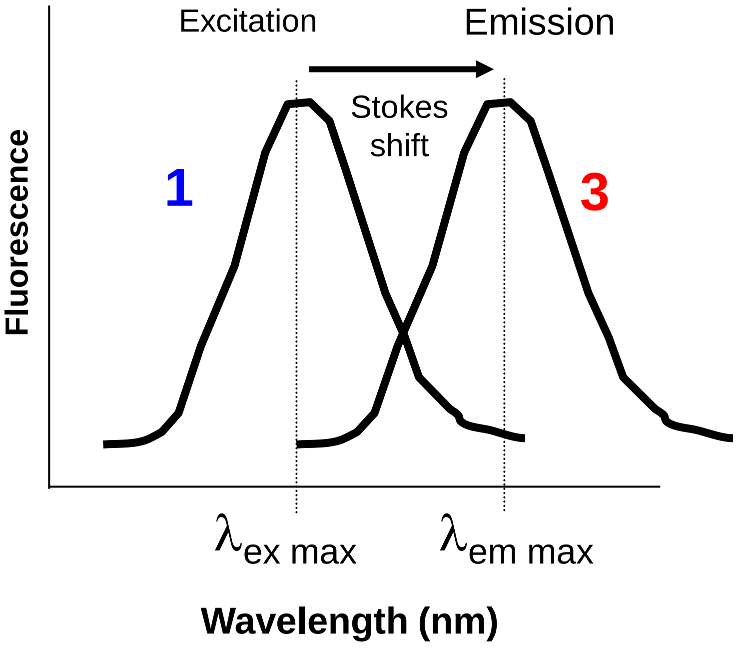
Detection

Optics for ABI 310



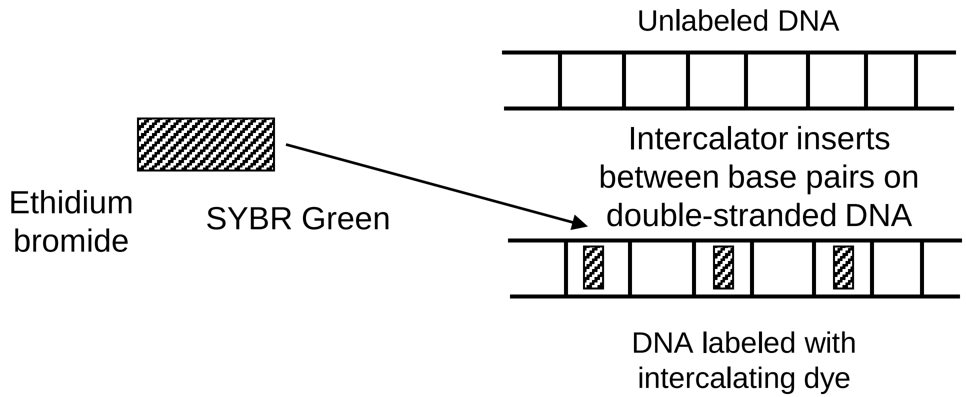
Fluorescence



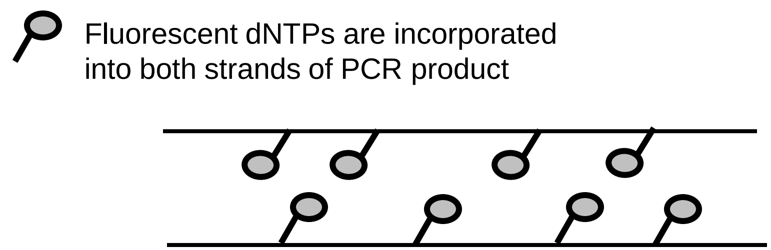


Methods for Fluorescently Labeling DNA

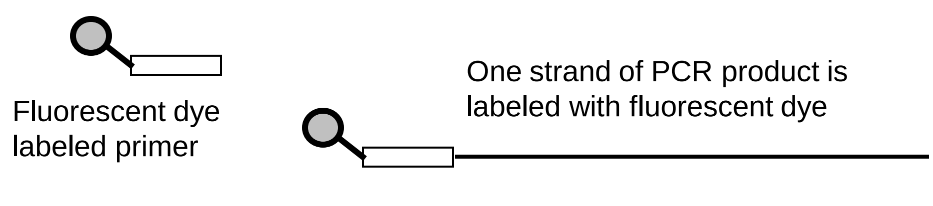
(a)



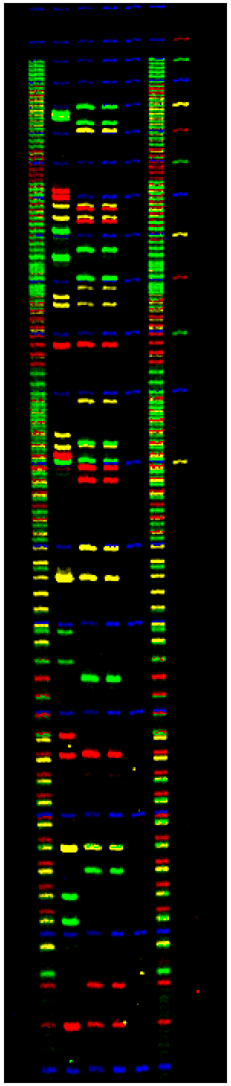
(b)



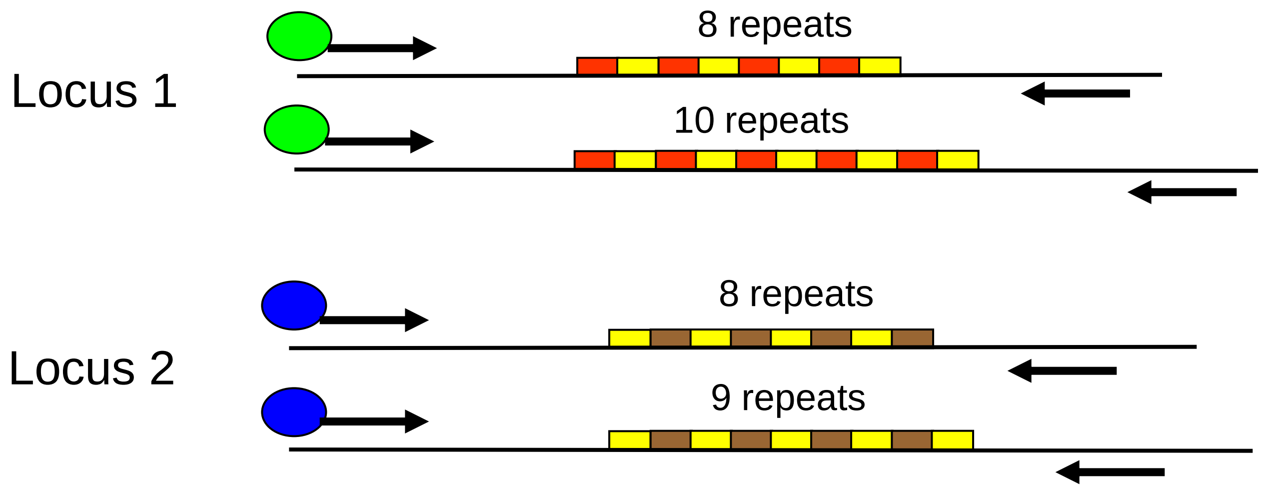
(c)

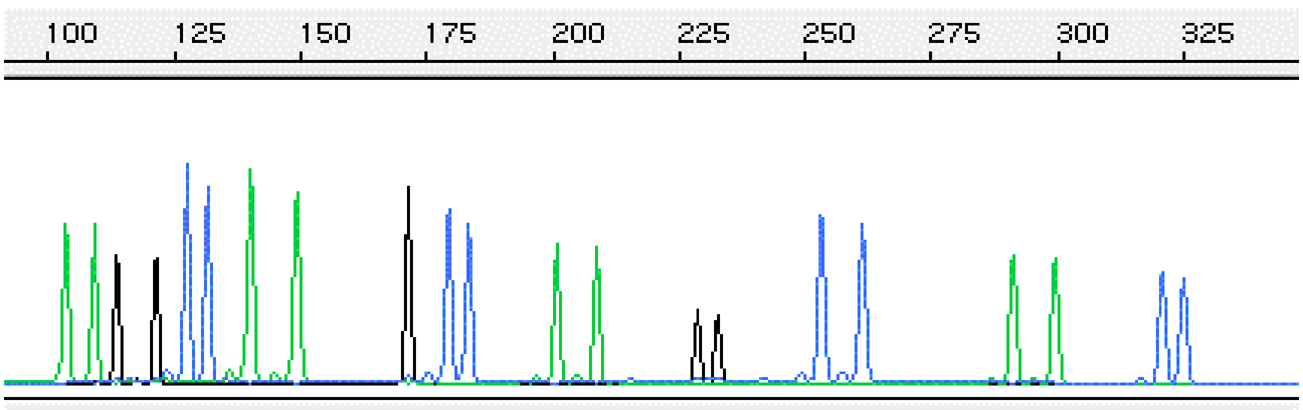


The polymerase chain reaction (PCR) is used to amplify STR regions and label the amplicons with fluorescent dyes using locus-specific primers



Scanned Gel Image

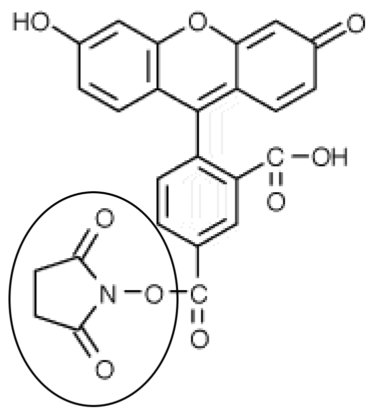




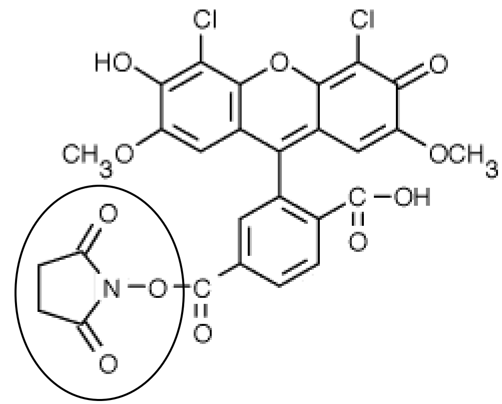
Capillary Electropherogram

ABI Fluorescent Dyes Used in Four-Color Detection

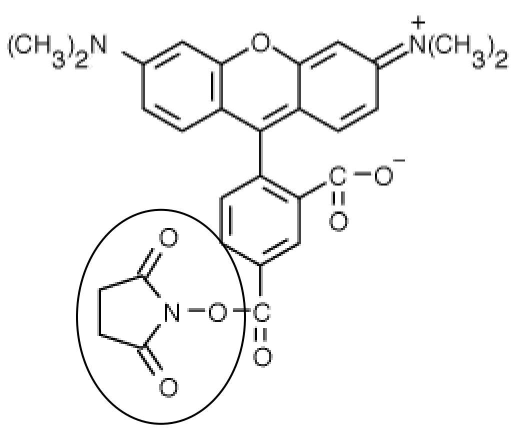
FAM
(blue)



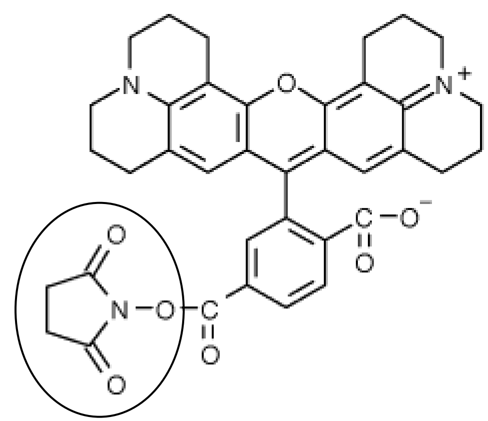
JOE
(green)



TAMRA
(yellow)

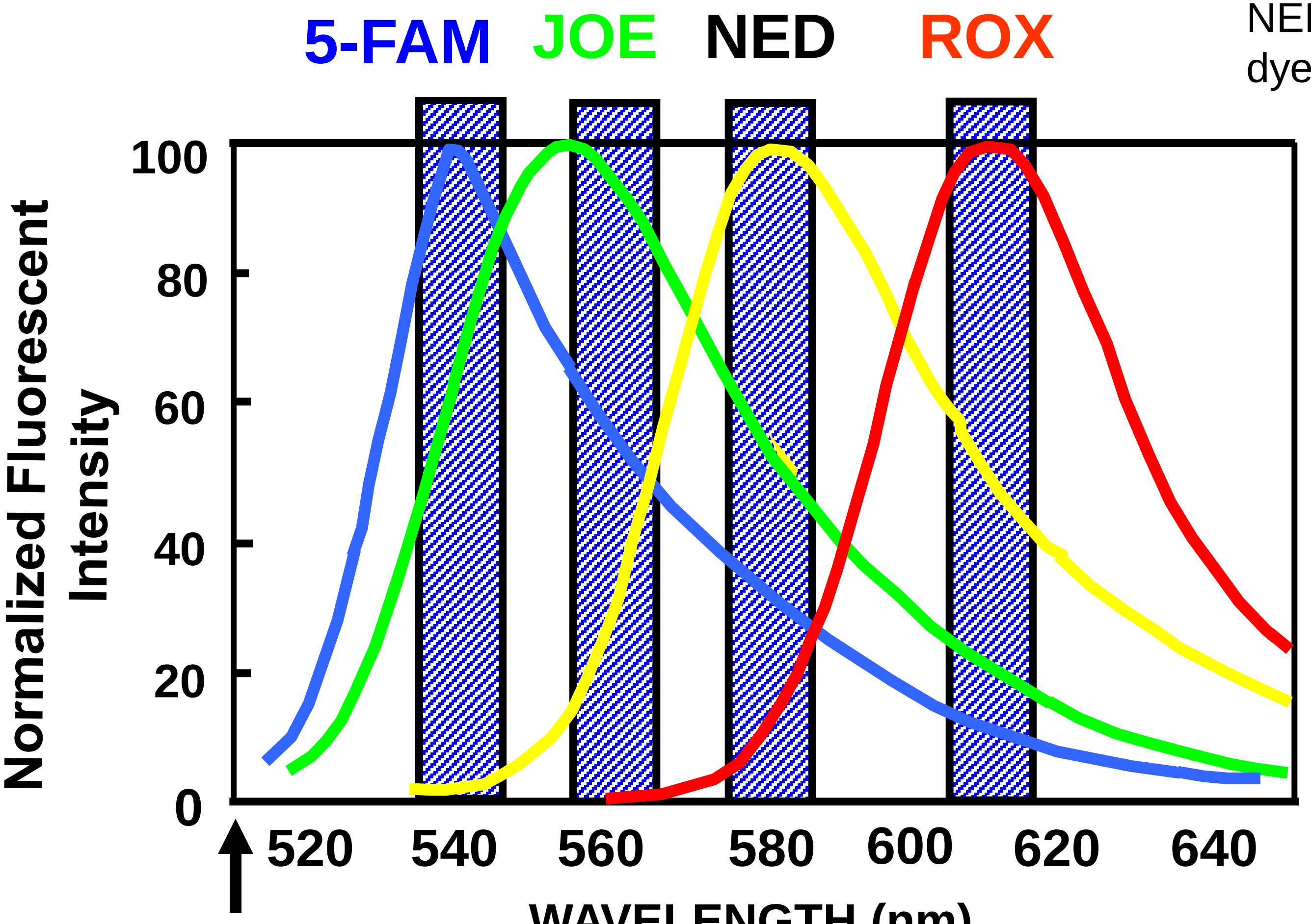


ROX
(red)



Fluorescent Emission Spectra for ABI Dyes

NED is a brighter dye than TAMRA



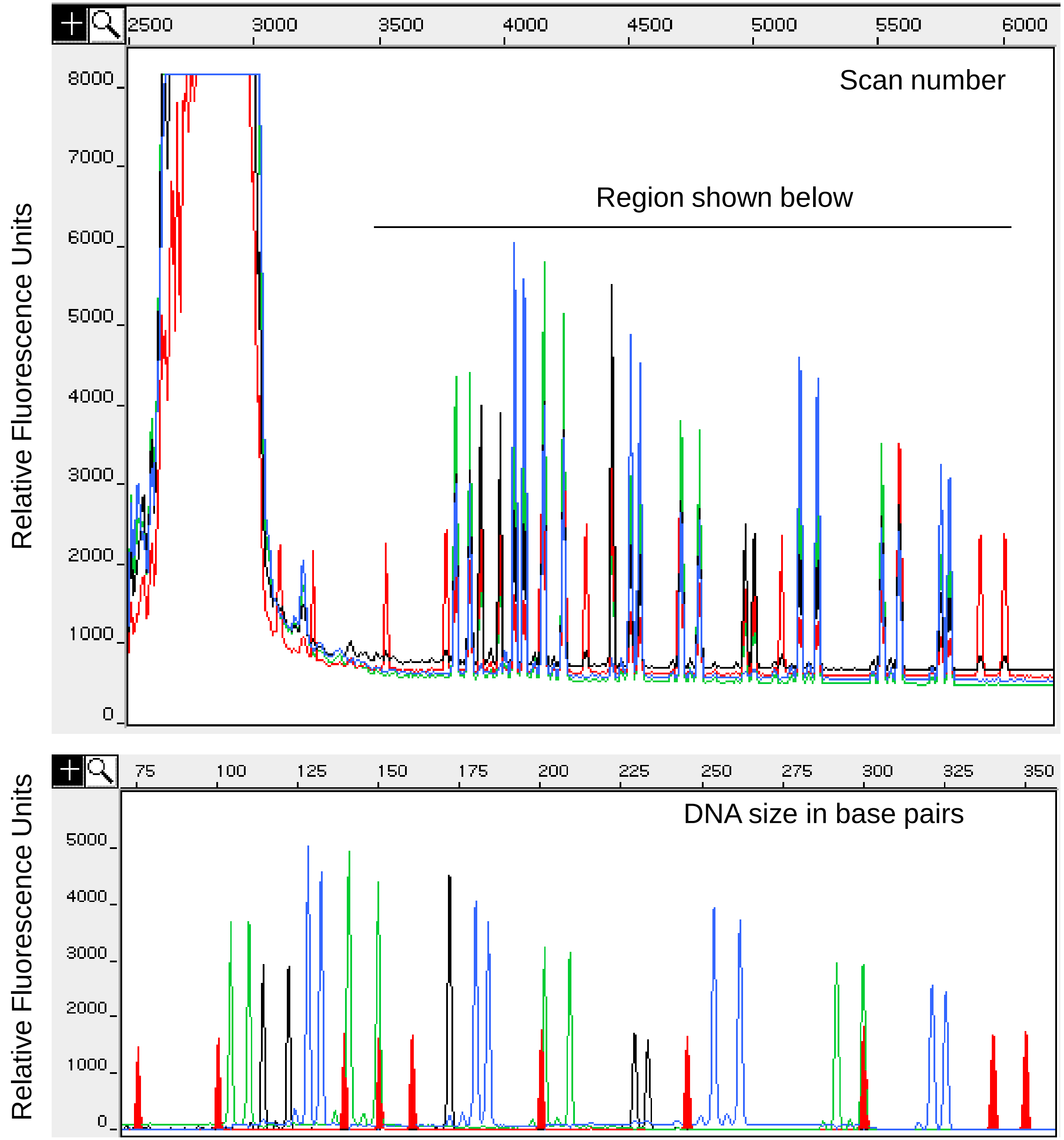
Laser excitation (488, 514.5 nm)

ABI 310 Filter Set F

Butler, J.M. (2001) *Forensic DNA Typing,* Figure 10.4, ©Academic Press

(a)

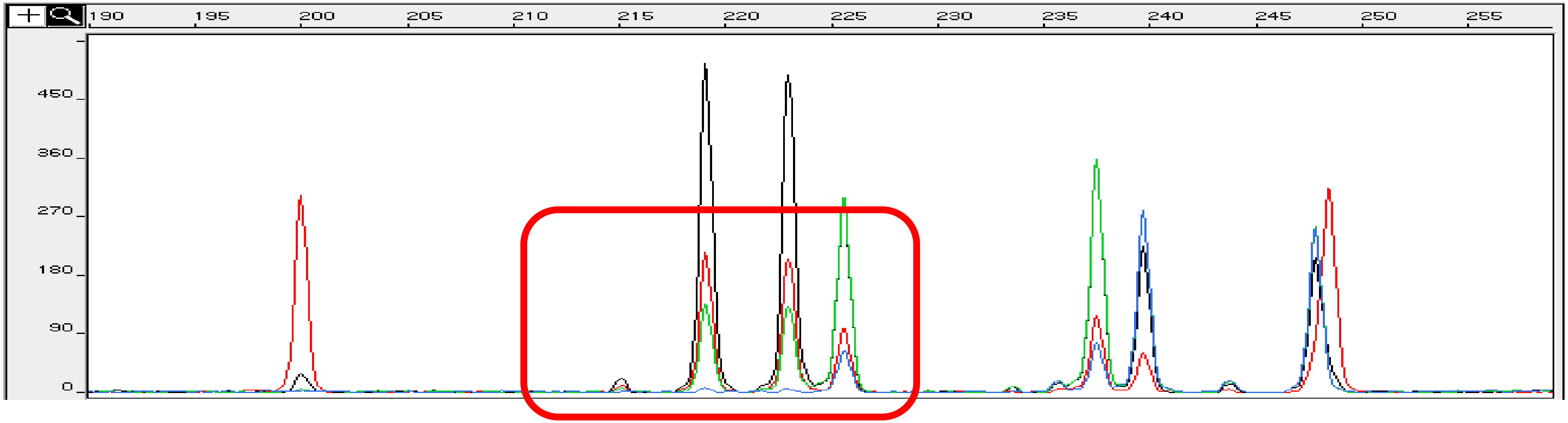
(b)



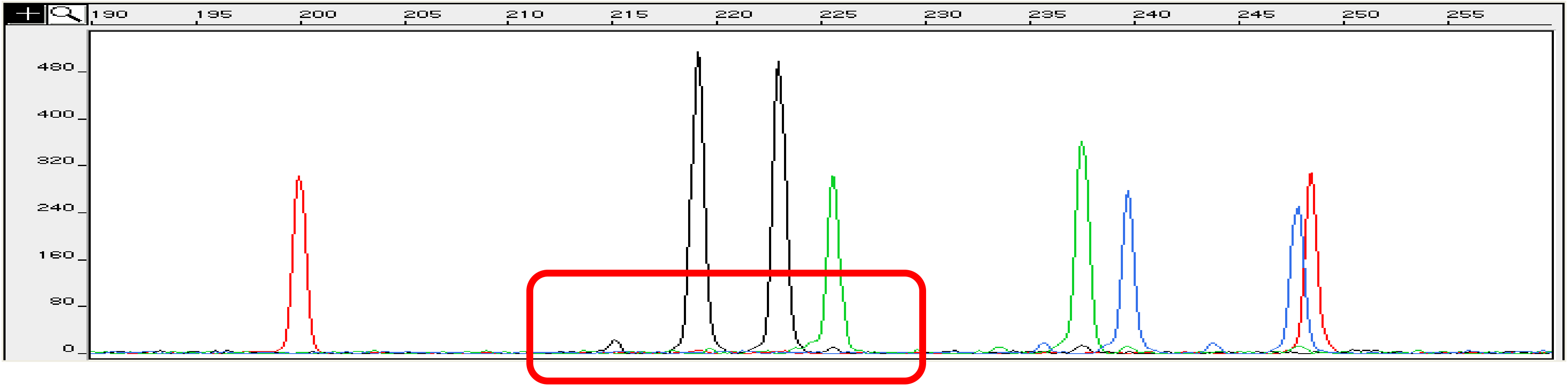
2500
3000
3500
4000
4500
5000
5500
6000
Scan number
Region shown below
8000
7000
6000
5000
4000
3000
2000
1000
0
Relative Fluorescence Units
75
100
125
150
175
200
225
250
275
300
325
350
DNA size in base pairs
5000
4000
3000
2000
1000
0
Relative Fluorescence Units

Importance of Spectral Calibration

Before Color Separation



After Color Separation



ABI 310 Data

Matrix with 4 Dyes on ABI 310

$I_{540}= bx_b + gy_b + yz_b + rw_b$ intensity of blue
$I_{560}= bx_g + gy_g + yz_g + rw_g$ intensity of green
$I_{580}= bx_y + gy_y + yz_y + rw_y$ intensity of yellow
$I_{610}= bx_r + gy_r + yz_r + yw_r$ intensity of red

Where
b is the %blue labeled DNA
g is the %green labeled DNA, etc.

x,y,z,w are the numbers in the matrix (sensitivity to each color)

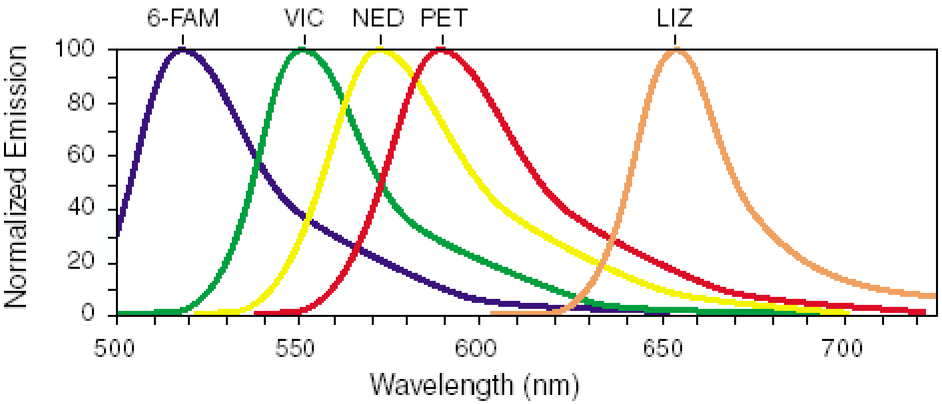
POP4STRMODF

Reactions

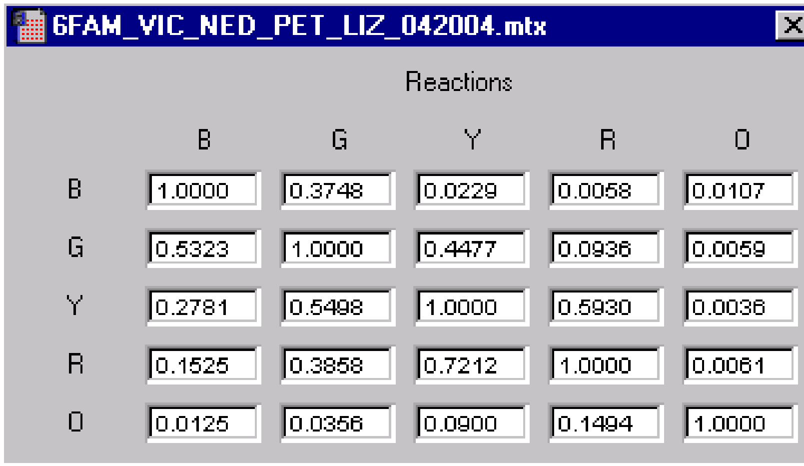
	B	G	Y	R
B	1.0000	0.8502	0.1380	0.0009
G	0.8300	1.0000	0.7622	0.0051
Y	0.6416	0.8324	1.0000	0.1102
R	0.4493	0.6484	0.7851	1.0000

If you solve xyzw for each dye individually
Then you can determine dye contribution for any mixture

5 x 5 matrix for 5-dye analysis on ABI 310

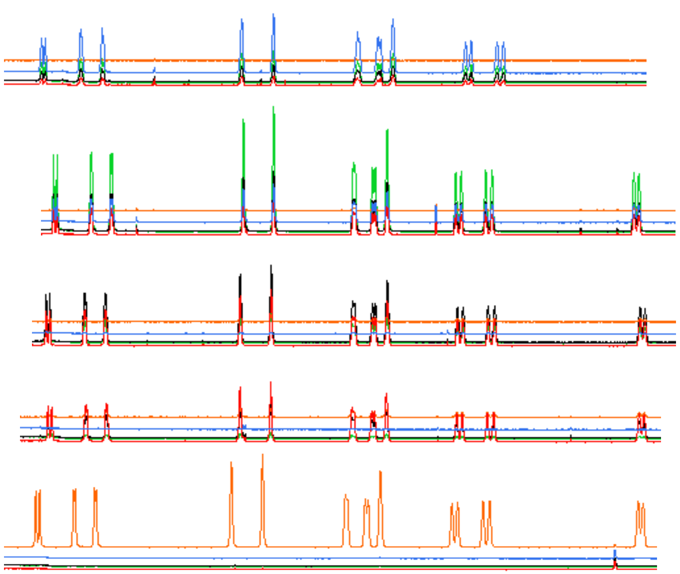


From Identifiler User's Manual

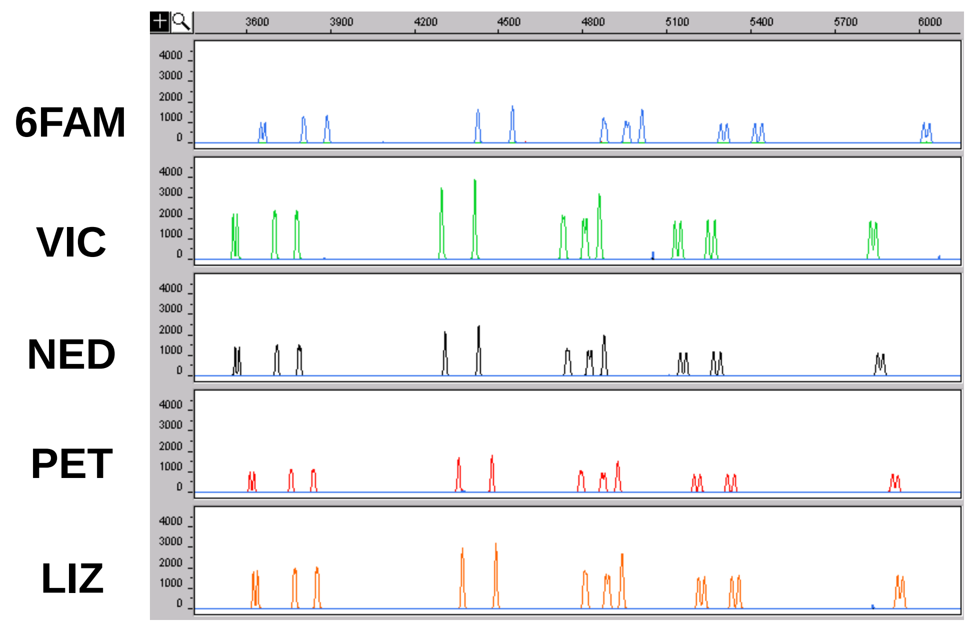


	B	G	Y	R	O
B	1.0000	0.3748	0.0229	0.0058	0.0107
G	0.5323	1.0000	0.4477	0.0936	0.0059
Y	0.2781	0.5498	1.0000	0.5930	0.0036
R	0.1525	0.3858	0.7212	1.0000	0.0061
O	0.0125	0.0356	0.0900	0.1494	1.0000

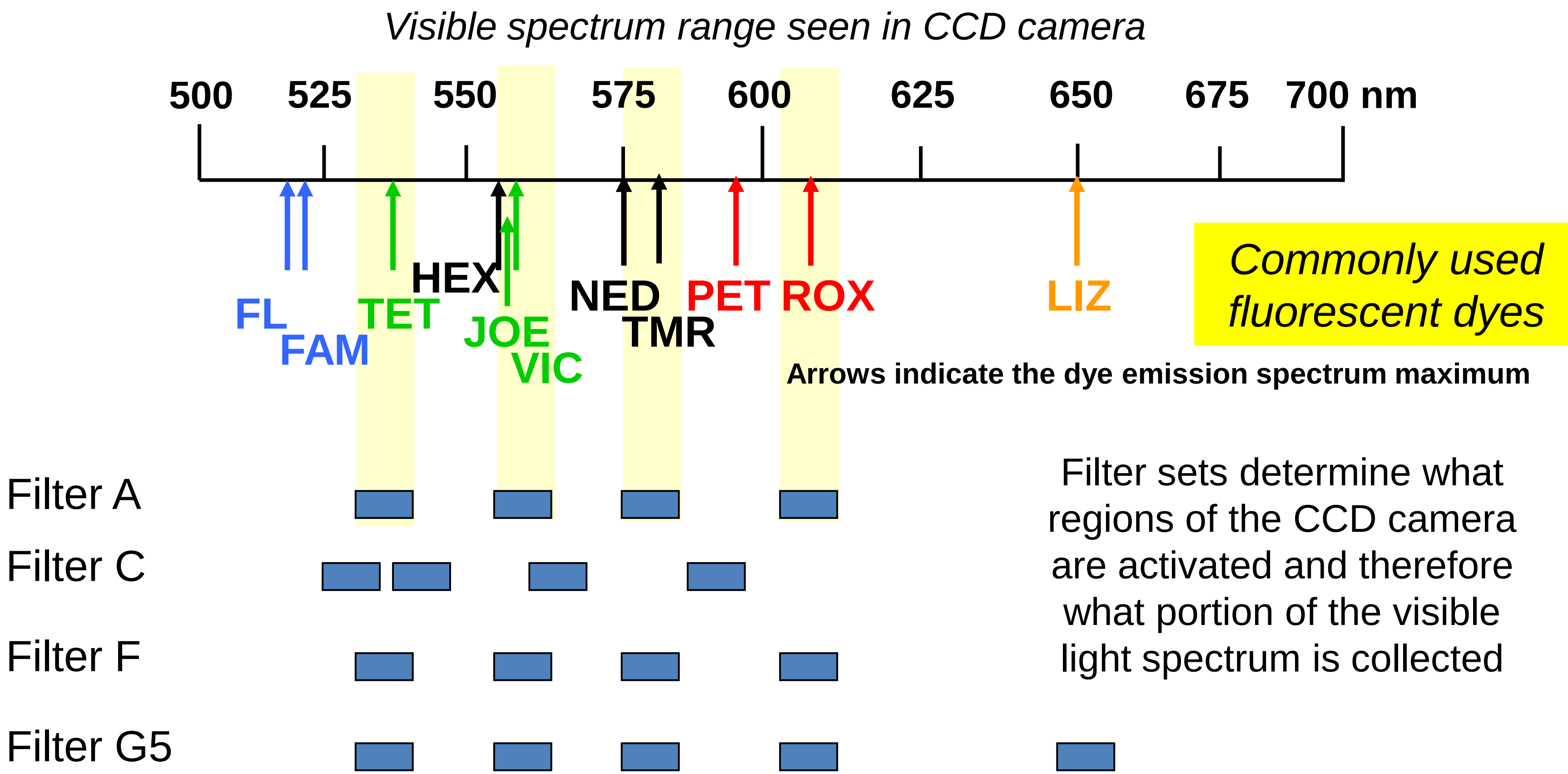
Raw Data for Matrix Standards



Processed Data (matrix applied with baselining)



Virtual Filters Used in ABI 310

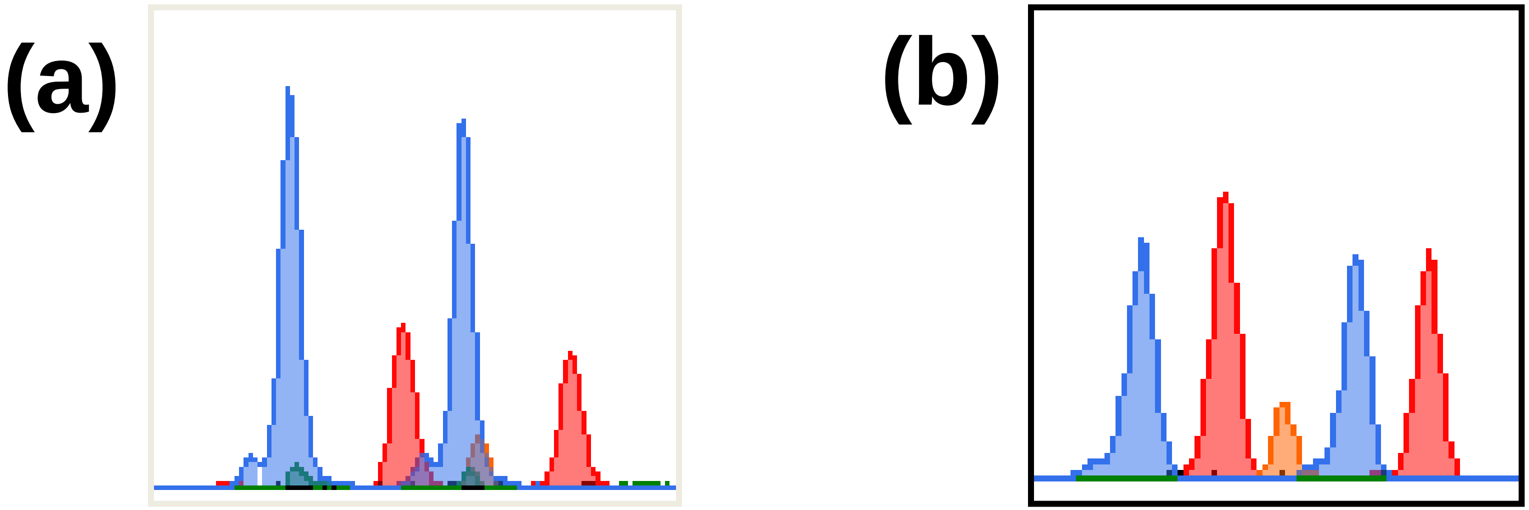


Filter sets determine what regions of the CCD camera are activated and therefore what portion of the visible light spectrum is collected

	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

Variable Binning Increases Red Peaks

Comparison of Data Collection Versions



ABI 3100
Data Collection v1.0.1

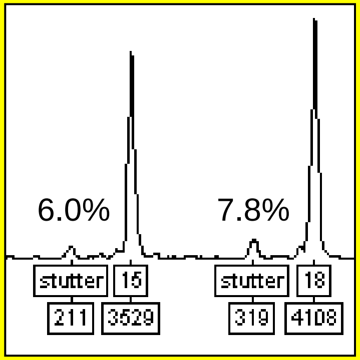
ABI 3130xl
Data Collection v3.0

The same PCR products examined with different data collection versions. In (a) there is an equal number of pixels of light collected from the CCD camera for the blue-labeled and red-labeled peaks. In (b) the signal increase in the red dye-labeled PCR products is accomplished with ‘variable binning’ where more pixels of light are collected from the CCD camera in the red-channel to help balance the less sensitive red dye with blue dye-labeled amplicons.

Deciphering Artifacts from the True Alleles

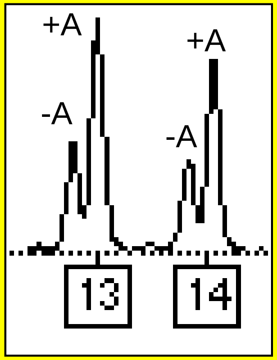
Biological (PCR) artifacts

Stutter products

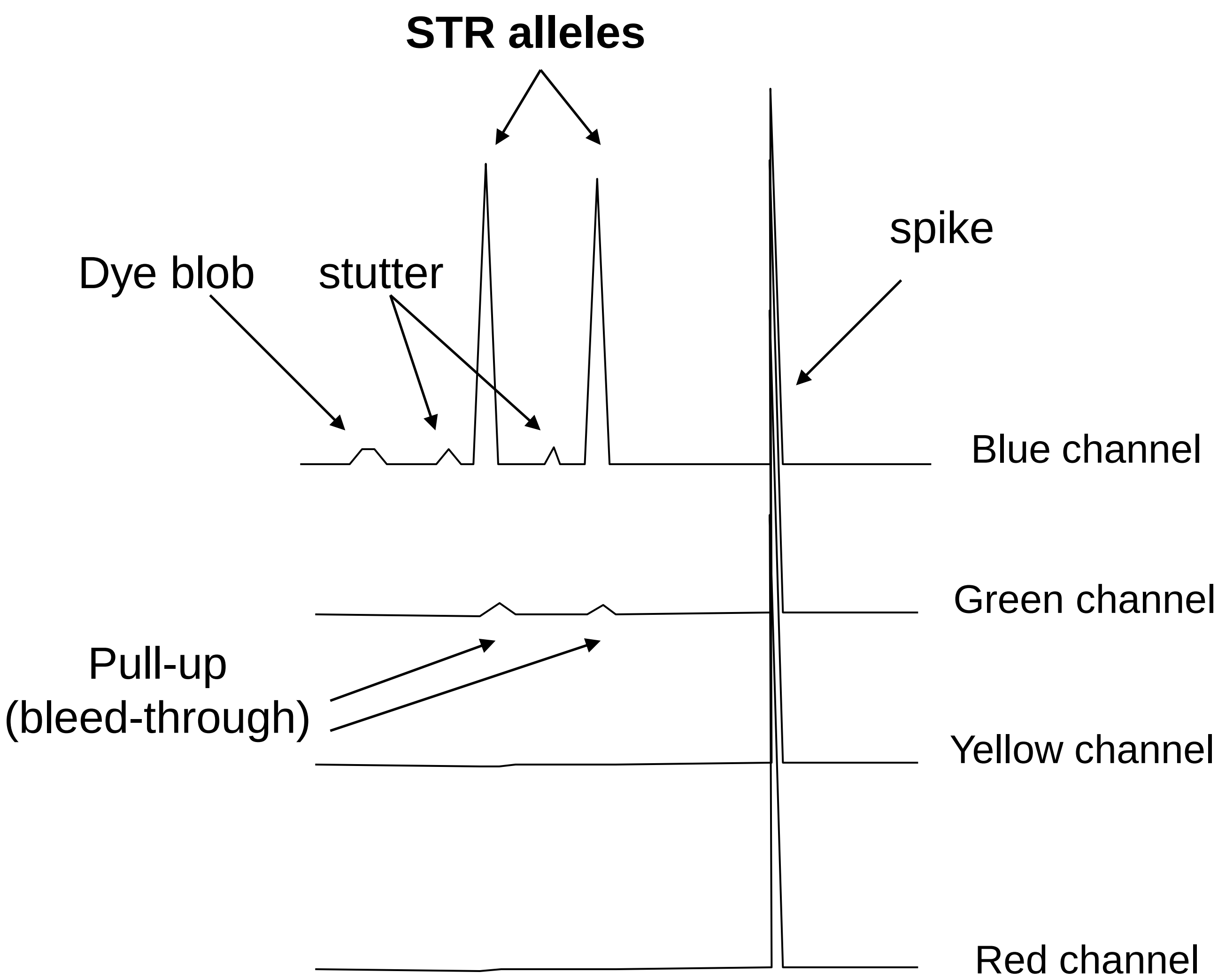


D3S1358

Incomplete adenylation

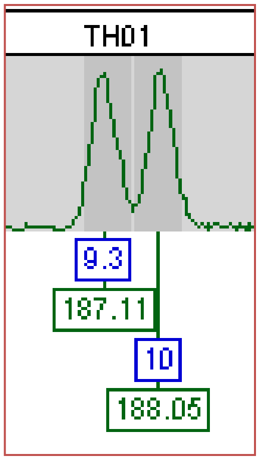


D8S1179

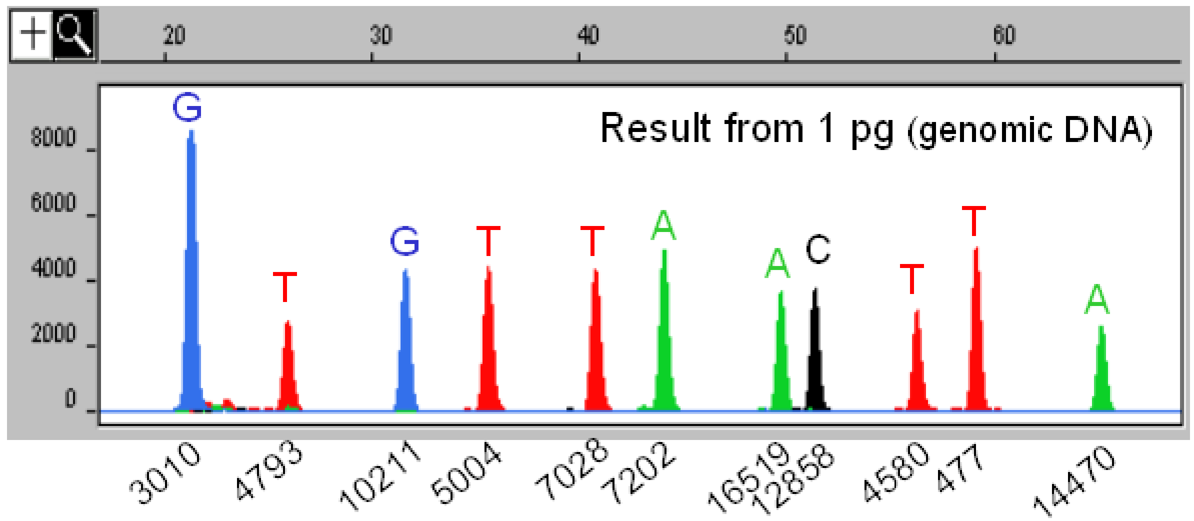


NIST ABI 3100 Analysis Using POP-6 Polymer

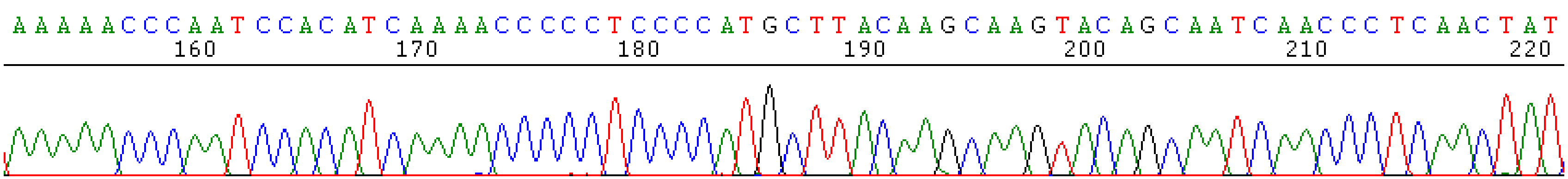
High Resolution
STR Typing



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



mtDNA Sequencing (HV1)



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**

ABI 3500
Genetic Analyzer

ABI 3500 Genetic Analyzer

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

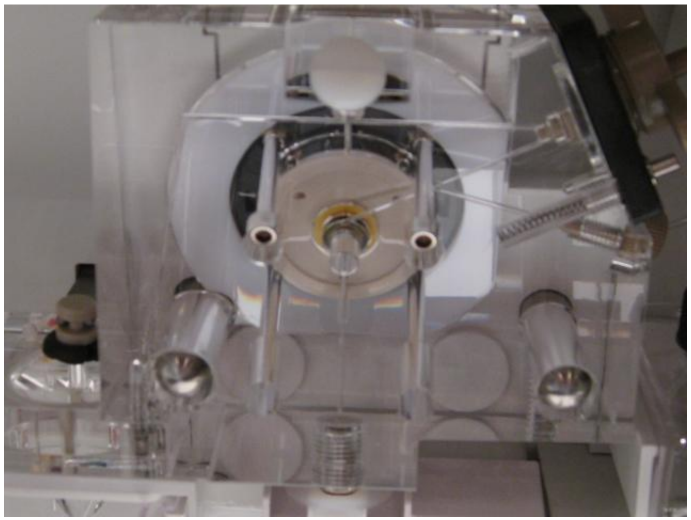
New Features of the ABI 3500 CE

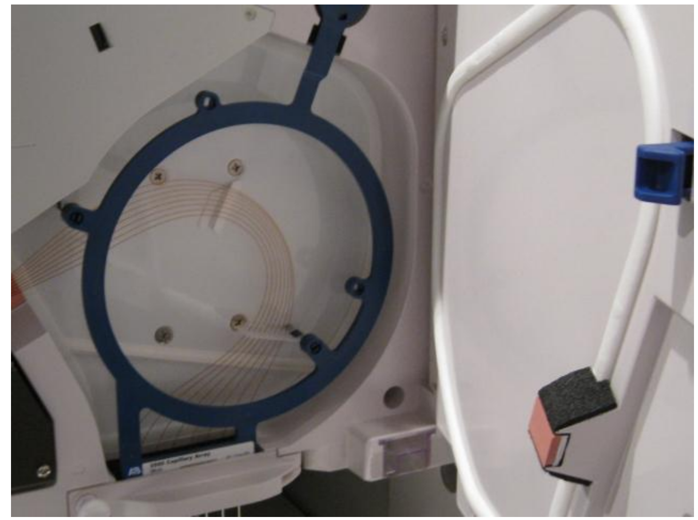
- an improved polymer delivery pump design,
- ready-to-use consumables and containers,
- Radio Frequency Identification (RFID) consumable tracking,
- quality control software features for rapid identification and re-injection of failed samples,
- increased throughput,
- new laser technology,
- reduced 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?)





Primary Differences Between 31xx and 3500

31xx Instruments

- Argon ion (Ar+) lasers with 488/514 nm wavelengths for fluorescence excitation
- 220V power requirement
- Optimal signal intensity 1500-3000 RFU
- **Data signal depressed 4-fold during data collection**
- Currently validated and operational in most forensic laboratories (.fsa files)

3500 Instruments

- Single-line 505 nm, solid-state long-life laser
- Smaller footprint
- 110V power requirement
- Optimal signal intensity can approach 20,000-30,000 RFU
- Normalization of instrument-to-instrument signal variability
  - Ability to increase or decrease overall signal
- Requires the use of GeneMapper IDX v1.2 (.hid files)

ABI 3500 'Dash Board' Data Collection

Gauges

POP7 Polymer	AB 3500 Buffer - (Anode)	AB 3500 Buffer - (Cathode)	50cm - 24 cap Array

634 Samples Remaining (34 Injections Remaining)	5 Days Remaining (50 Injections Remaining)	5 Days Remaining (30 Injections Remaining)	43 Injections Performed

Consumable	Name	Status	Days on Instrument	Expiration Date	Lot Number	Part Number
Polymer	POP7	634 Samples Remaining	0	04-Sep-2009 11...	51A007	4393714
Anode Buffer	AB 3500 Buffer	5 Days Remaining	2	08-May-2009 1...	51-B-34007	4393718
Cathode Buffer	AB 3500 Buffer	5 Days Remaining	2	05-Jun-2009 11...	8751-6TH-B	4408256
Capillary Array	50cm - 24 cap	117 Injections Remainin	0	01-Jan-2010 11...	80K005	4404688 - Serial # 80K2450

Tracks the numbers of samples for 'QC purposes'

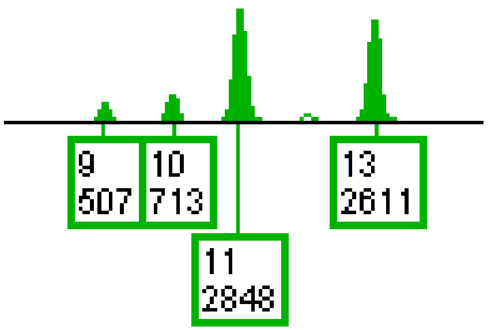
Maintenance Notifi

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

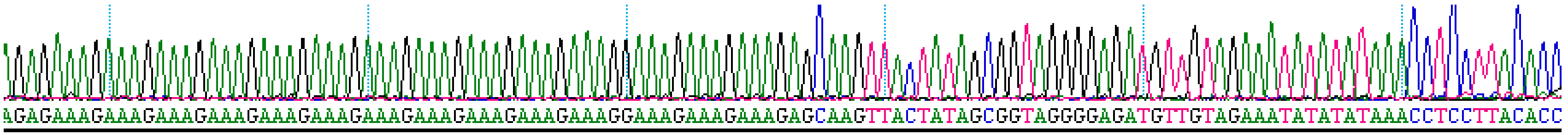
https://www3.appliedbiosystems.com/cms/groups/portal/documents/web_content/cms_064299.jpg

ABI 3500 Generates Excellent Data

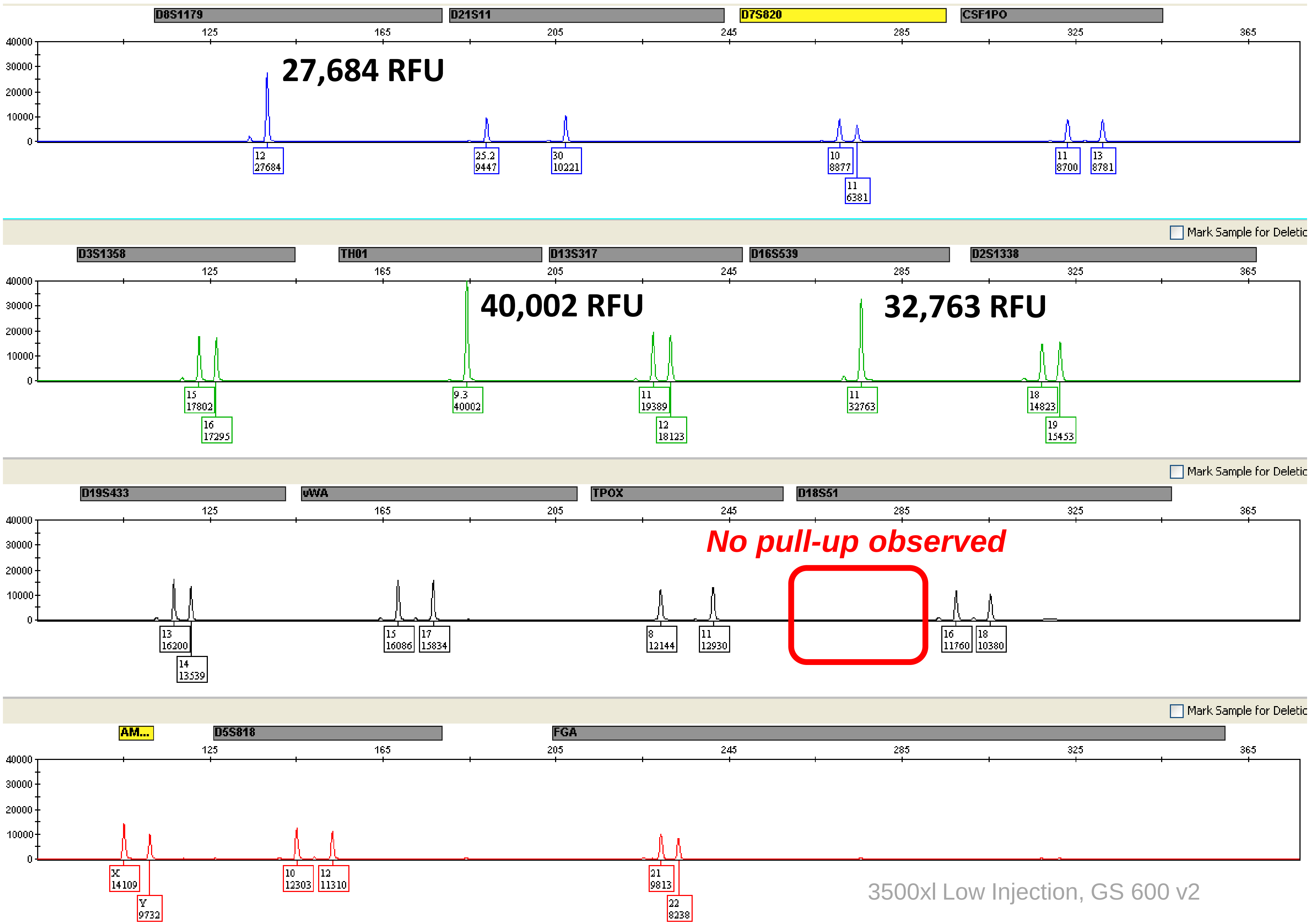
STR typing with a 1:7 mixture using **36 cm array and POP4**



DNA sequencing of an SE33 allele using **50 cm array and POP7**



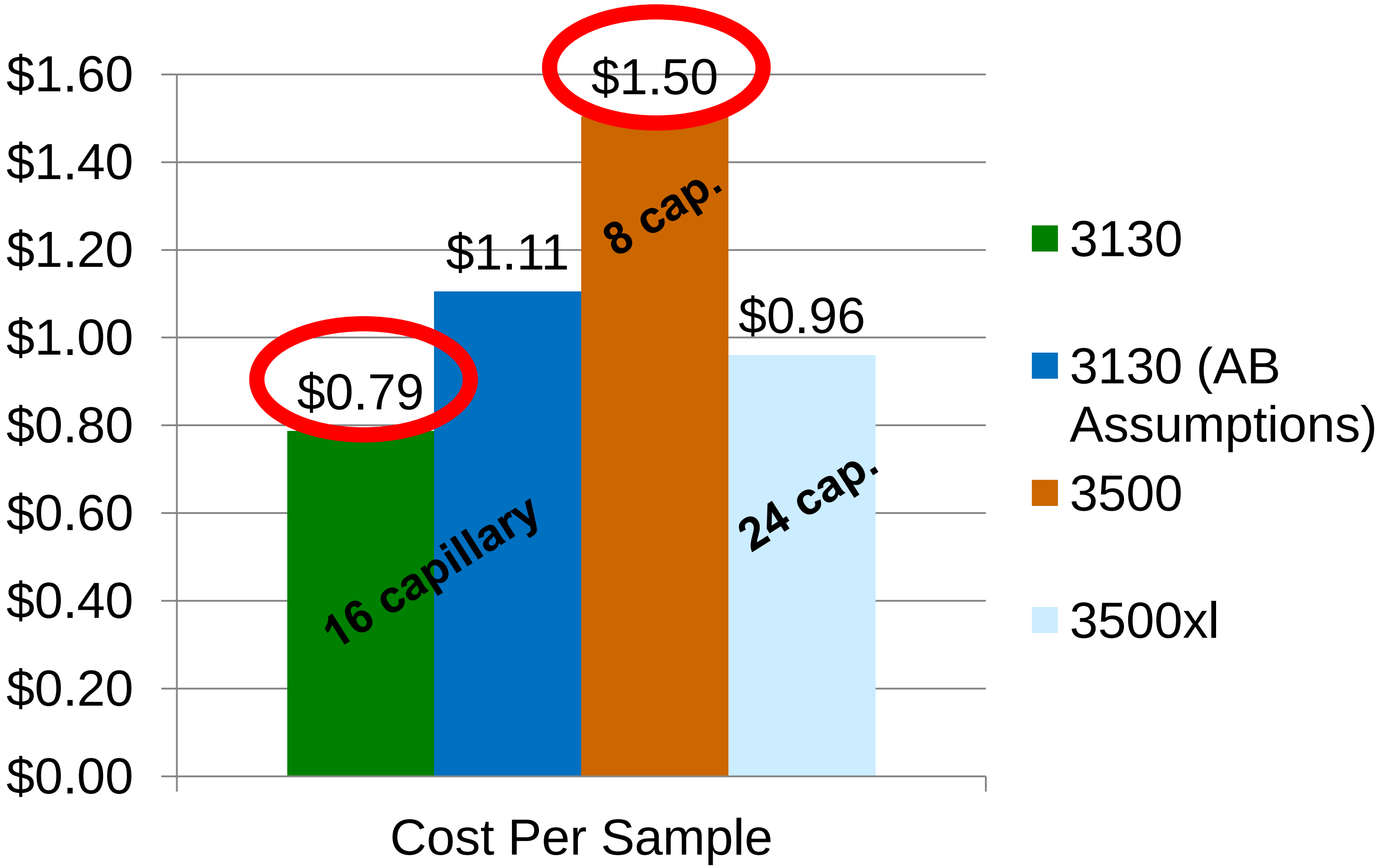
Identifiler Result on ABI 3500xl



3500xl Low Injection, GS 600 v2

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

Running two plates per day (10 plates per week)



Troubleshooting:

Strategies and Solutions

Bruce McCord's *Profiles in DNA* Article

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.

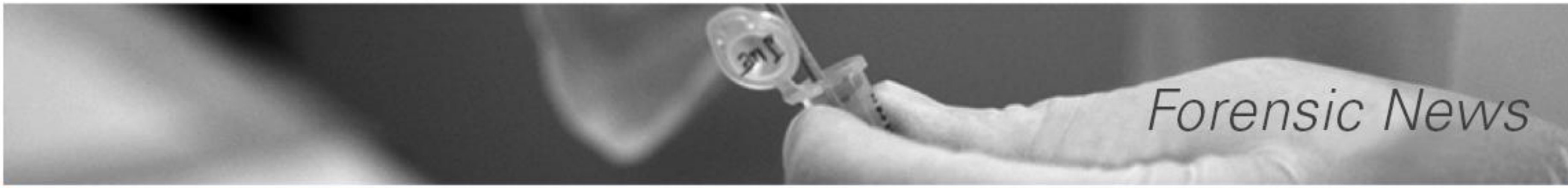
INTRODUCTION

The development of capillary electrophoresis (CE) has played a key role in bringing about the modern application of DNA typing. Forensic laboratories are the beneficiaries of this new technology, but many practitioners are not fully aware of the underlying principles of the CE system. This article attempts to address the important issues in CE separations to aid analysts in troubleshooting problematic separations. The key to producing good DNA separations is to understand the principles underlying the injection, separation and detection of each allele. These points are addressed below.

SEPARATION

DNA analysis by CE is performed using entangled polymer buffers (Figure 1). These buffers can be easily pumped into a capillary prior to a separation and pumped out at its conclusion, providing a fresh separation matrix for each run. A typical buffer for forensic DNA separation contains 4% polydimethyl acrylamide (pDMA), buffered to pH 8

Applied Biosystems



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

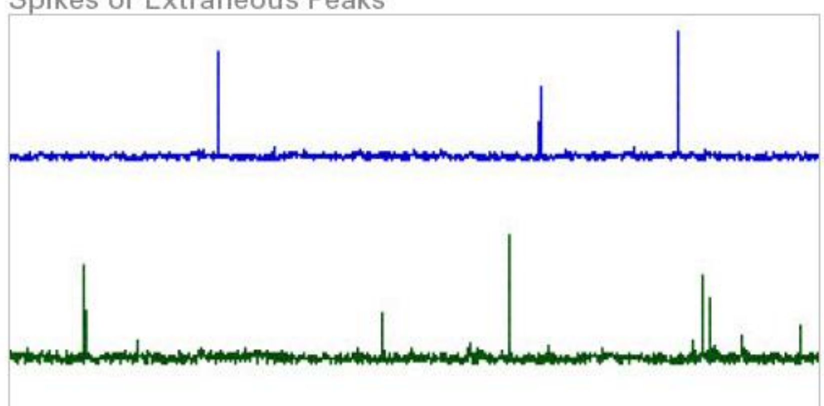
One of the key responsibilities of our Human Identification Field Application Specialists is to troubleshoot results obtained using any of the AmpFℓSTR® kits on any Applied Biosystems validated instrument platform.

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

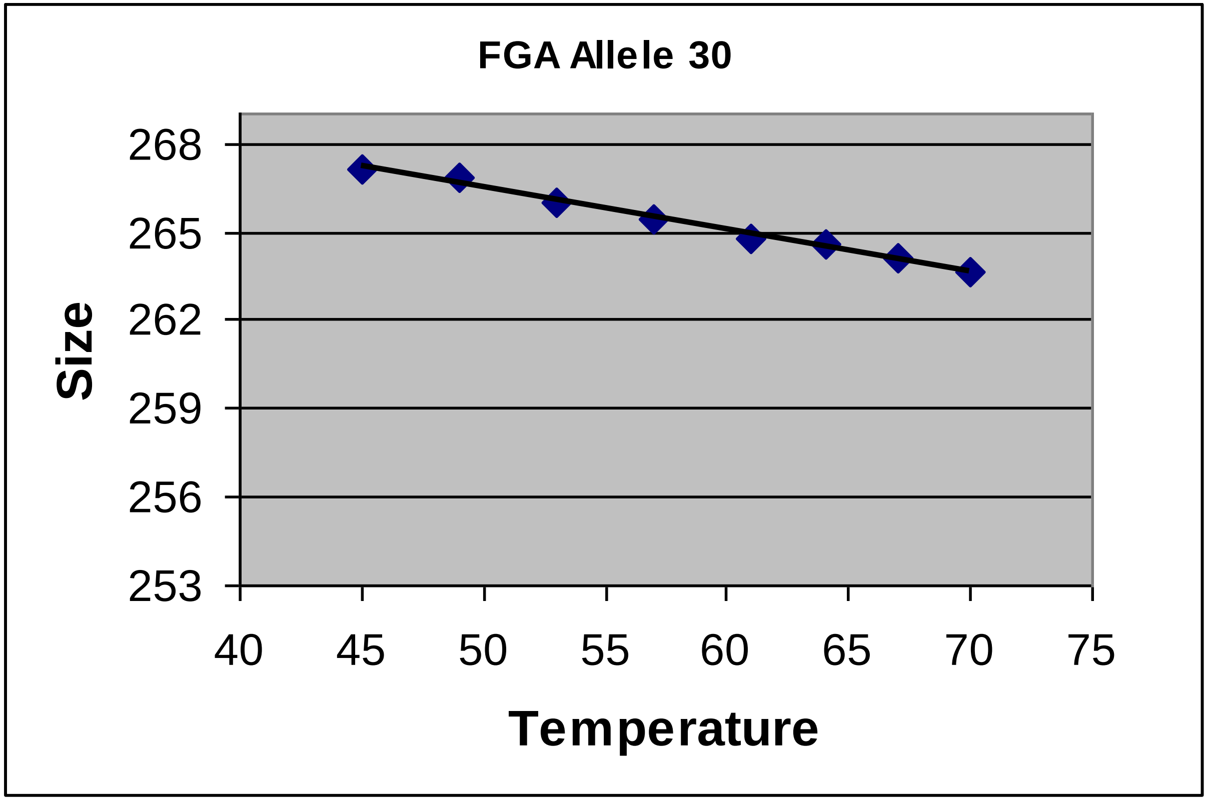
Spikes or Extraneous Peaks



External Factors

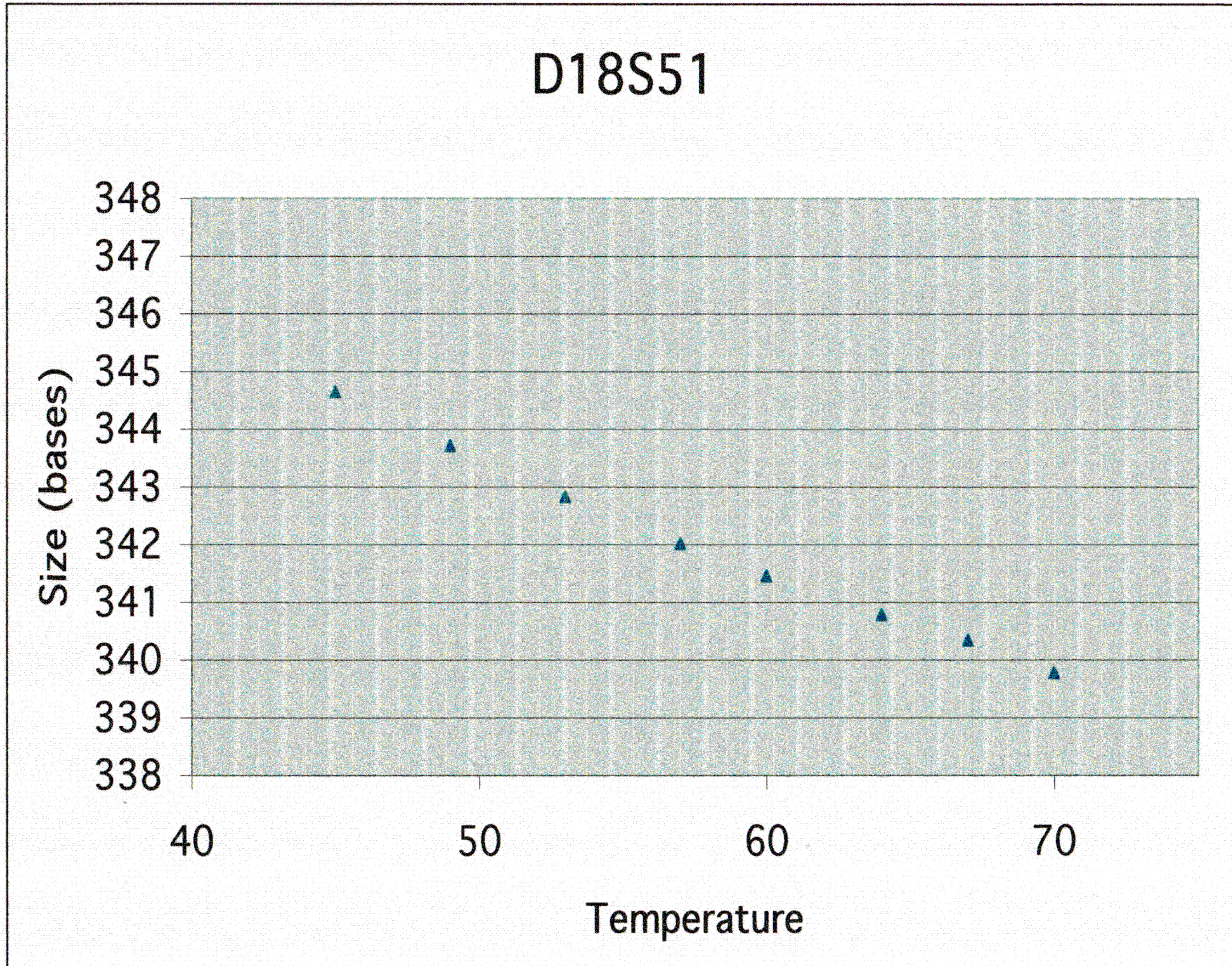
- Room temperature
  - Variations in room temperature can cause mobility shifts with band shifts and loss of calibration
  - Temperature is also important due to effects of high humidity on electrical conductance
- Cleanliness
  - Urea left in sample block can crystallize and catalyze further crystal 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.

Effect of temperature on allele size



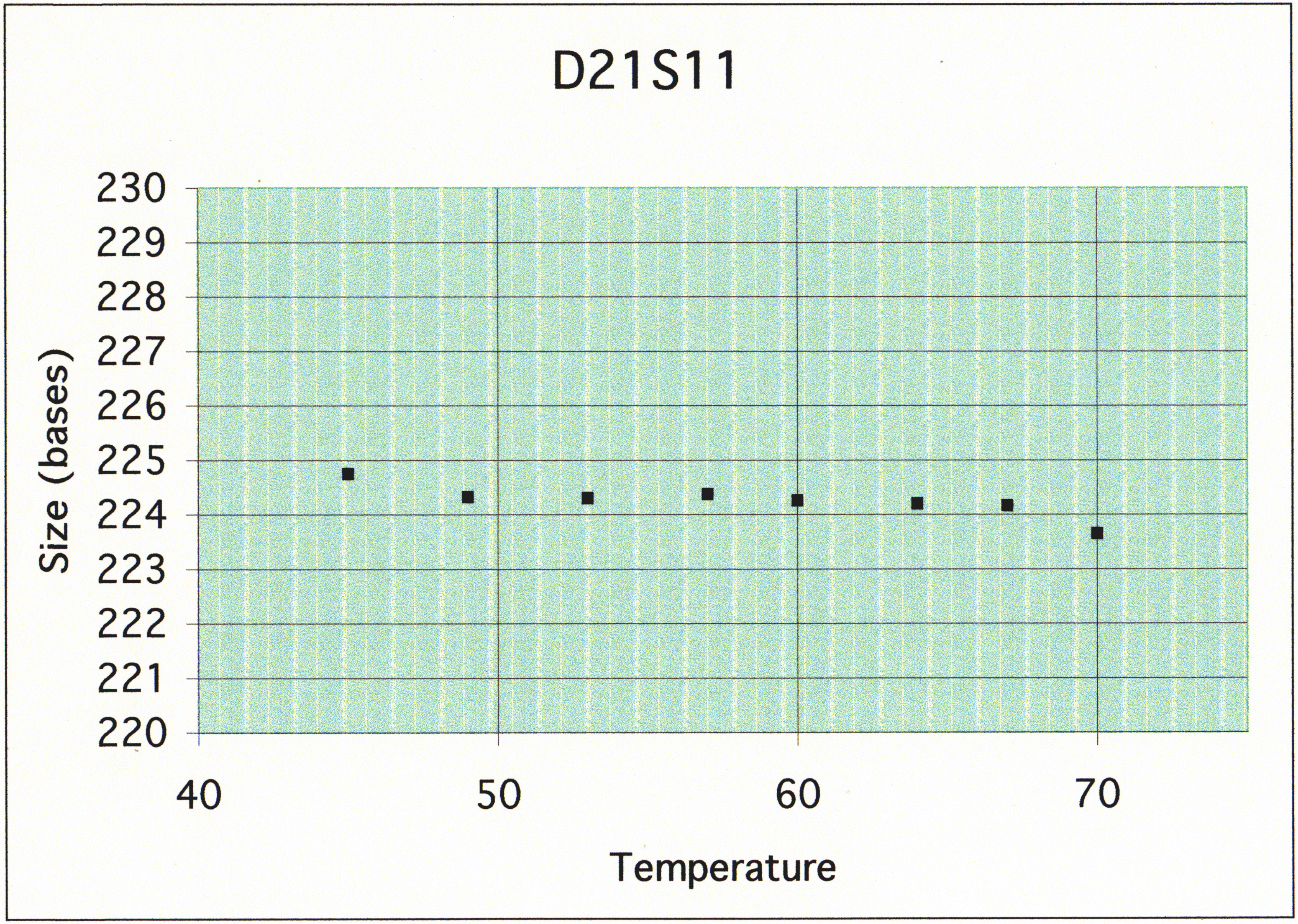
Slope is 0.14 bases/degree centigrade
Therefore a small change in temperature has a big effect
(A 1-2 degree shift in temperature of the heat plate can produce an OL allele)

Hartzell, B., et al. (2003). Response of short tandem repeat systems to temperature and sizing methods. *Forensic Science International, 133,* 228-234.



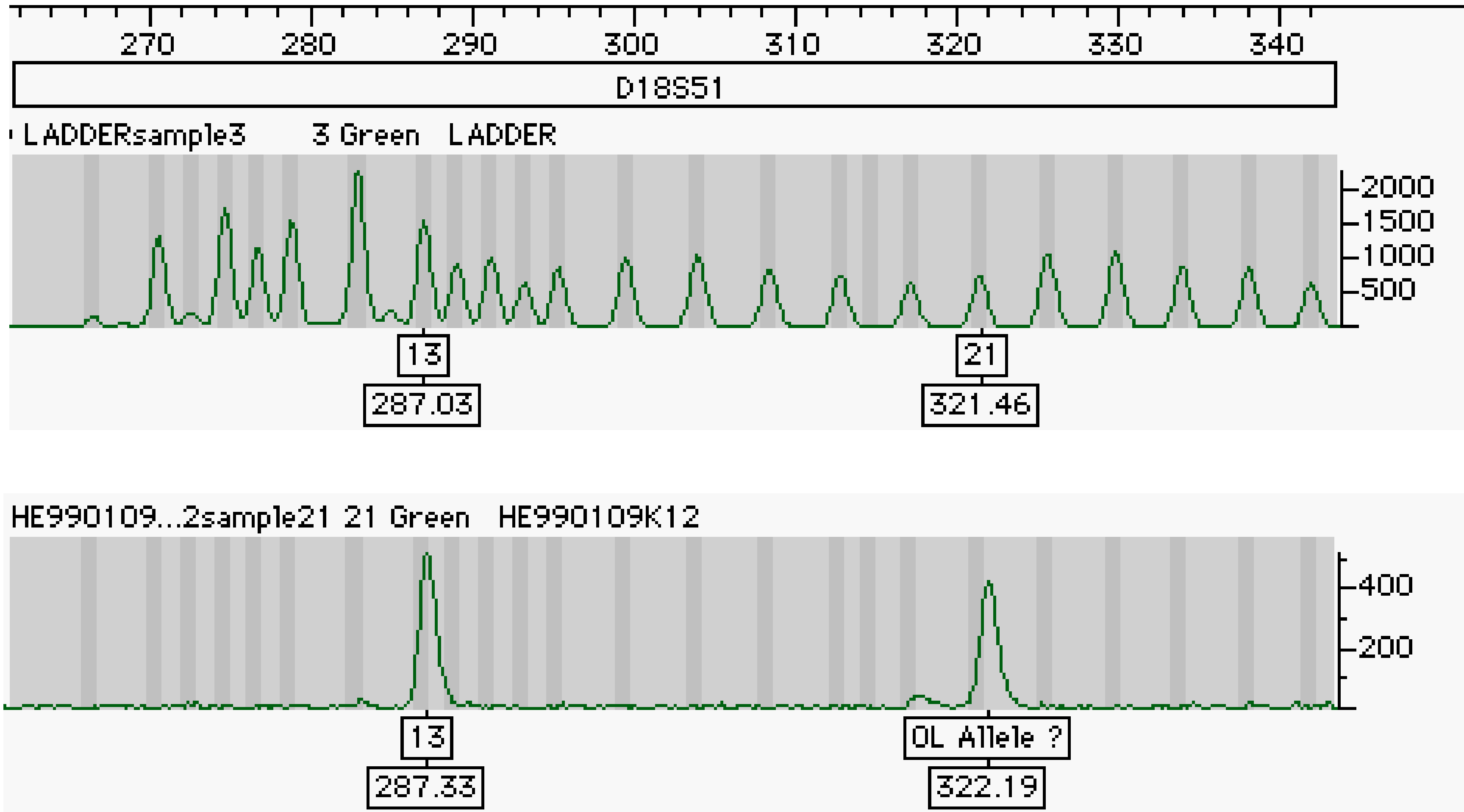
D18S51
Size (bases)
348
347
346
345
344
343
342
341
340
339
338
40
50
60
70
Temperature

allele 34

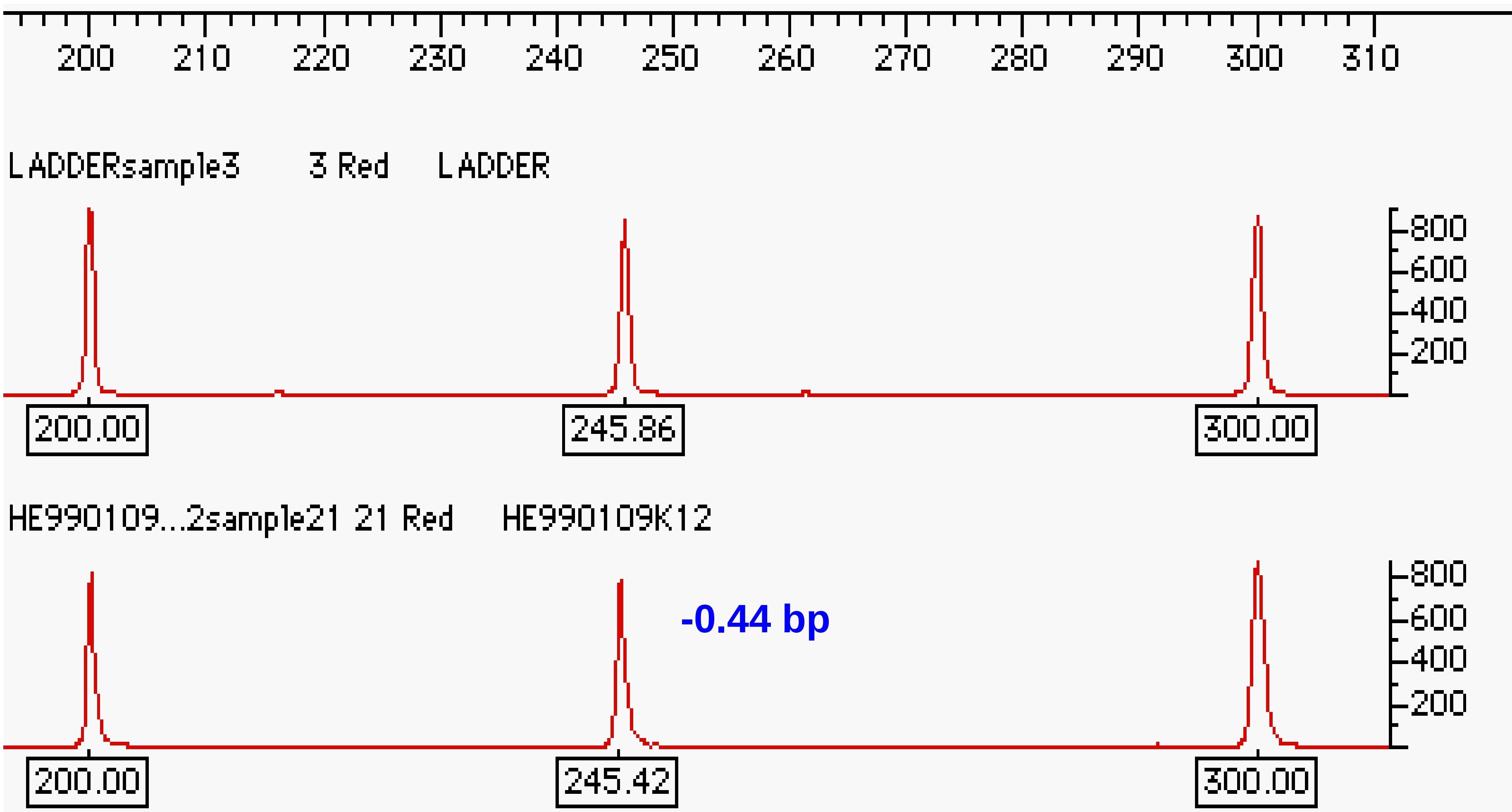

D21S11
230
229
228
227
226
225
224
223
222
221
220
Size (bases)
40
50
60
70
Temperature

Temperature Effects

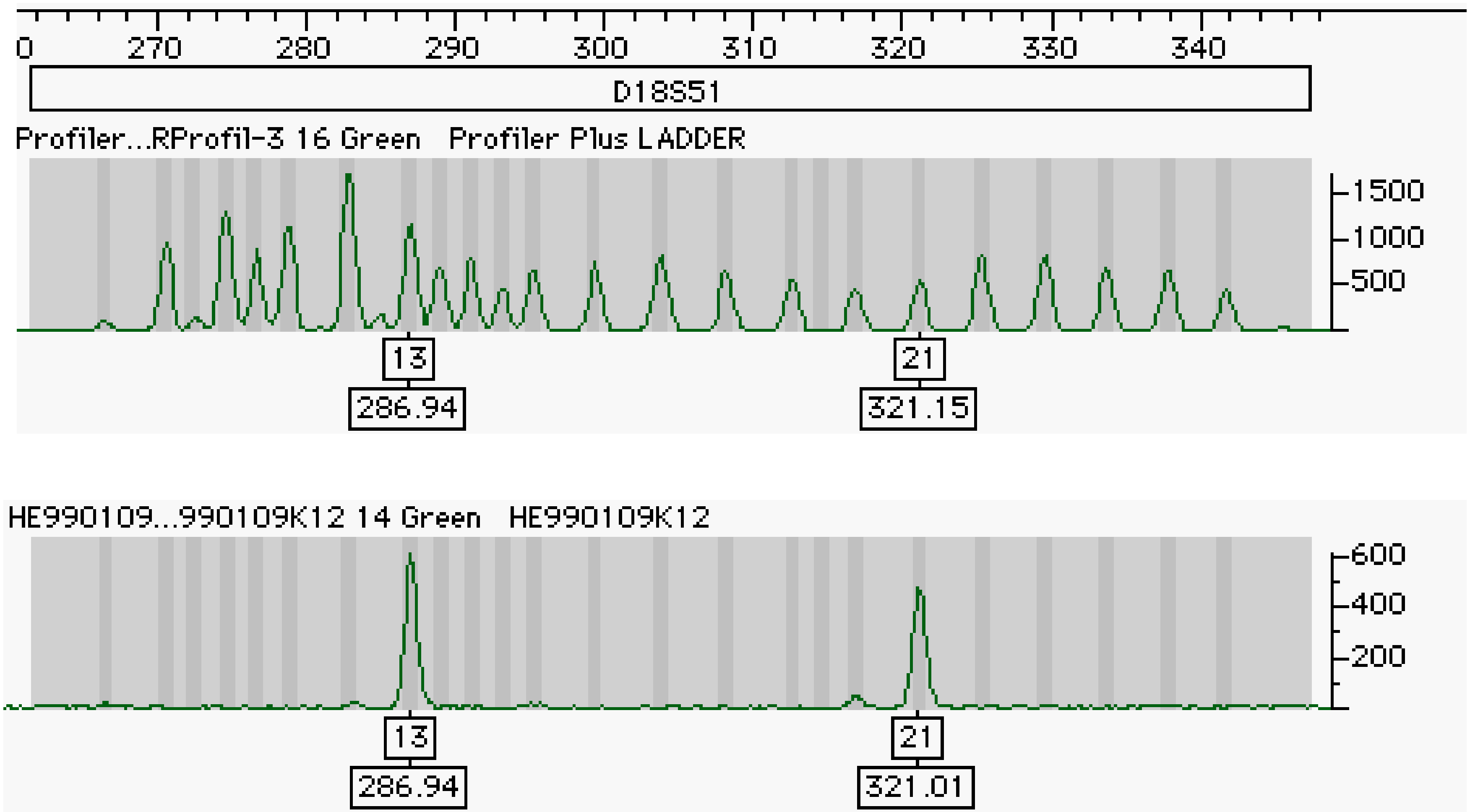
Off-Ladder “OL Alleles”



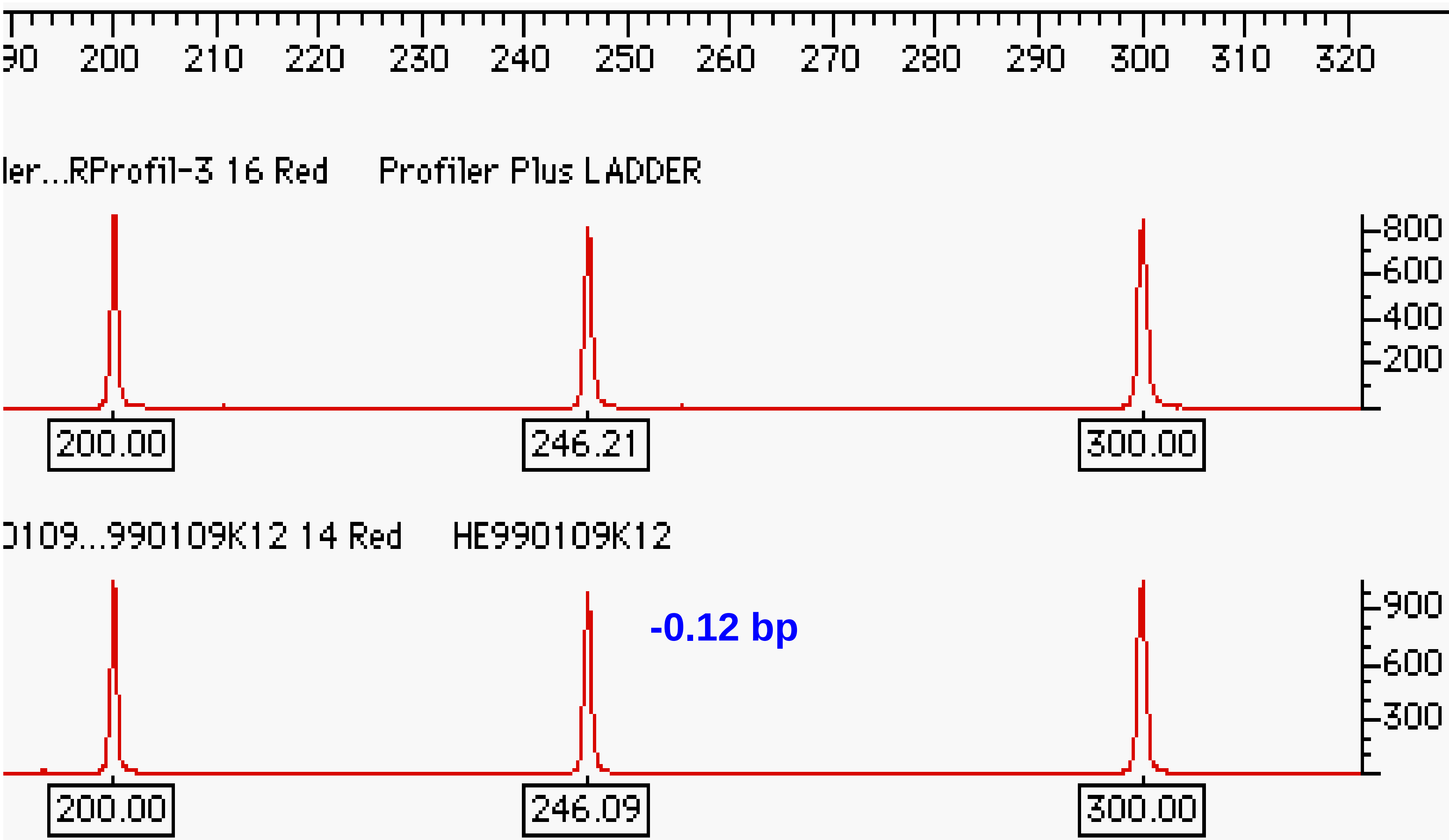
“OL alleles ” - look at the 250 peak



“OL allele re-injected”



And the 250 peak...

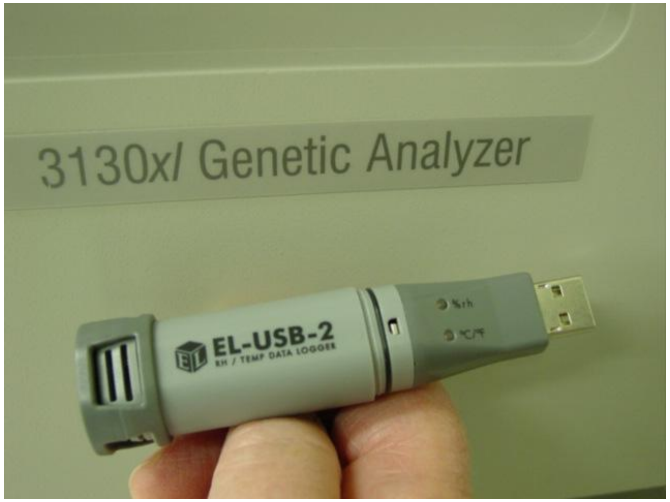


Temperature Probes

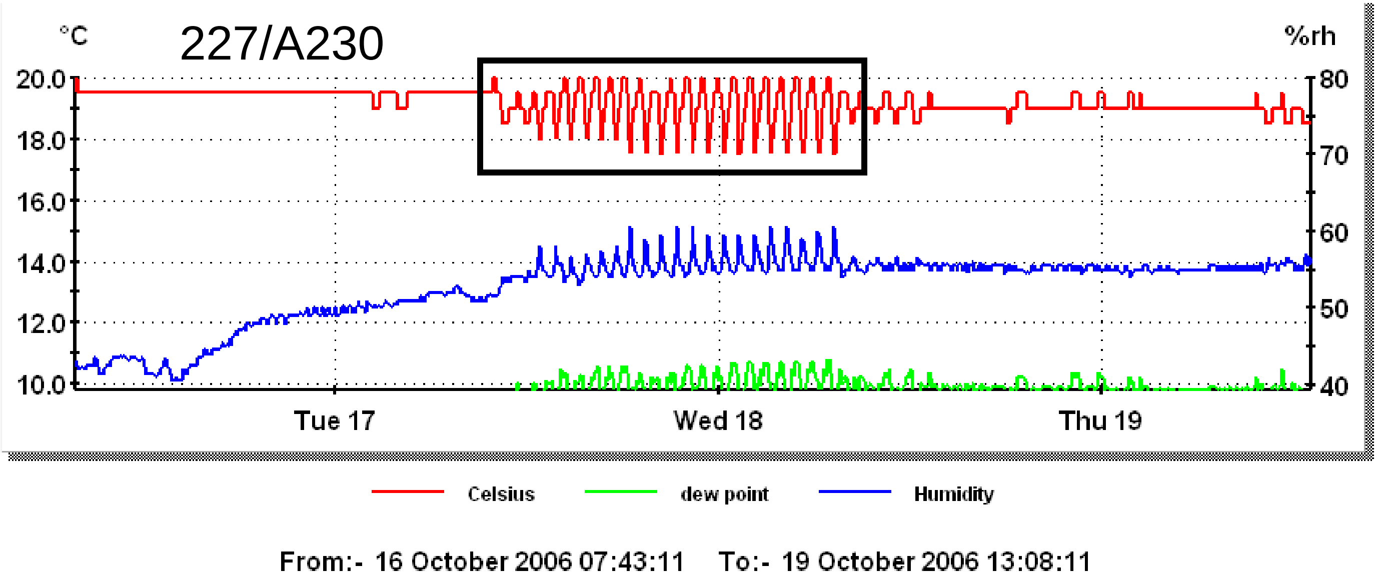
Refrigerator and freezer monitoring

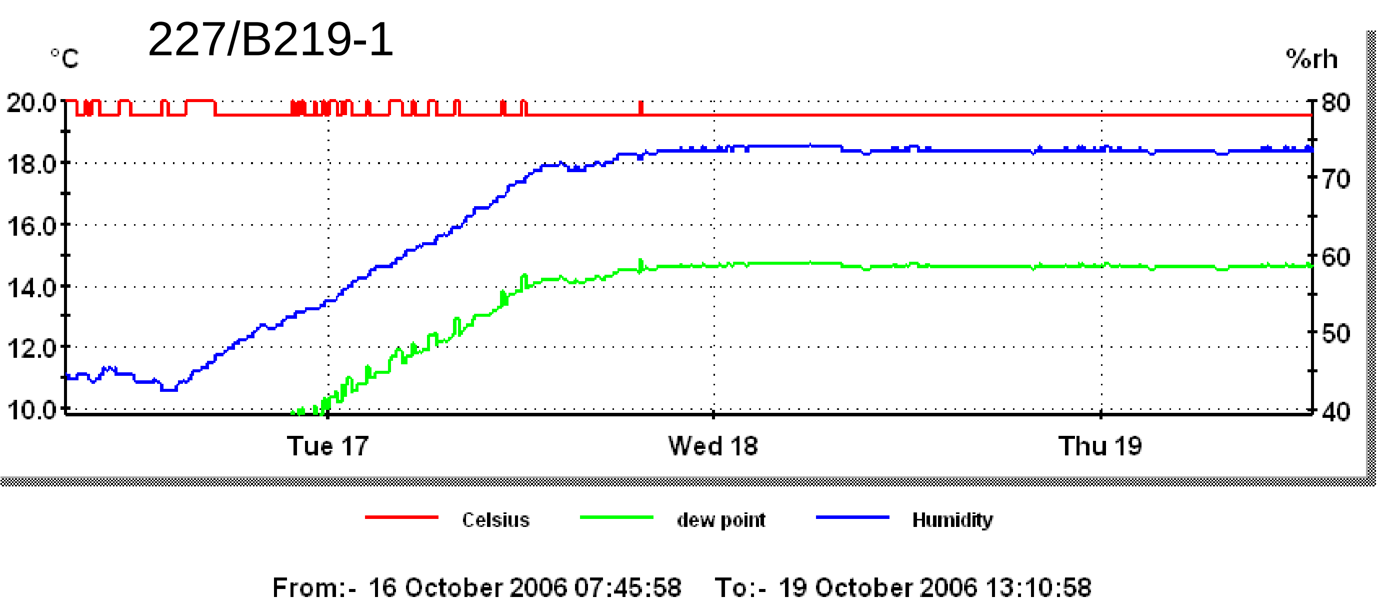
Frig/Freeze Monitors $240 #DT-23-33-80 – USB Temperature Datalogger PLUS Software $79.00 (#DT-23-33-60)
Room Monitors, # DT-23039-52 – USB Temperature-Humidity Datalogger $91.00 (Cole Parmer, Vernon Hills IL)

Room temperature monitoring



Monitoring Instrument Room Temperature Fluctuations

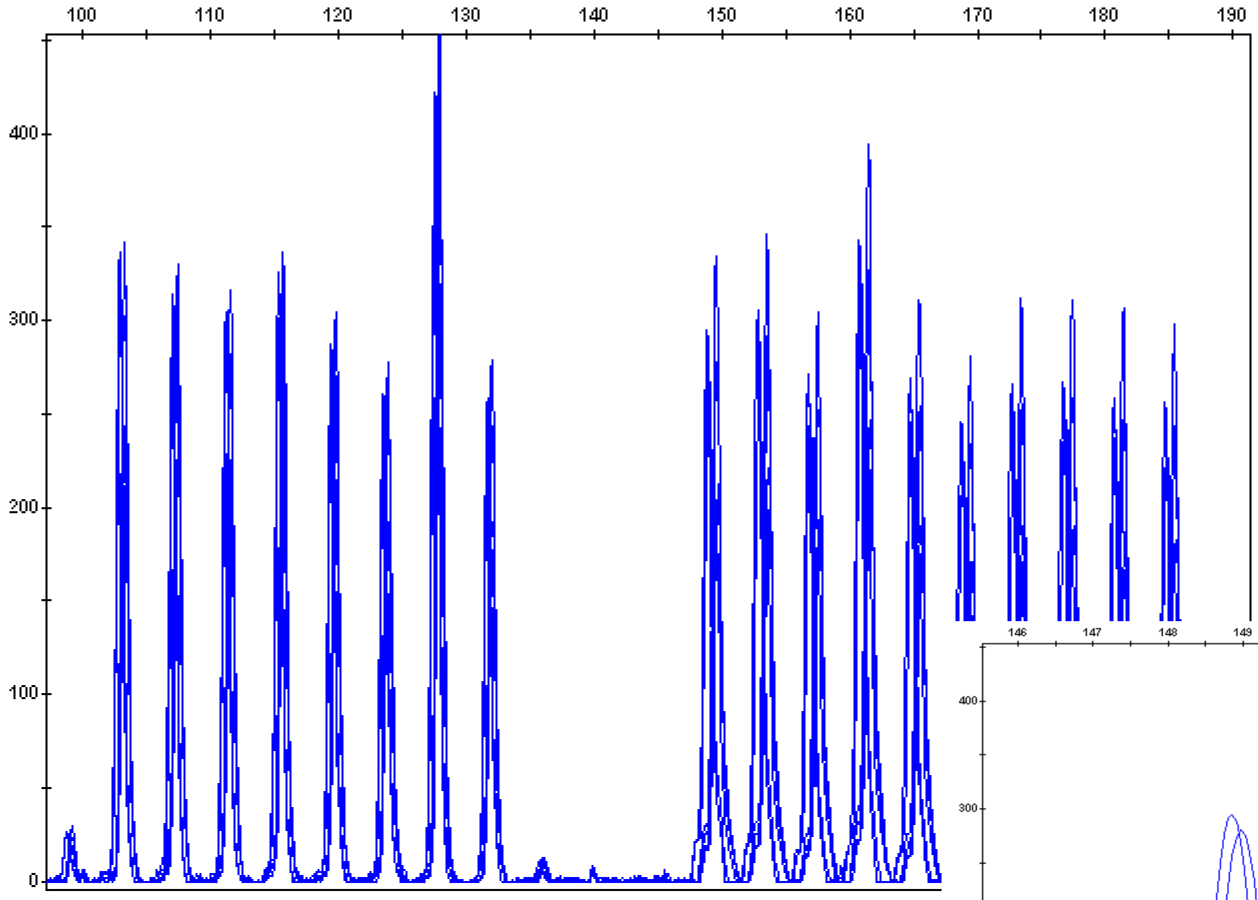


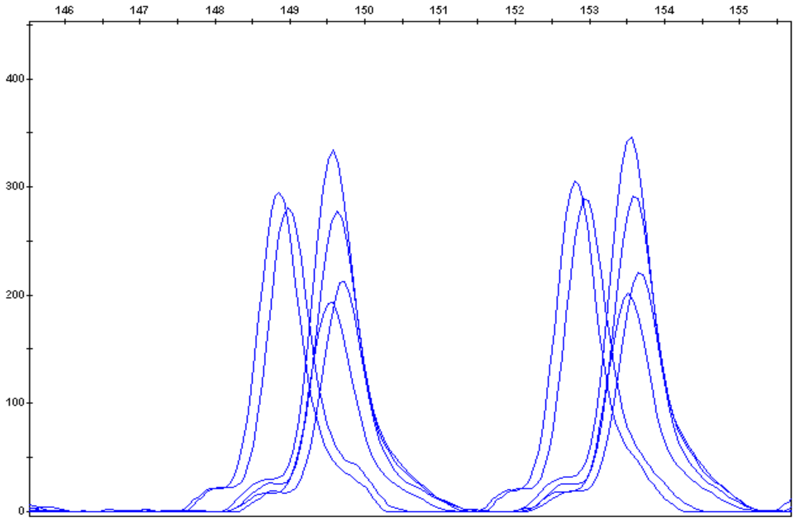


Temperature Monitoring of two separate instrument rooms.

Box area is a 24 hour period where temperature control is not stable.

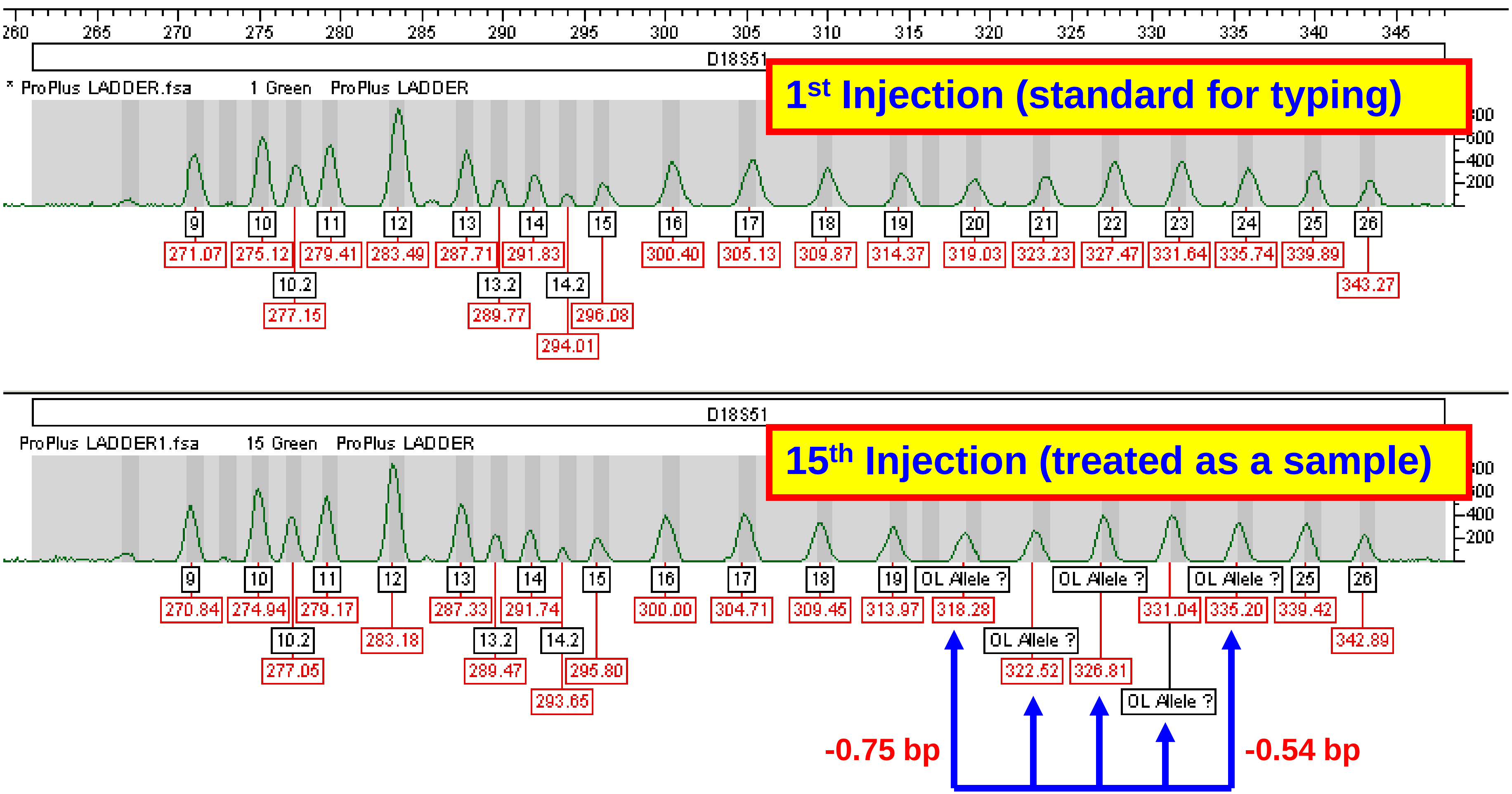
Poor Temperature Control Causes DNA Sizing Imprecision





Ladder Overlay, 6FAM
Combo1, 3130xl

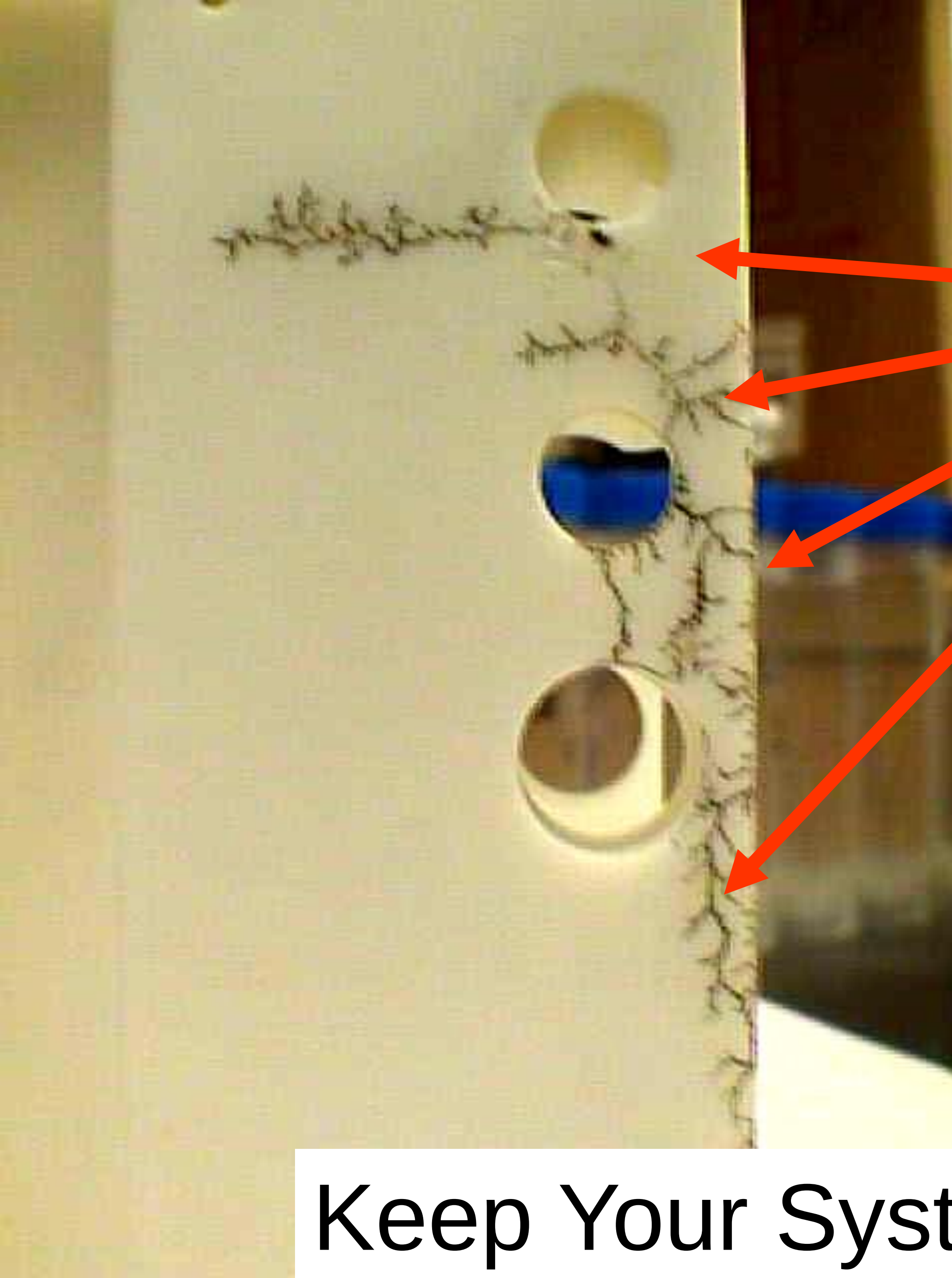
Use of Second Allelic Ladder to Monitor Potential Match Criteria Problems



These alleles have drifted outside of their genotyping bins due to temperature shifting over the course of the sample batch

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



Carbon Trails

High Humidity or wet buffer vials can create other paths to ground

Keep Your System Clean!

Instrumental Factors

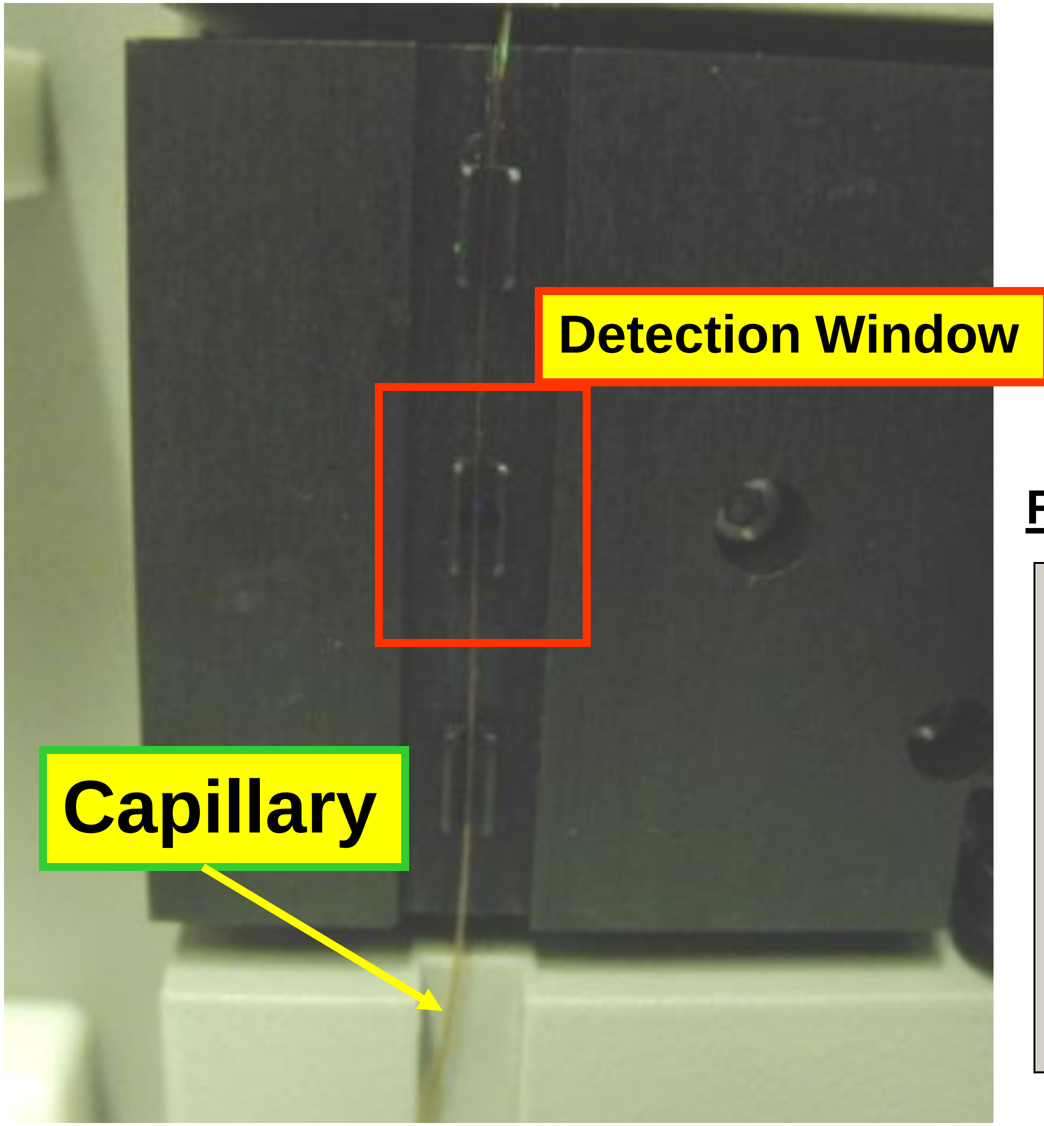
- Optical System
  - Sensitivity changes with age, capillary diameter, capillary cleanliness, instrument calibration

- 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)

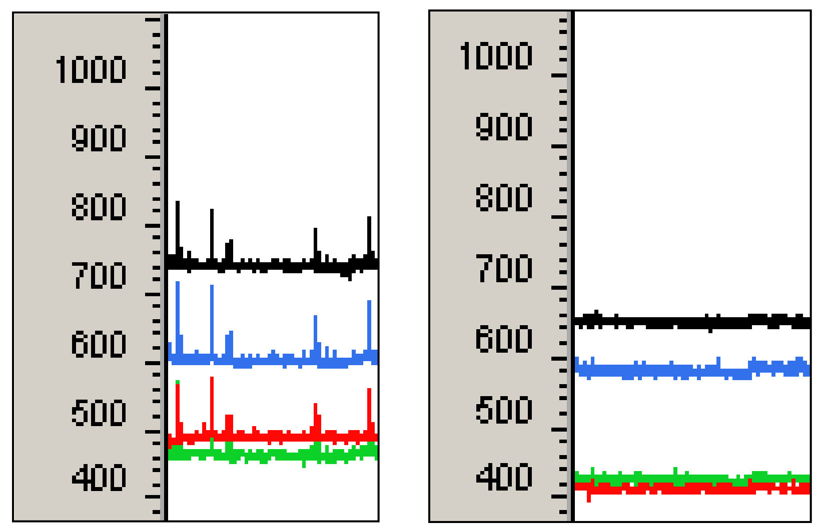
The Detection Window



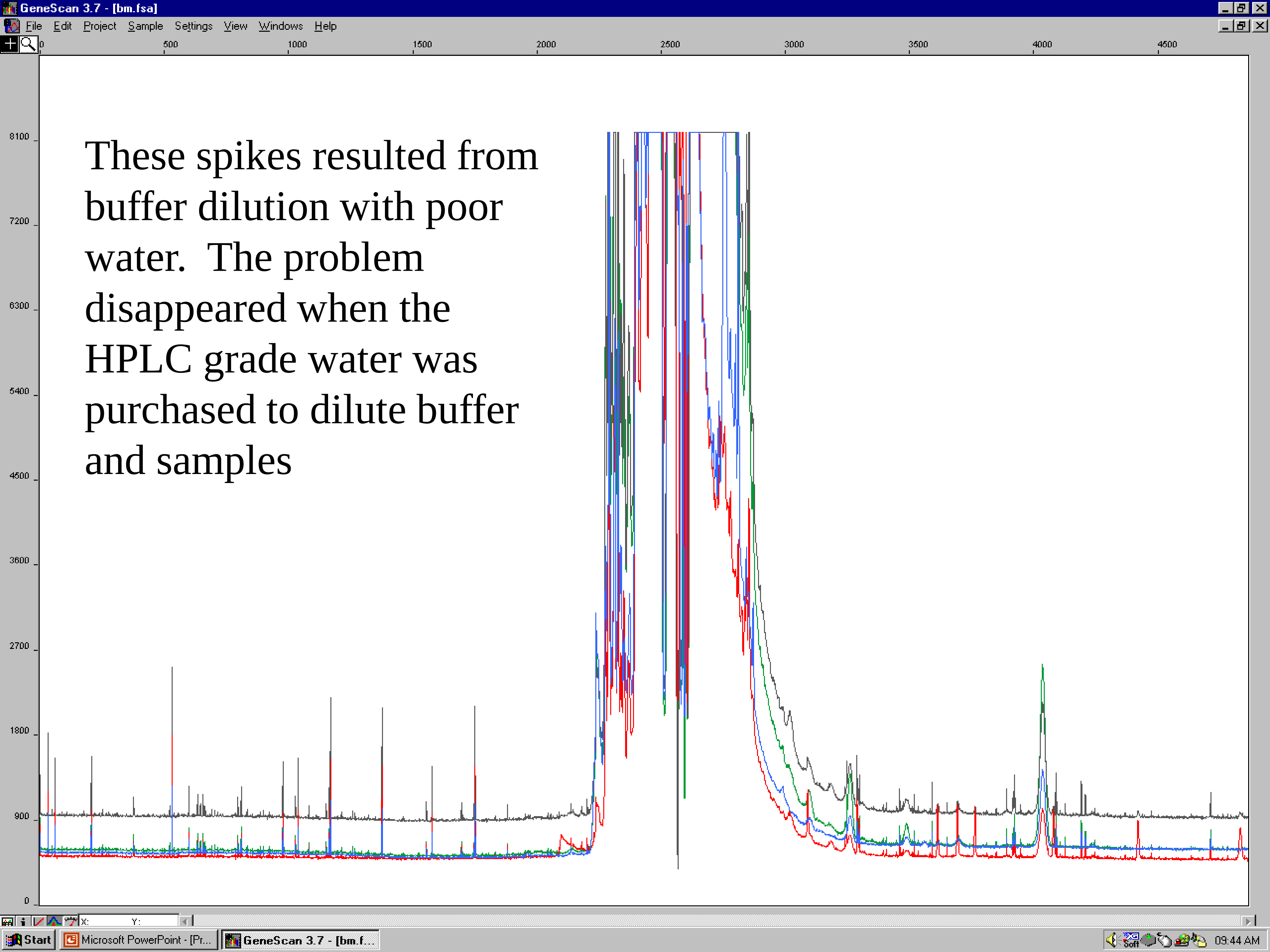
Make sure that the capillary window is lined up (if it is not, then no peaks will be seen)

Window may need to be cleaned with ethanol or methanol

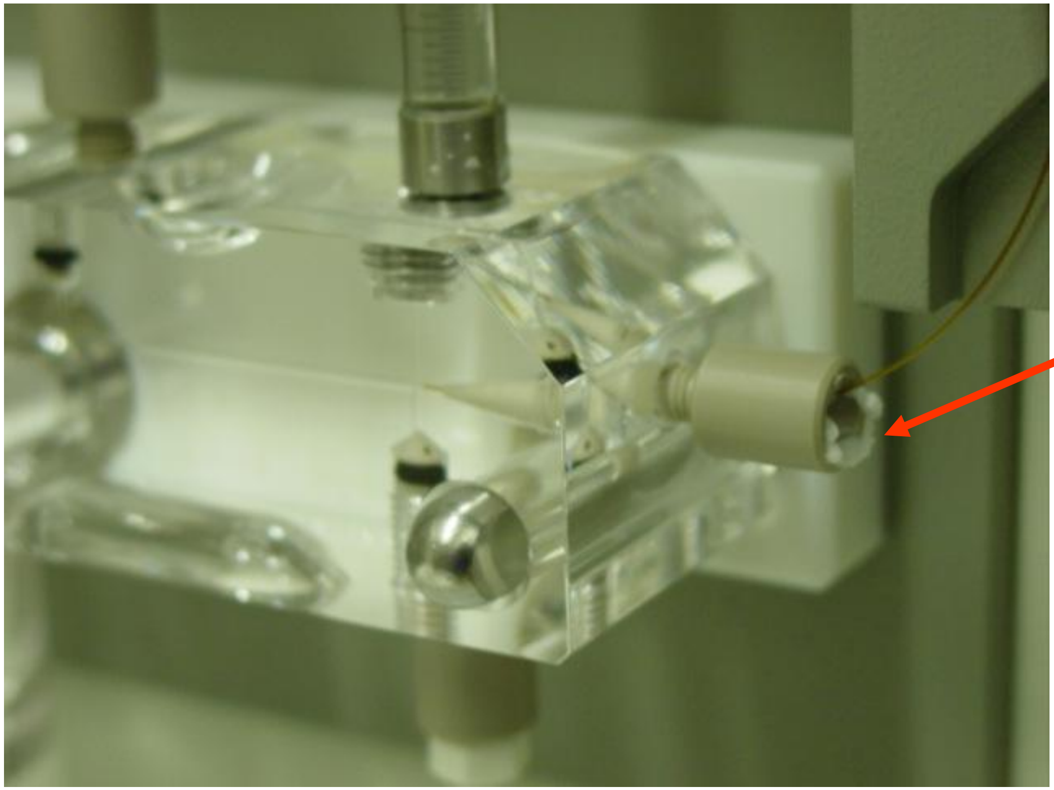
<u>Review Start of Raw Data Collection</u>



Little spikes indicate need to change buffer… check current


GeneScan 3.7 - [bm.fsa]
File Edit Project Sample Settings View Windows Help
0
500
1000
1500
2000
2500
3000
3500
4000
4500
8100
7200
6300
5400
4500
3600
2700
1800
900
0
These spikes resulted from buffer dilution with poor water. The problem disappeared when the HPLC grade water was purchased to dilute buffer and samples
X:
Y:
Start
Microsoft PowerPoint - [Pr...
GeneScan 3.7 - [bm.f...
09:44 AM

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

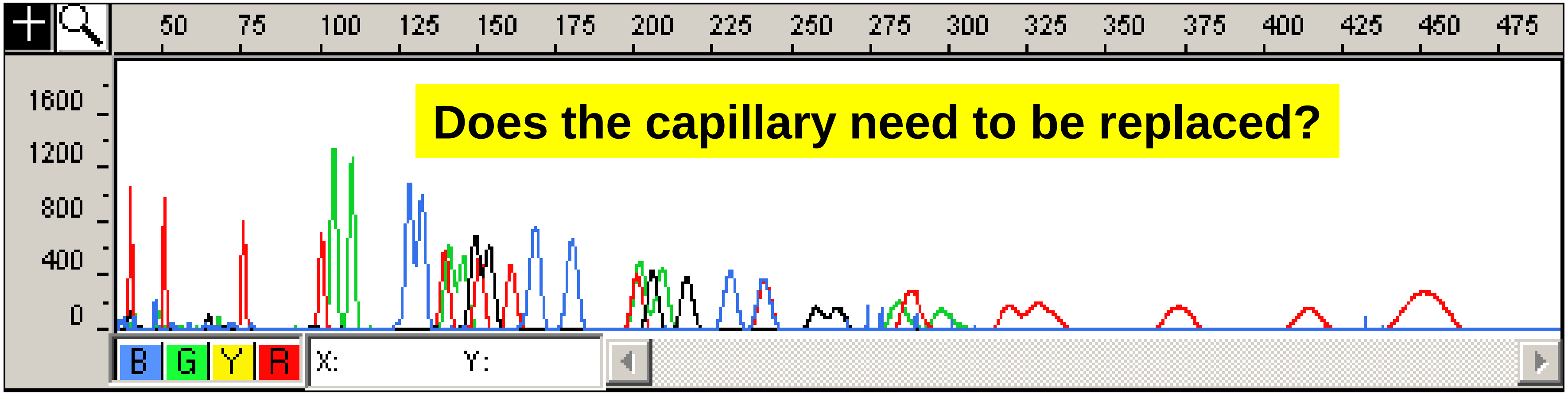
Pump block should be well cleaned to avoid problems with urea crystal formation

Buffer Issues

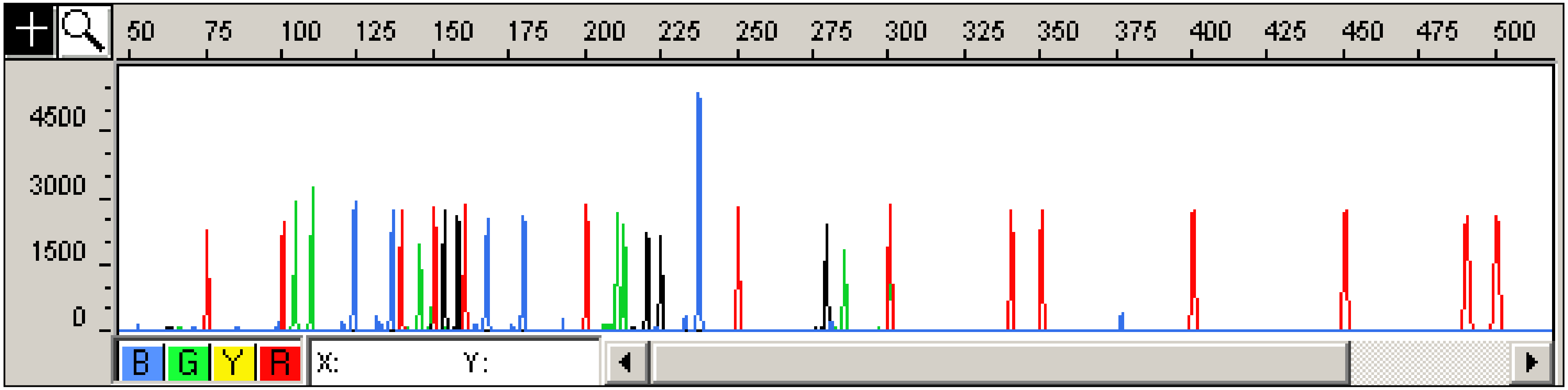
- The buffer and polymer affect the background fluorescence- affecting the matrix
- Urea crystals and dust may produce spikes
- High salt concentrations may produce reannealing of DNA
- High salt concentrations affect current
- Low polymer concentrations affect peak resolution

Meltdowns can be permanent or transitory

as we have seen these may result from sample contamination effects



No! The next injection looks fine…



Meltdowns may be the result of

- Bad formamide
- Excess salt in sample/renaturation
- 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

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)

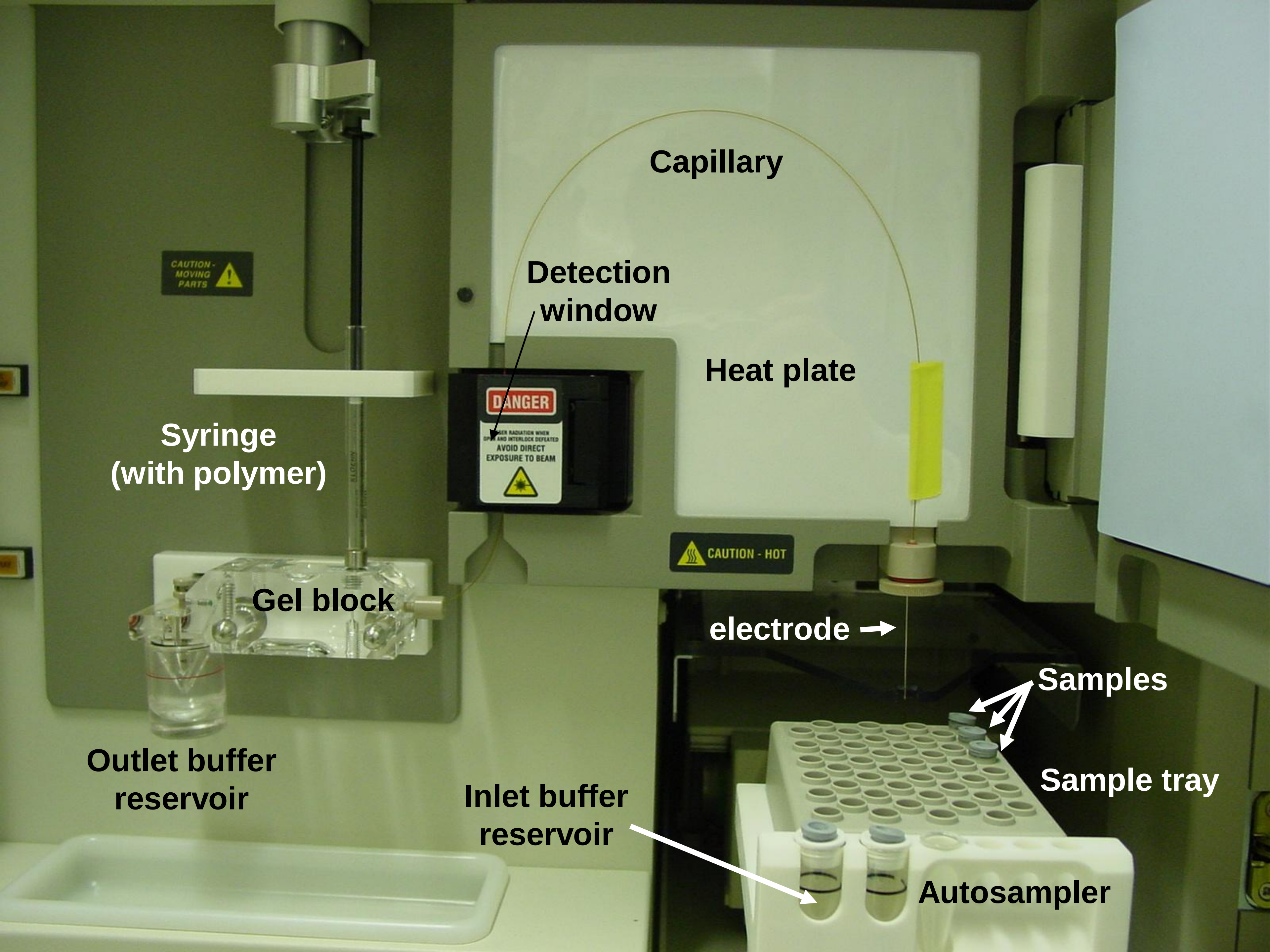
Measurement of Current

- 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)

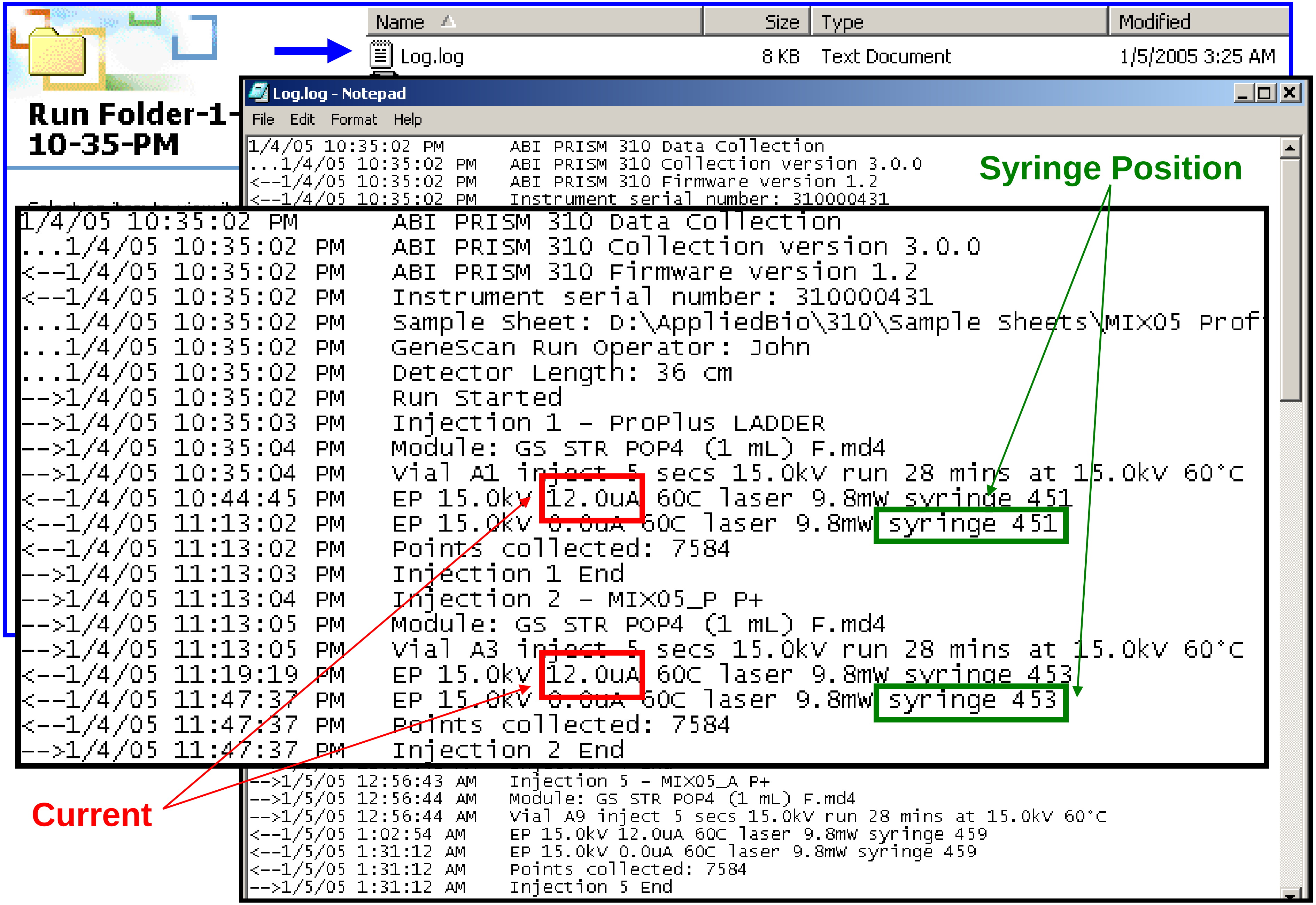


Capillary
Detection window
Heat plate
Syringe (with polymer)
Gel block
electrode
Samples
Sample tray
Outlet buffer reservoir
Inlet buffer reservoir
Autosampler
CAUTION - MOVING PARTS
DANGER
AVOID DIRECT EXPOSURE TO BEAM
CAUTION - HOT

Syringe Travel

- The ABI 310 instrument also keeps track of the position of the syringe (in the log file)
- Depending on the resistance to flow, the syringe will travel different lengths
- Syringe leaks may be reflected in a longer distance traveled prior to each injection
- These leaks occur around the barrel of the syringe and at the connection to the capillary block

Use of ABI 310 Log File to Monitor Current and Syringe Travel

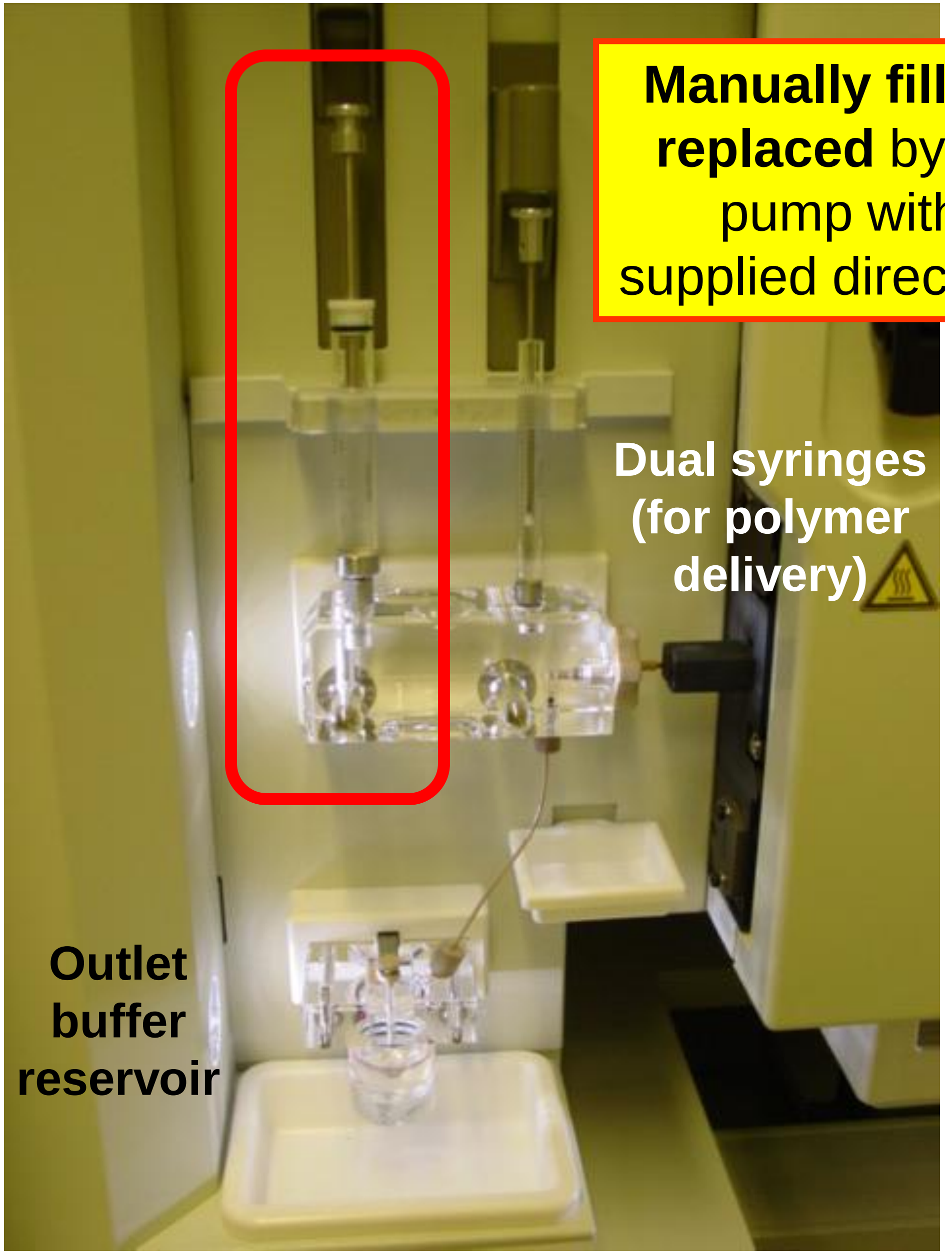


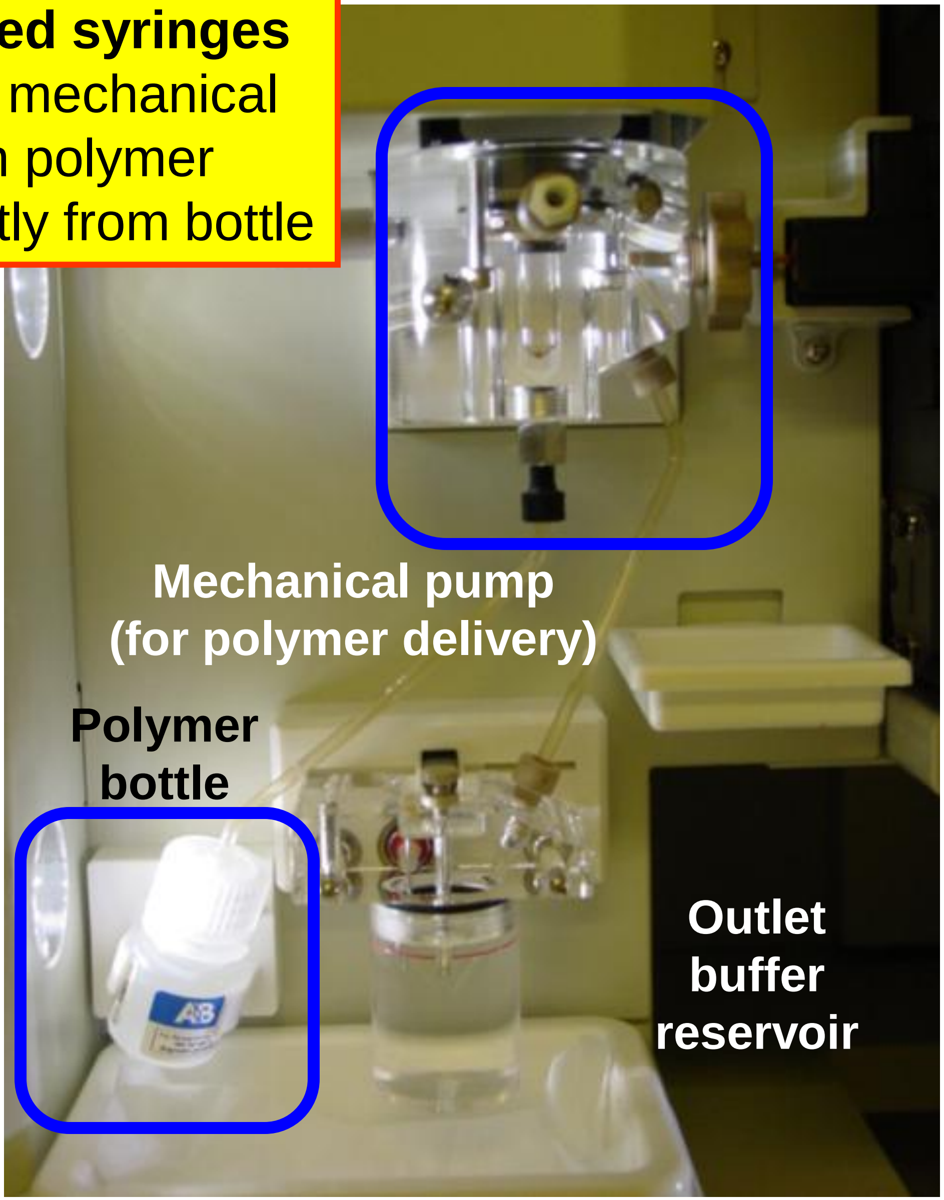
ABI 3100

ABI 3130xl

(upgraded from 3100)

Manually filled syringes replaced by mechanical pump with polymer supplied directly from bottle





Dye Blobs in the Negative Control Sample

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.

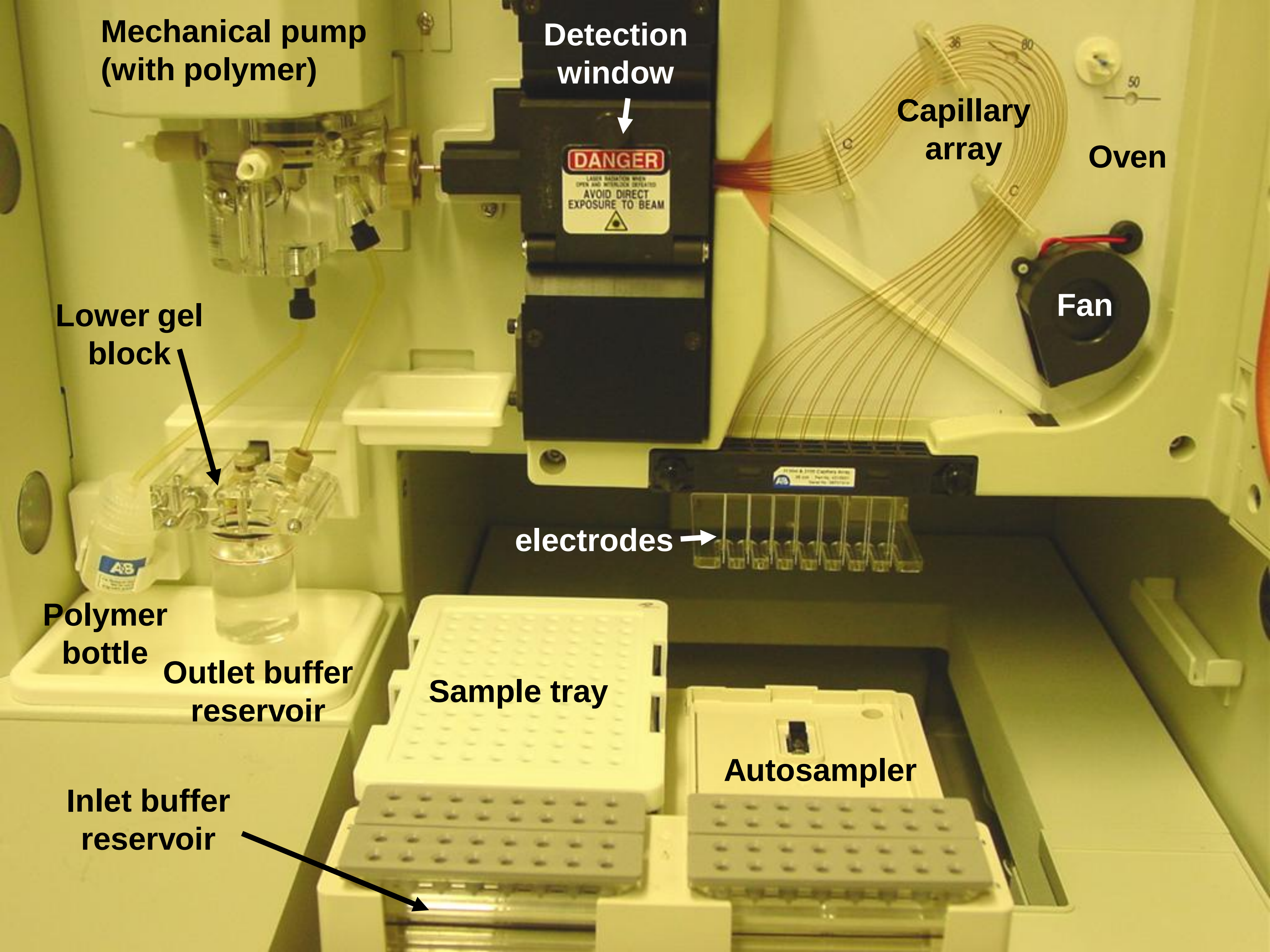
Do not ever open a cold bottle of formamide. Water will condense inside and aid in the formation of conductive formic acid.

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



Mechanical pump (with polymer)
Detection window
DANGER
AVOID DIRECT EXPOSURE TO BEAM
Capillary array
Oven
Fan
Lower gel block
electrodes
Polymer bottle
Outlet buffer reservoir
Sample tray
Autosampler
Inlet buffer reservoir

Acknowledgments

NIST Human Identity Project Team

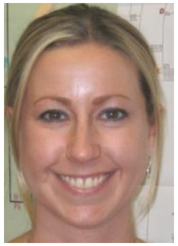
Leading the Way in Forensic DNA…

John Butler

Erica Butts

Mike Coble

Dave Duewer



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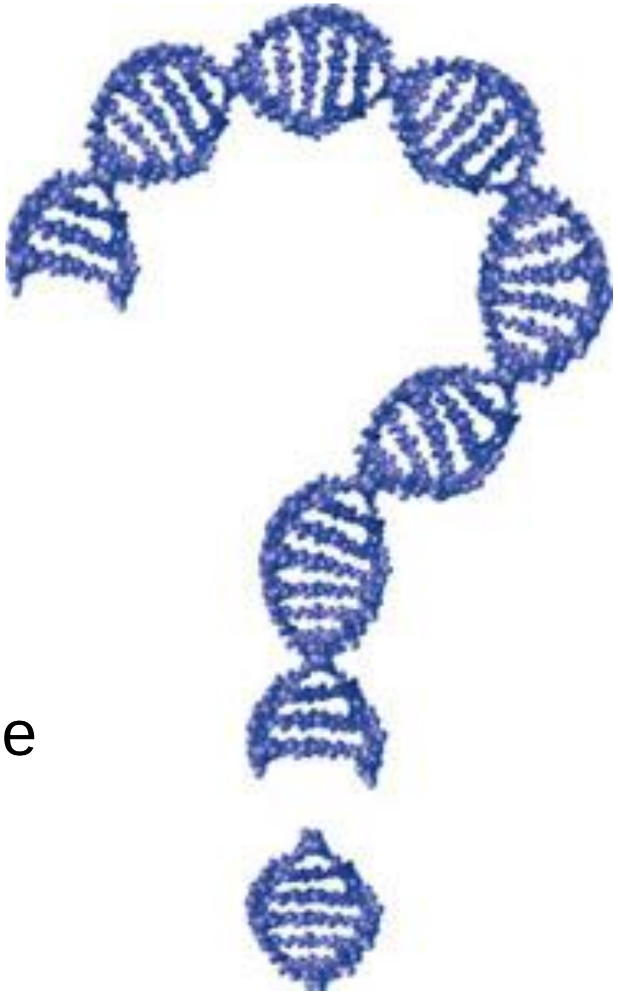
Thank you for your attention



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