Capillary Electrophoresis in DNA Analysis

Troubleshooting CE Systems

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Outline for Workshop

- Introductions
- STR Analysis
- Introduction to CE and ABI 310
- Validation and Interlaboratory Studies
- Real-time qPCR and miniSTRs
- Stats and Higher Throughput Approaches
- Y-Chromosome Analysis
- Troubleshooting CE Systems
- Review and Test

Troubleshooting

1. Chemistry problems- stutter, quantitation, PCR
2. External factors – power supply, room temperature
3. Sample and buffer problems – formamide, urea, dirt and dust
4. Instrument problems – age, capillary clogging, syringe leaks, voltage leaks

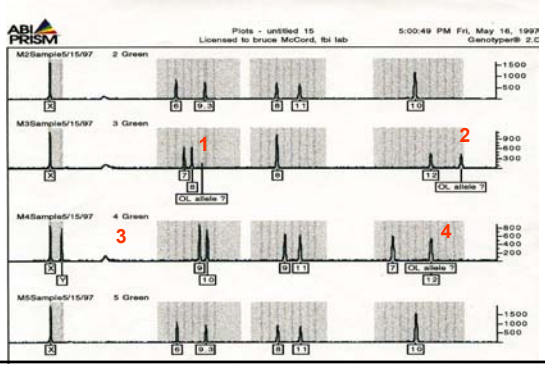
Bug a Boos…..

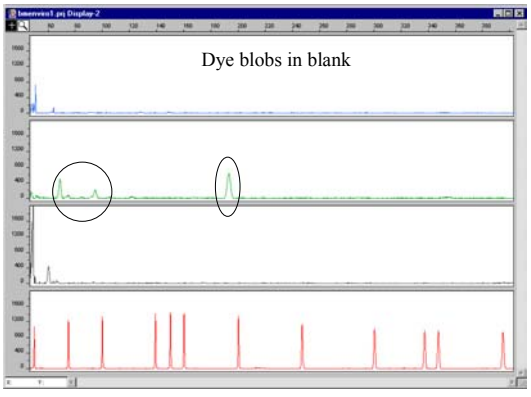
1. **Adenylation - PCR issue, particularly when overamplifying –effect on peak height**
2. **Stutter – also a PCR issue – a big problem with low and high template conc.**
3. **Free Dye – a manufacturing problem**
   Contaminants from primer dye manufacture
4. **Voltage Spikes – instrument or buffer problem –**
   Filtration or centrifugation will work sometimes
5. **Pull – up - Consider the effect of the capillary window, the buffer and the CCD on the matrix**

Off-ladder alleles

Four types

1. Spike
2. OL Allele
3. Free Dye
4. Noise

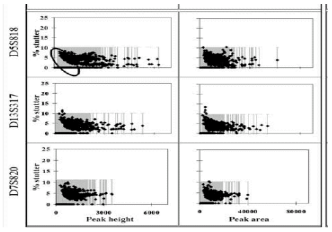




Question: What is a real blank?

- Because of the stacking effect, injections of pure water or formamide can produce extreme sensitivity leading to a false impression that carry-over is a problem
- Instead, inject ROX plus formamide as your blank

% Stutter vs Peak Height

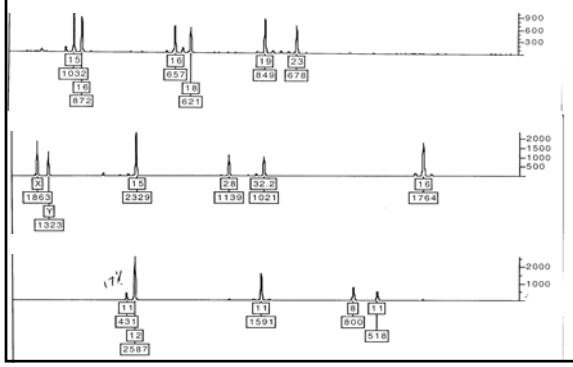


At low concentrations stutter increases – a stochaistic effect?

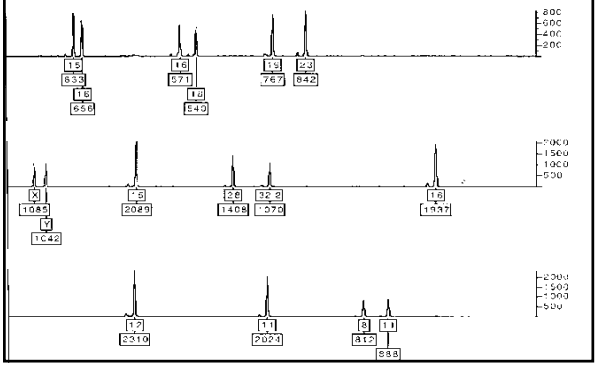
Poor quality samples from casework may also cause problems due to inhibition.

J Forensic Sci, Sept. 2004, Vol. 49, No. 5

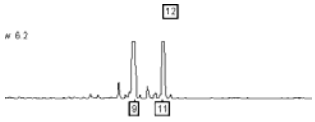
Extract of Pistol Grip and Trigger Amplified using Profiler+ Multiplex



Suspect Blood Sample Amplified using Profiler+ Multiplex



Overloaded peaks will also show relatively high stutter



Truncated peaks give wrong ratios for peak stutter

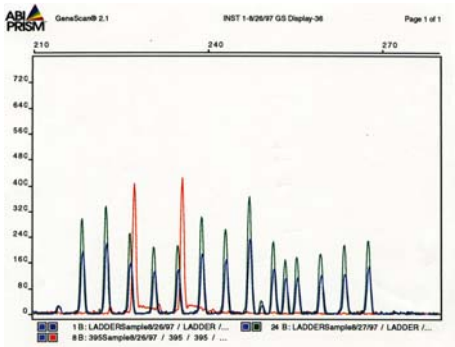
Why else is overloading bad?

1. **raised baseline**
2. **non specific amplification**
3. **peak height ratios**

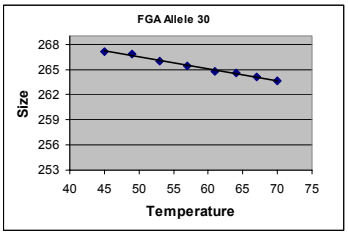
Temperature effects

- 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
  - $P = VI = I^2R$
  - Increased current → internal temperature rise→ diffusion → band broadening

Band shift in CE Analysis of the FGA locus
Likely the result of temperature or viscosity induced mobility change

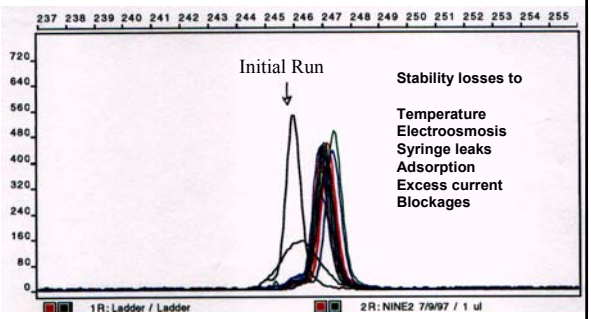


Effect of Temperature on allele size

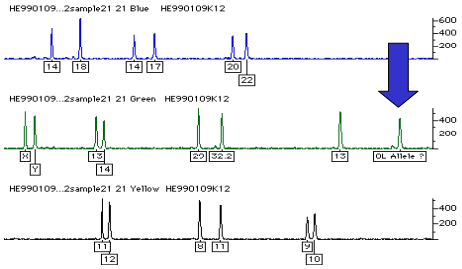


Slope is 0.14 bases/degree centigrade
Standard deviation is O. 17 or so
Therefore a small change in temperature has a big effect

Due to its structure and its non-calibration, the "250"peak can be used to indicate stability



Temperature Effect: Electrophoretic Mobility Shift



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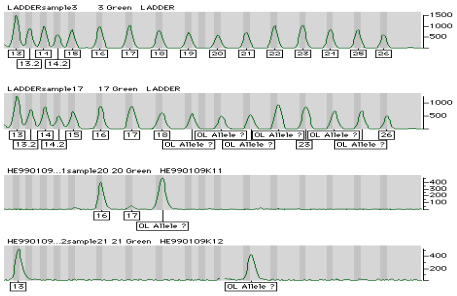

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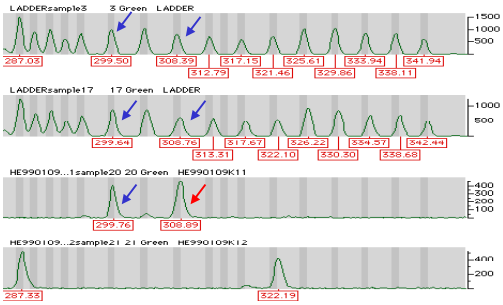
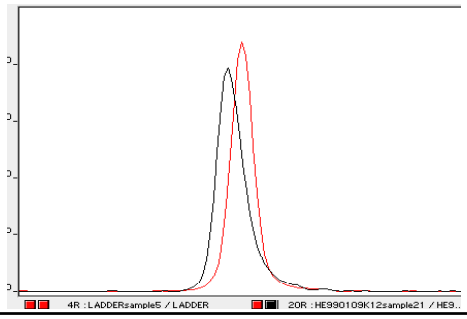
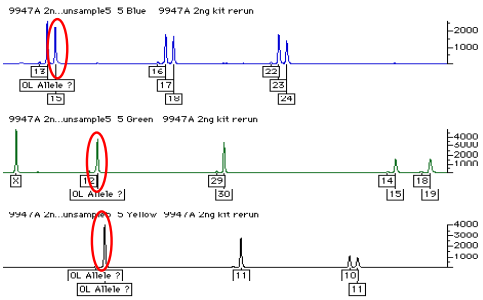


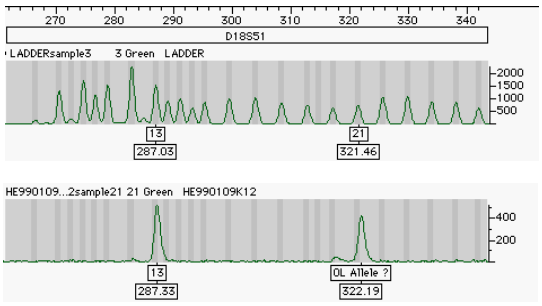
GS500: 250 Peak (K12 v Ladder)



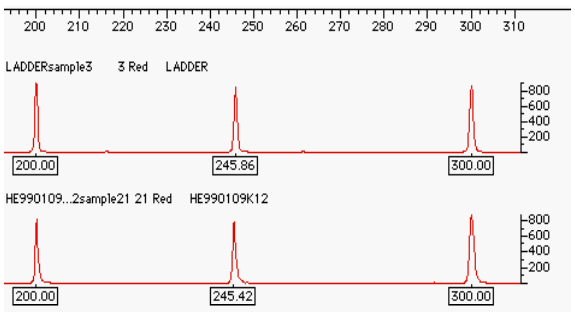
Temperature Effects: "OL" Alleles



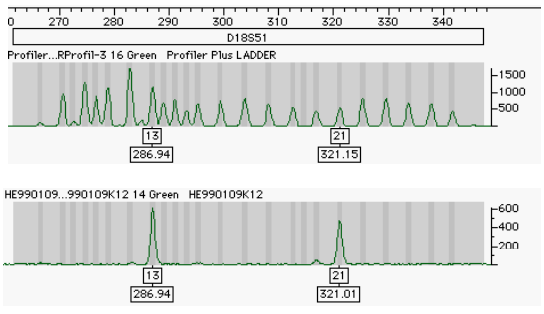
"OL Alleles"



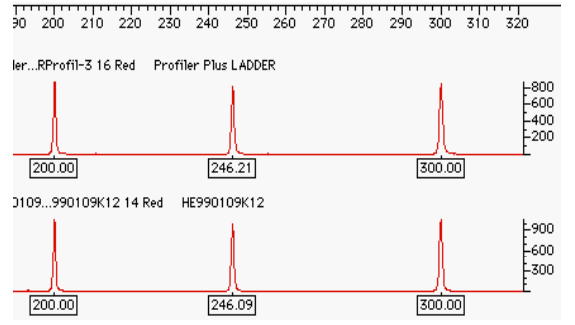
"OL alleles " - look at the 250 peak



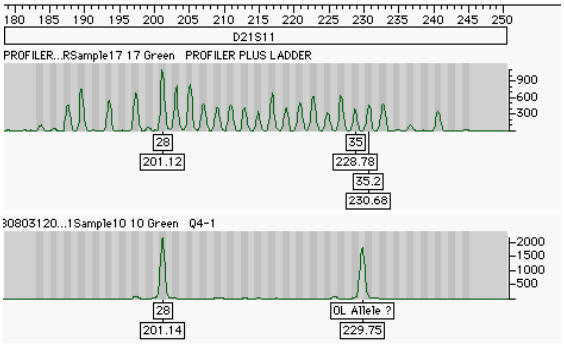
"OL allele re-injected"



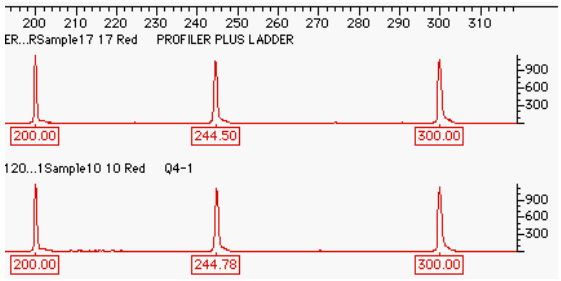
And the 250 peak...



True off-ladder alleles



True off-ladder allele: the 250 peak



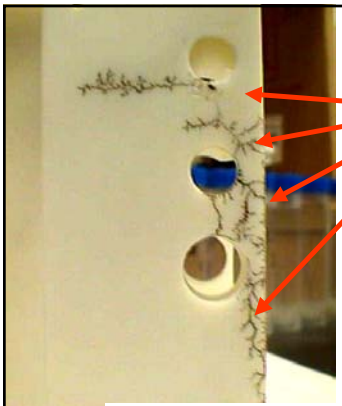
Monitoring Room Temperature Over Time

What to do if calibration is lost?

- If protocol permits
  - Go to the next ladder
  - Rerun sample
  - Check current
  - Check allelic ladder
- Always check the Rox ladder
  - 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

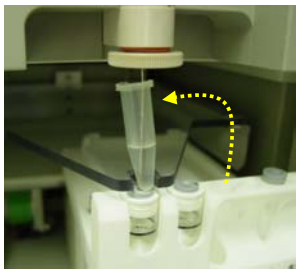


Carbon Trails

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

Keep Your System Clean!

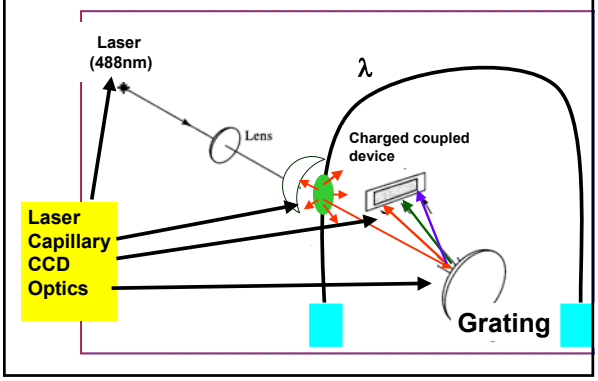
Storage when ABI 310 is not in use



Remember that the water in the open tube will evaporate over time…

- 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

Consider the optical system



Issues with the Optical System

- Pay attention to signal to noise, not absolute peak intensity
- Argon Ion lasers outgas and eventually loose intensity. Take note of laser current
- Fluorescence expression:

$I_f = I_0 k \varepsilon b C \varphi$ - changes in input intensity, I_0

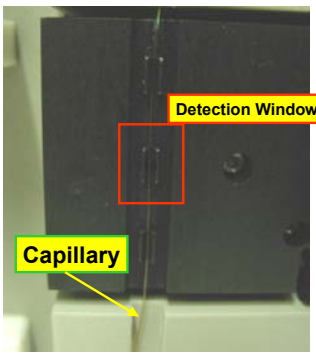
- changes in capillary diameter, b

- cleanlyness of capillary, k

All these things directly affect peak RFUs, however, baseline noise is more affectected by detector.

Thus by monitoring signal to noise, you can get a better picture of your optical system.

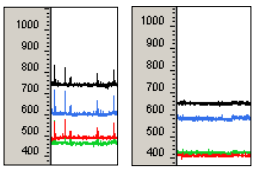
The Detection Window



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

Review Start of Raw Data Collection



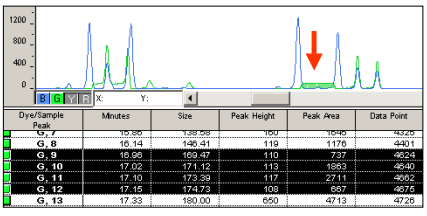
Little spikes indicate need to change buffer… check current

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

Raised Baseline Problem

- 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

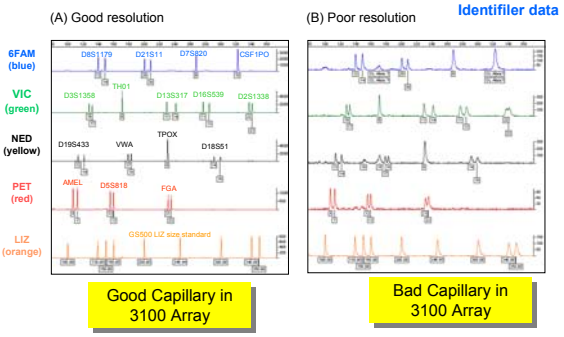


Dye/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point
G, 7	15.85	138.58	150	1546	4325
G, 8	16.14	146.41	119	1176	4401
G, 9	16.98	169.47	110	737	4624
G, 10	17.02	171.12	113	1883	4640
G, 11	17.10	173.39	117	2711	4662
G, 12	17.15	174.73	108	667	4675
G, 13	17.33	180.00	650	4713	4726

Some Other Problems

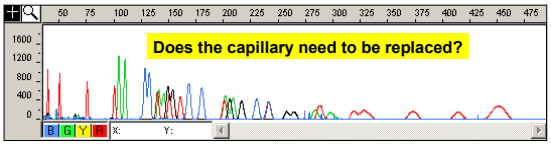
- Capillary with poor resolution
- "Melt downs" – sample contaminants
- Syringe leak or bottoming – peak broadening and mobility shifts
- Formamide conductivity gives low sensitivity or excessive sensitivity

Capillary Resolution Differences

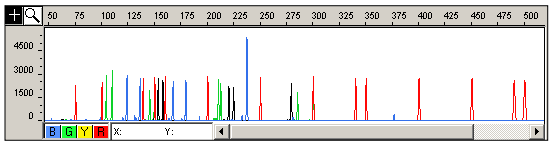


Butler, J.M., Buel, E., Crivellente, F., McCord, B.R. (2004) Forensic DNA typing by capillary electrophoresis: using the ABI Prism 310 and 3100 Genetic Analyzers for STR analysis. *Electrophoresis*, 25: 1397-1412.

Meltdowns can be permanent or transitory



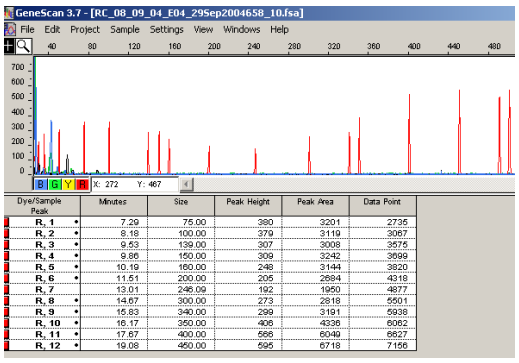
No! The next injection looks fine…



Meltdowns may be the result of

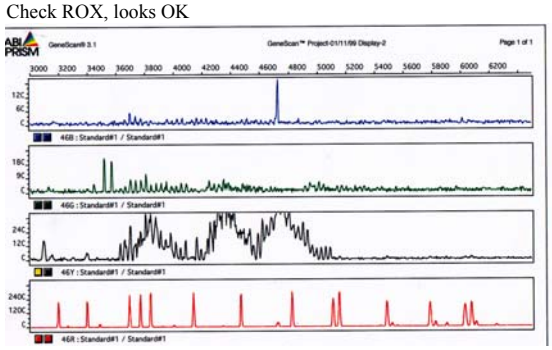
- Bad formamide
- Excess salt in sample/renaturation
- Water in the polymer buffer
- Syringe leak or bottom out
- Poisoned capillary
- Conductive polymer buffer due to urea degradation
- Crack/shift in capillary window

Golden Gate Effect
Attributed to poor formamide



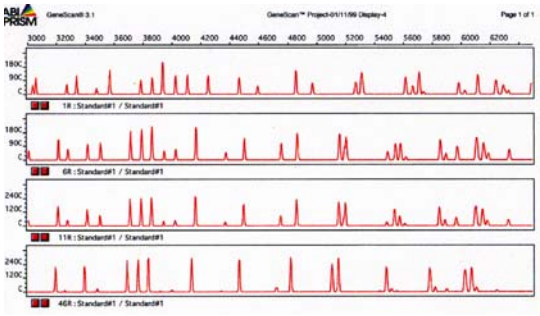
Dye/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point
R, 1	7.29	75.00	390	3201	2735
R, 2	8.18	100.00	379	3119	3067
R, 3	9.53	139.00	307	3008	3575
R, 4	9.86	150.00	309	3242	3699
R, 5	10.19	160.00	248	3144	3820
R, 6	11.51	200.00	205	2694	4318
R, 7	13.01	246.09	192	1950	4877
R, 8	14.67	300.00	273	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

Sample Problem?.
Check ROX, looks OK



320 V/cm 47 cm uncoated capillary
POP4 Polymer

Answer: Incomplete denaturation of standard due to poor quality formamide



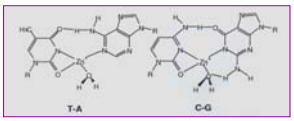
320 V/cm 47 cm uncoated capillary POP4 Polymer

A permanent loss of resolution may mean

- Adsorptive sites on a capillary
- Initiation of electroosmotic flow
- Conductivity changes in buffer
- Wrong molecular weight or concentration of sieving polymer (viscosity)

Transition metal ions

Metal cations present in degraded samples represent a different type of contamination

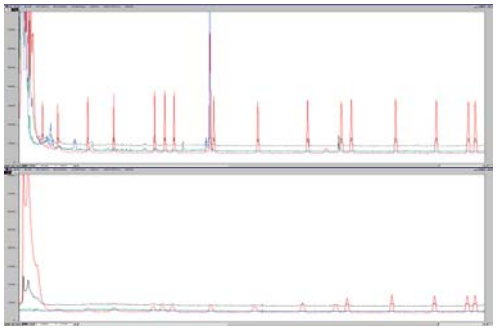


Zn^{2+}, Co^{2+}, and Ni^{2+} form DNA-metal ion complexes, termed M-DNA.at pH conditions above 8,

These cations produce severe effects in CE injection and analysis

Hartzell and McCord, *Electrophoresis*, in press

CE: Effect of pH 7 vs. 8.3

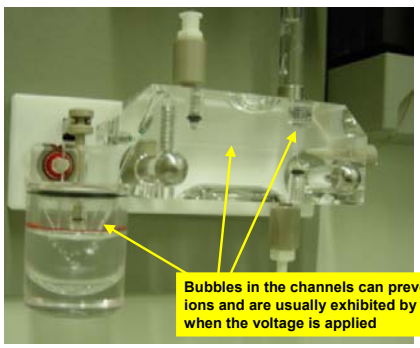


1 µl TH01 added to 10 µl of 3.0 mM $NiCl_2$ in 10 mM Tris, pH 7 or pH 8.3. Sample allowed to interact for 1 hr and then 1 µl added to ROX/formamide.

Another cause of band broadening can be poor capillary loading

- Syringe leaks
  - At the barrel
  - At the capillary nut
  - At the capillary window
- Viscosity changes
  - Water in the block
  - Bubbles
  - Temperature
- Capillary conditioning
  - Preelectrophoresis
  - clogging

Remove all bubbles from the channels



Beware of Urea Crystals

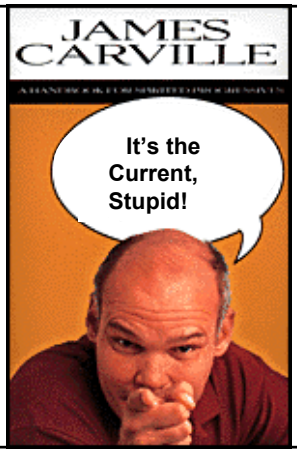
Urea crystals have formed due to a small leak where the capillary comes into the pump block

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

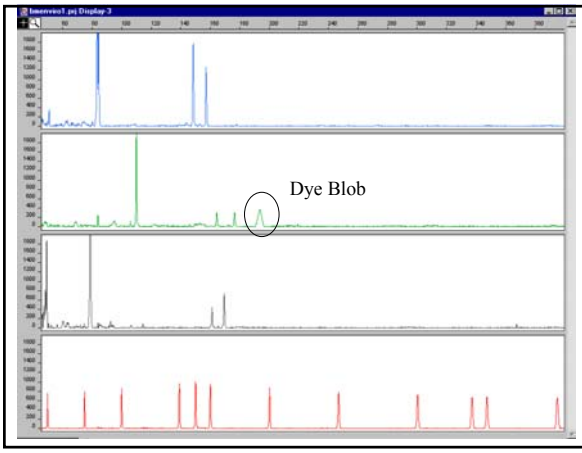
Troubleshooting is more than following the protocols

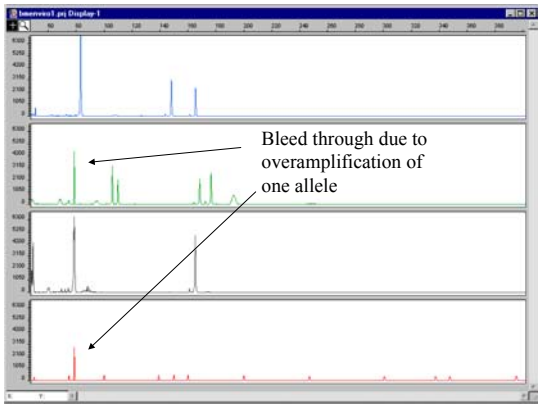
It means keeping watch on all aspects of the operation

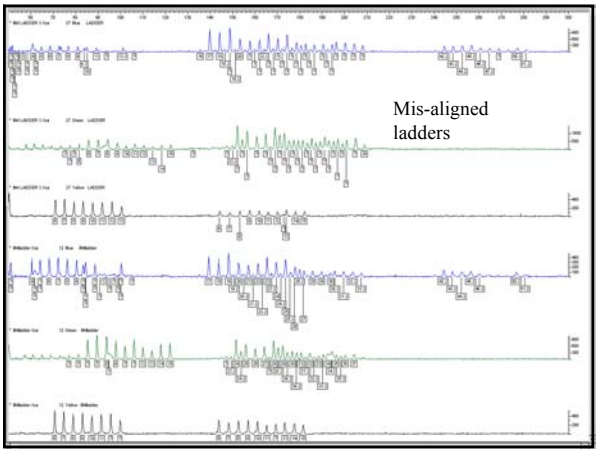
1. Monitoring conductivity of sample and formamide
2. Keeping track of current and syringe position in log.
3. Watching the laser current
4. Watching and listening for voltage spikes
5. Monitoring room temperature and humidity

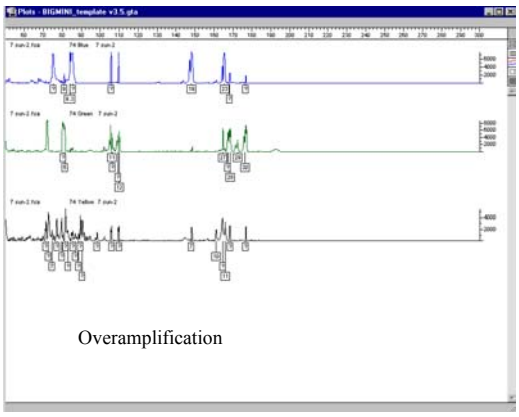


Just Remember

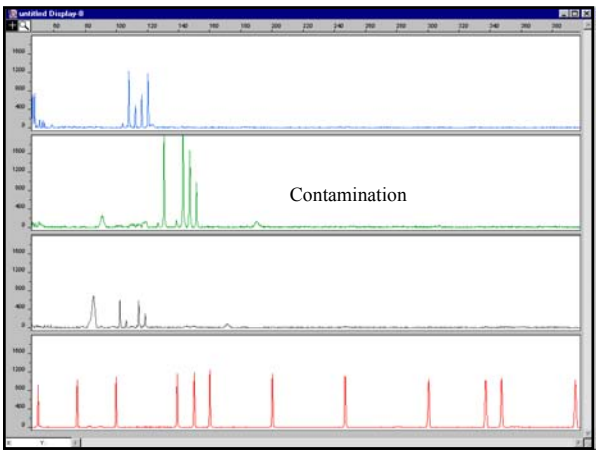




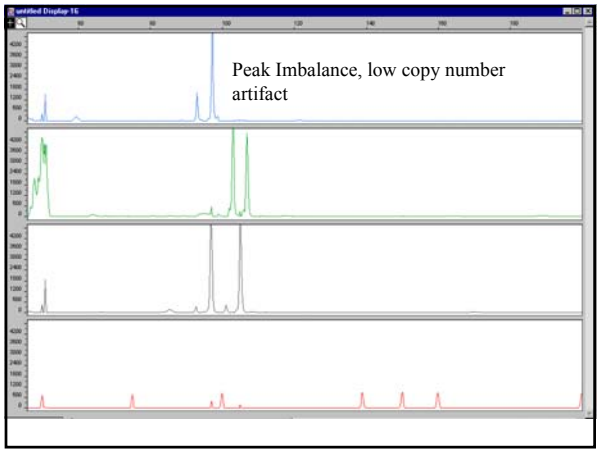




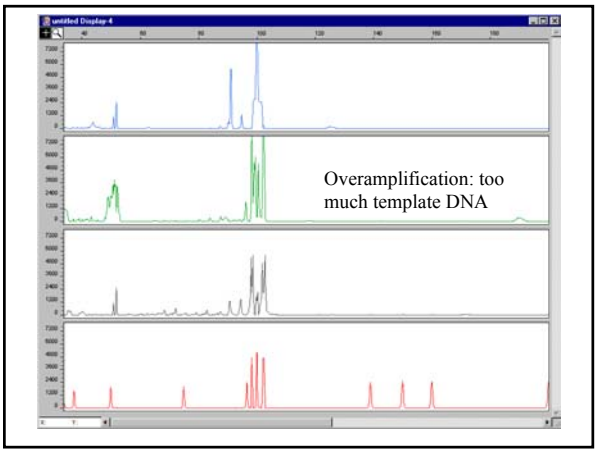
Overamplification



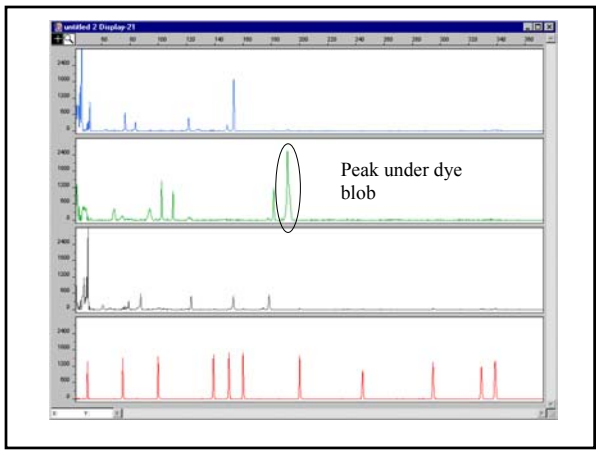
Contamination



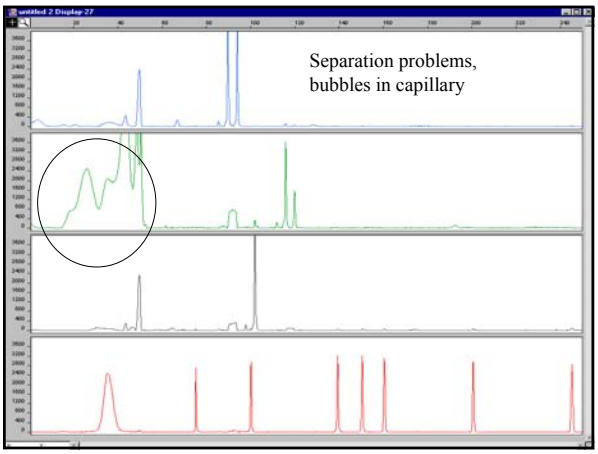
Peak Imbalance, low copy number artifact



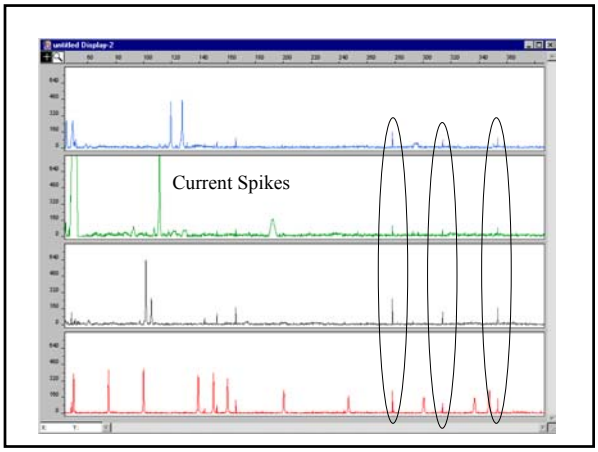
Overamplification: too much template DNA



Peak under dye blob



Separation problems, bubbles in capillary


Current Spikes