Capillary Electrophoresis in DNA Analysis

Troubleshooting CE Systems

DNA Academy Workshop
Albany, NY
June 13-14, 2005
Dr. John M. Butler
Dr. Bruce R. McCord

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

Dye blobs in blank

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
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 stochastic effect?

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

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
  - \( \mu_p = \frac{q}{6\tau \eta r} \)
- Diffusion – band broadening
- DNA
- Conformation – DNA size based sieving
  - vs \( \sim \)
  - \( \mu_p = \frac{q}{6\tau \eta r} \)
- Current – Power
  - \( P = VI = I^2R \)
  - Increased current \( \rightarrow \) internal temperature rise
  - diffusion \( \rightarrow \) 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 0.17 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

Stability losses to
Temperature
Electroosmosis
Syringe leaks
Adsorption
Excess current
Blockages
GS500: 250 Peak (K12 v Ladder)
True off-ladder alleles

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 sublimes 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

Keep Your System Clean!

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Storage when ABI 310 is not in use

- 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 lose intensity. Take note of laser current
- Fluorescence expression: \[ I = I_0 k b C \phi \]
  - changes in input intensity, \( I_0 \)
  - changes in capillary diameter, \( b \)
  - cleanliness of capillary, \( k \)

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

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
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

(A) Good resolution

(B) Poor resolution

Some Other Problems

- Meltdowns can be permanent or transitory

Meltdowns can be permanent or transitory

Does the capillary need to be replaced?

No! The next injection looks fine...

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Capillary Resolution Differences

(A) Good resolution

(B) Poor resolution

Identifiler data

Golden Gate Effect

Attributed to poor formamide

Sample Problem?

Check ROX, looks OK

320 V/cm 47 cm uncoated capillary

POP4 Polymer

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Answer: Incomplete denaturation of standard due to poor quality formamide

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

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
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.

It’s the Current, Stupid!

Bleed through due to overamplification of one allele.

Dye Blob

Mis-aligned ladders.
Overamplification:
- Too much template DNA
- Peak under dye blob
- Separation problems, bubbles in capillary

Contamination:
- Low copy number artifact