Advanced Topics in Forensic DNA Analysis

**ABI 310/3100/3130**

**Capillary Electrophoresis Fundamentals**

New Jersey State Police
Training Workshop

Hamilton, NJ
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Presentation Outline

- Historical perspective
- STR loci and kits
- CE history and background
- Injection and sample preparation
- Separation
- Detection

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Historical Perspective on DNA Typing

- **1989**: DQA1 & PM (dot blot)
- **1990**: CODIS loci defined
- **1992**: Capillary electrophoresis of STRs first described
- **1994**: First STRs developed
- **1994**: PowerPlex® 16 (6 loci in single amp)
- **1995**: First commercial fluorescent STR multiplex
- **1996**: mtDNA
- **1998**: FSS Quadruplex
- **1999**: CODIS loci defined
- **2000**: STR typing with CE is fairly routine
- **2002**: mtDNA
- **2004**: V-STRs
- **2006**: DNA is an important part of the criminal justice system

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

STR typing is here to stay for a few years because of DNA databases that have grown to contain millions of profiles.

Short Tandem Repeat (STR) Markers

An accordion-like DNA sequence that occurs between genes

TCCCAAGCTTCTCTTCTTCTCTCTTAGTAATACAGCAGACAGACAGA
GGTAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGAT
TAGATAGATACATTGAAAGACAAAACAGAGATGGATGATAGAT
ACATGCTTACAGATACAC

= 12 GATA repeats ("12" is all that is reported)

The number of consecutive repeat units can vary between people

The FBI has selected 13 core STR loci that must be run in all DNA tests in order to provide a common currency with DNA profiles.

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

http://www.cstl.nist.gov/biotech/strbase/training.htm
Types of STR Repeat Units

Requires size based DNA separation to resolve different alleles from one another

- **Di** nucleotide  
  (CA)(CA)(CA)(CA)

- **Trinucleotide**  
  (GCC)(GCC)(GCC)

- **Tetranucleotide**  
  (AATG)(AATG)(AATG)

- **Penta** nucleotide  
  (AGAAA)(AGAAA)

- **Hexanucleotide**  
  (AGTACA)(AGTACA)

Short tandem repeat (STR) = microsatellite = simple sequence repeat (SSR)

Categories for STR Markers

<table>
<thead>
<tr>
<th>Category</th>
<th>Example Repeat Structure</th>
<th>13 CODIS Loci</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple repeats – contain units of identical length and sequence</td>
<td>(GATA)(GATA)(GATA)</td>
<td>TPOX, CSF1PO, D5S818, D13S317, D19S433</td>
</tr>
<tr>
<td>Simple repeats with non-consensus alleles (e.g., TH01 9.3)</td>
<td>(GATA)(GAY)(GATA)</td>
<td>TH01, D18S51, D7S820</td>
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<tr>
<td>Compound repeats – comprise two or more adjacent simple repeats</td>
<td>(GATA)(GACA)</td>
<td>VWA, PGA, D3S1358, D8S1179</td>
</tr>
<tr>
<td>Complex repeats – contain several repeat blocks of variable unit length</td>
<td>(GATA)(GACA)(CA)(CATA)</td>
<td>D2S111</td>
</tr>
</tbody>
</table>

These categories were first described by Urquhart et al. (1994) Int. J. Legal Med. 107:13-20

How many STRs in the human genome?

- The efforts of the Human Genome Project have increased knowledge regarding the human genome, and hence there are many more STR loci available now than there were 10 years ago when the 13 CODIS core loci were selected.

- More than 20,000 tetranucleotide STR loci have been characterized in the human genome (Collins et al. An exhaustive DNA microsatellite map of the human genome using high performance computing. Genomics 2003;82:10-19)

- There may be more than a million STR loci present depending on how they are counted (Lander et al. Initial sequencing and analysis of the human genome. Nature 2001;409:860-921).


http://www.cstl.nist.gov/biotech/strbase/training.htm
Multiplex PCR
(Parallel Sample Processing)

- Compatible primers are the key to successful multiplex PCR
- STR kits are commercially available
- 15 or more STR loci can be simultaneously amplified

Advantages of Multiplex PCR
- Increases information obtained per unit time (increases power of discrimination)
- Reduces labor to obtain results
- Reduces template required (smaller sample consumed)

Challenges to Multiplexing
- Primer design to find compatible primers (no program exists)
- Reaction optimization is highly empirical often taking months

Information is tied together with multiplex PCR and data analysis

Position of Forensic STR Markers on Human Chromosomes
13 CODIS Core STR Loci

http://www.cstl.nist.gov/biotech/strbase/training.htm
Characteristics of Core STR Loci

<table>
<thead>
<tr>
<th>Locus</th>
<th>Chromosomal Location</th>
<th>Physical Position (May 2004; NCBI build 35)</th>
</tr>
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<tr>
<td>TPOX</td>
<td>5q23.2</td>
<td>Chr 2; 1.472 Mb</td>
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<tr>
<td>D3S1358</td>
<td>3p21.31</td>
<td>Chr 3; 45.507 Mb</td>
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<tr>
<td>FGA</td>
<td>alpha f-actin, 5’ exons</td>
<td>Chr 4; 155.866 Mb</td>
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<tr>
<td>D5S818</td>
<td>5q31.2</td>
<td>Chr 5; 123.139 Mb</td>
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<tr>
<td>CSF1PO</td>
<td>5q33.1</td>
<td>Chr 5; 149.438 Mb</td>
</tr>
<tr>
<td>D7S820</td>
<td>7q21.11</td>
<td>Chr 7; 93.821 Mb</td>
</tr>
<tr>
<td>D8S1179</td>
<td>4q24.2</td>
<td>Chr 8; 120.913 Mb</td>
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<td>TH01</td>
<td>11q16.5</td>
<td>Chr 11; 21.249 Mb</td>
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<td>1q13.31</td>
<td>Chr 11; 21.249 Mb</td>
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<td>1q21.1</td>
<td>Chr 12; 81.620 Mb</td>
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<td>16q22.1</td>
<td>Chr 16; 84.044 Mb</td>
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Commercial STR 16plex Kits

Identifiler™ kit (Applied Biosystems) multiplex STR result

PowerPlex® 16 kit (Promega Corporation) multiplex STR result

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

http://www.cstl.nist.gov/biotech/strbase/training.htm
Reduced Volume PCR Amplifications

**Advantages**
- **Lower cost** since kit contents are stretched
- Improved sensitivity perceived due to use of concentrated PCR products (since 1 uL out of a 5 uL reaction is 20% while 1 uL out of a 50 uL reaction is 2%)

**Disadvantages**
- Less volume of input DNA
  - Tighter control (improved precision) required in DNA quantitation
  - If low amount of DNA, then potential for allelic dropout (LCN conditions)
  - If PCR inhibitor is present, then less opportunity for dilution of inhibitor
- Evaporation impacts PCR amplification performance

**Publications**

**Identifiler 5 µL PCR Protocol**
Identifiler PCR amplification was carried out on a GeneAmp® 9700 using 1 ng of DNA according to kit protocols with the exception of reduced volume reactions (5 µL instead of 25 µL) and reduced cycles (26 instead of 28).

Amplification products were diluted 1:15 in Hi-Di™ formamide and GS500-LIZ internal size standard (0.3 uL) and analyzed on the 16-capillary ABI Prism® 3100 Genetic Analyzer without prior denaturation of samples.

PPOP™-6 (3700 POPES) rather than POP™-4 was utilized for higher resolution separations.

Allele calls were made in Genotyper® 3.7 by comparison with kit allelic ladders using the Kazaam macro (20% filter).

**Identifiler 5 µL PCR (lower 3100 injection, 5s@2kV instead of 10s@3kV)**

**Total cost per sample = $3.87 (Fall 2002)**

http://www.cstl.nist.gov/biotech/strbase/training.htm
Steps in DNA Analysis

- Collection
- Extraction
- Quantitation
- Genotyping
- Interpretation of Results
- Database Storage & Searching

STR Typing Technologies

- Gels
- Capillary Electrophoresis
- Capillary Arrays
- Microchip CE
- Mass Spectrometry
- Hybridization Arrays

Typical Instruments Used for STR Typing

- Thermal Cycler for PCR Amplification
- Capillary electrophoresis instruments for separating and sizing PCR products

- single capillary
- 16-capillary array

- ABI 310
- ABI 3100

http://www.cstl.nist.gov/biotech/strbase/training.htm
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
In the early 1990s the real question was how to transition from a gel to a capillary

- Cross-linked acrylamide gel filled capillaries were tried first
  - Reusable?
  - Bubble formation
  - Thermal degradation

- Alternative was to not use a gel at all
  - Refillable sieving polymers
  - However, resolution was poor early on

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**Early Work with CE and STRs**

- Barry Karger’s group (1988-1990)
  - Utilized gel-filled capillaries to separate ssDNA
  - Introduced sieving polymers in the form of linear polyacrylamide to separate restriction digests

- Beckman P/ACE 2050 is introduced in 1992 as the first commercially available CE coupled to a laser to enable fluorescence detection

- John Butler and Bruce McCord (1993-1995)
  - First STR typing with single color CE using intercalating dyes and dual bracketing internal size standards

  - First STR typing with multi-color CE (and multi-capillary) using dye-labeled primers

- ABI 310 is introduced in July 1995 as the first commercially available multi-color CE

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**First Rapid STR Typing with Capillary Electrophoresis**

Single color detection with dual internal size standards

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

- TH01 allelic ladder

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


- Reliable sizing over a 75-500 bp size region
- High run-to-run precision between processed samples to permit comparison of allelic ladders to sequentially processed STR samples
- Effective color separations of different dye sets used to avoid bleed through between 4 or 5 different colors
- Resolution of at least 1 bp to >350 bp to permit reliable detection of microvariant alleles

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ABI Prism 310 Genetic Analyzer

Syringe with polymer solution
Outlet buffer
Autosampler tray
Injection electrode
Inlet buffer
Capillary

Close-up of ABI Prism 310 Sample Loading Area

Electrode (cathode)
Capillary
Samples
Autosampler Tray

End of capillary should be near end of electrode (and autosampler position should be calibrated to these tips)
Mixture of dye-labeled PCR products from multiplex PCR reaction

**Steps in STR Typing with ABI 310**

- Color Separation
- Size Separation
- Sample Separation
- Sample Preparation
- Sample Injection
- Sample Detection
- Sample Interpretation

**Irradiation for Capillary Array Detection**

- Side irradiation (on-capillary)
- Sheath flow detection
- Fixed laser, moving capillaries

**ABI Genetic Analyzer Usage at NIST**

- ABI 310 x 2 (originally with Mac, then NT)
  - 1st was purchased in 1996
  - 2nd was purchased in June 2002

- ABI 3100 (Data collection v1.0.1)
  - Purchased in June 2002
  - Original data collection software retained

- ABI 3130xl upgrade (Data collection v3.0)
  - Purchased in April 2001 as ABI 3100
  - Upgraded to ABI 3130xl in September 2005
  - Located in a different room
Our Use of the ABI 3100

- Data collection software, version 1.0.1
- **POP-6** with 36 cm capillary array
- STR kits and in-house assays for autosomal STRs, Y-STRs, and miniSTRs
- SNaPshot assays for mtDNA SNPs, Y-SNPs, and autosomal SNPs
- DNA sequencing for mtDNA and STR repeat sequencing

We can routinely get more than 400 runs per capillary array by not changing the polymer between applications.

NIST ABI 3100 Analysis Using POP-6 Polymer

High Resolution STR Typing

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

mtDNA Sequencing (HV1)

Inside the 3100

1 mL syringe
Loads polymer

5 mL syringe
Polymer reservoir

Detection window
Buffer reservoir

Oven
Seal
Better temp control

Capillary array
Oven fan
Autosampler

http://www.cstl.nist.gov/biotech/strbase/training.htm
ABI 3100 and 3130xl Differences

- **Polymer Block**
  - No more manually filled syringes for the 3130xl
- **Polymer solution**
  - POP-7 vs. POP-4 and POP-6
- **Data Collection software**
  - New, user-friendly features in the upgraded software
  - Compensation for the red dye channel (variable binning – not present in v1.0.1)

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**ABI 3130xl uses pump rather than syringe**

5 mL polymer reserve syringe

250 µL array-fill syringe

Tubing where bubbles hide

Upper Polymer Block

Lower Polymer Block

Anode Buffer reservoir

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**ABI 3100**

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

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http://www.cstl.nist.gov/biotech/strbase/training.htm
Benefits of the 3130xl Upgrade
(Compared to the original 3100, Data Collection 1.0.1)

- Takes much less time to change the polymer
- User-friendly wizards to install capillary arrays and change polymer
- Can easily duplicate plate templates
- Creation of results group to determine the format of how the data is saved
- Can easily import data, analysis methods, bins and panels, and size standard info into GeneMapper ID
- Data can be analyzed in GeneScan/Genotyper with “GeneMapper Generic” application setting

GeneMapper Generic Setting from Data Collection v3.0

GeneMapper ID
GeneScan/Genotyper
GeneMapper ID only

We always choose the “GeneMapper – Generic” setting as the Application in our plate templates so we can use both types of data analysis software - allows more flexibility

Pre-Set Templates cannot be changed

No template for 36 cm, POP-6

http://www.cstl.nist.gov/biotech/strbase/training.htm
Comparison of ABI 3100 Data Collection Versions

Same DNA sample run with Identifiler STR kit (identical genotypes obtained)

**ABI 3100** (36 cm array, POP-6)
Data Collection v1.0.1
5s@2kV injection

**ABI 3130xl** (50 cm array, POP-7)
Data Collection v3.0
5s@2kV injection

Relative peak height differences are due to “variable binning” with newer ABI data collection versions.

Difference in the STR allele relative mobilities (peak positions) are from using POP-6 vs. POP-7.

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### POP-7 Observations

- POP-7 is included in the 3130xl upgrade package
- Shorter run times compared to POP-6
- Similar resolution to POP-6
- Slightly lower precision compared to POP-6
- Mobility differences relative to POP-6, particularly for smaller DNA fragments used in SNaPshot assays

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Comparison of SNaPshot Data

**ABI 3100** (36 cm array, POP-6)
Data Collection v1.0.1
5s@2kV injection

**ABI 3130xl** (50 cm array, POP-7)
Data Collection v3.0
5s@2kV injection

Due to drastic size differences with POP-7, we decided to continue using POP-6 for SNaPshot assays so we wouldn’t have to rewrite the Genotyper macros.
Process Involved in 310/3100 Analysis

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

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**Injection**

CE Injection Methods

- **Hydrodynamic (pressure)**
- **Electrokinetic (voltage)**


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http://www.cstl.nist.gov/biotech/strbase/training.htm
**Electrokinetic Injection Process**

- **Amount of DNA injected** is inversely proportional to the ionic strength of the solution.
- Salty samples result in poor injections.

**Sample Conductivity Impacts Amount Injected**

\[
[D\text{NA}_{\text{inj}}] = \frac{Et(\pi r^2) (\mu_{\text{ep}} + \mu_{\text{eof}}) [D\text{NA}_{\text{sample}}]}{\lambda_{\text{sample}}}.
\]

- \([D\text{NA}_{\text{inj}}]\) is the amount of sample injected.
- \(E\) is the electric field applied.
- \(t\) is the injection time.
- \(r\) is the radius of the capillary.
- \(\mu_{\text{ep}}\) is the mobility of the sample molecules.
- \(\mu_{\text{eof}}\) is the electroosmotic mobility.
- \([D\text{NA}_{\text{sample}}]\) is the concentration of DNA in the sample.
- \(\lambda_{\text{buffer}}\) is the buffer conductivity.
- \(\lambda_{\text{sample}}\) is the sample conductivity.

**Two Major Effects of Sample Stacking**

1. Sample is preconcentrated. Effect is inversely proportional to ionic strength.
2. Sample is focused. Ions stop moving in low electric field.
3. Mobility of sample = \(\mu_{\text{ep}} = \text{velocity} / \text{electric field}\).
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

Typical Sample Preparation for ssDNA

1. Perform PCR with dye-labeled primers
2. Dilute 1 µL PCR product with 24 µL deionized formamide; add 1 µL ROX-labeled internal sizing standard
3. Denature 2 minutes at 95 °C with thermocycler
4. Cool to 4 °C in thermocycler or ice bath
5. Sample will remain denatured for at least 3 days

Comments on Sample Preparation

- **Use high quality formamide (<100 µS/cm)!**
  - ABI sells Hi-Di formamide
  - regular formamide can be made more pure with ion exchange resin
- **Deionized water vs. formamide**
  - water works fine but samples are not stable as long as with formamide; water also evaporates over time...
- **Denaturation with heating and snap cooling**
  - use a thermal cycler for heating and cold aluminum block for snap cooling
  - heat/cool denaturation step is necessary only if water is substituted for formamide...
January 6, 2005 Letter from Applied Biosystems to ABI 310 Customers

- "Testing has shown that Hi-Di Formamide denatures DNA without the need to heat samples..."
- In other words, no heat denaturation and snap cooling needed!

Applied Biosystems Okays Use of Deionized Water for DNA Sequencing

Technical Bulletin #1  Issued August 2006
Applied Biosystems 3730/3730xl DNA Analyzer

Subject: Influence of Sequencing Injection Solution on 3730/3730xl DNA Analyzer Performance

In this Bulletin:
- Three Loading Solutions Tested on Page 1
- Loading Solution Test Data on Page 2
- Recommendations on Page 3
- Guidelines for Use on Page 6

Three Loading Solutions Tested

Loading Solution Background:
Applied Biosystems generally recommends the use of 85% Formamide to the sample-loading solution for all Applied Biosystems DNA sequencers to ensure sample preservation and resistance to evaporation. However, many users of the 3700 desire either deionized water or sterile, EDTA solution. These choices are driven largely by cost and safety/hazards material considerations.
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

\[ \mu_{ep} = \frac{q}{6\pi\eta r} \]

small ions with high charge move fastest

<table>
<thead>
<tr>
<th>A</th>
<th>T</th>
<th>G</th>
<th>C</th>
</tr>
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<tbody>
<tr>
<td>PO⁻</td>
<td>PO⁻</td>
<td>PO⁻</td>
<td></td>
</tr>
</tbody>
</table>

As size increases so does charge!

Separation Issues

- **Capillary wall coating** — dynamic coating with polymer
  - Wall charges are masked by methyl acrylamide

- **Electrophoresis buffer** —
  - Urea for denaturing and viscosity
  - Buffer for consistent pH
  - Pyrididione for denaturing DNA
  - EDTA for stability and chelating metals

- **Polymer solution** — POP-4 (but others work also)

- **Run temperature** — 60 °C helps reduce secondary structure on DNA and improves precision.
  - (Temperature control affects DNA sizing)

Capillary Coating

- Dynamic coating of charged sites on fused silica capillary is accomplished with POP-4 polymer

http://www.cstl.nist.gov/biotech/strbase/training.htm
Capillary Wall Coatings Impact DNA Separations

**Electrophoretic flow**

- DNA

**Electroosmotic flow (EOF)**

- DNA

Solvated ions drag solution towards cathode in a flat flow profile

DNA Separations in Entangled Polymer Sieving Solutions

- Size based separation due to interaction of DNA molecules with entangled polymer strands
- Polymers are not cross-linked (as in slab gels)
- "Gel" is not attached to the capillary wall
- Pumpable -- can be replaced after each run
- Polymer length and concentration determine the separation characteristics

Entangled Polymer Solutions

- Polymers are not cross-linked (above entanglement threshold)
- "Gel" is not attached to the capillary wall
- Pumpable -- can be replaced after each run
- Polymer length and concentration determine the separation characteristics
- Examples:
  - 1% HEC (hydroxyethyl cellulose)
  - 4% polyvinyl pyrrolidinone
  - POP-4 and POP-6
  - POP4  Polymer
  - Polydimethyl acrylamide

http://www.cstl.nist.gov/biotech/strbase/training.htm
Transient Pores Are Formed Above the Entanglement Threshold.

\[ C < C^* \quad C = C^* \quad C > C^* \]

Otgon Sieving  
Reptation  
Entanglement

\[ \mu \sim \mu e^{-NC} \quad \mu \sim \frac{1}{N} \quad \mu \sim f(1/CN) \]

What is in POP-4 and Genetic Analyzer Buffer?

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

Running buffer contains 100 mM TAPS and 1 mM EDTA (adjusted to pH 8.8 with NaOH) TAPS = N,N'-Tris(hydroxymethyl)methyly-3aminopropyl-sulfonic acid

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

Improved single-strand DNA sizing accuracy in capillary electrophoresis

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

Detection

Detection Issues

• Fluorescent dyes
  – spectral emission overlap
  – relative levels on primers used to label PCR products
  – dye “blobs” (free dye)
• Virtual filters
  – hardware (CCD camera)
  – software (color matrix)

Filters determine which wavelengths of light are collected onto the CCD camera
Laser Used in ABI 310

- Argon Ion Laser
- 488 nm and 514.5 nm for excitation of dyes
- 10 mW power
- Lifetime ~5,000 hours (1 year of full-time use)
- Cost to replace ~$5,500
- Leads to highest degree of variability between instruments and is most replaced part
- Color separation matrix is specific to laser used on the instrument

Methods for Fluorescently Labeling DNA

- Intercalating Dyes (post-PCR)
- Dye-labeled nucleotide insertion during PCR
- Dye-labeled primer insertion during PCR

Fluorescent Labeling of PCR Products

- Dyes are attached to one primer in a pair used to amplify a STR marker
- Dyes are coupled to oligonucleotides (primers) through NHS-esters and amine linkages on the 5’ end of the primer: Dye-(CH₂)₆-primer
- Dye-labeled oligonucleotides are incorporated during multiplex PCR amplification giving a specific color “tag” to each PCR product
- PCR products are distinguished using CCD imaging on the 310
Amine Reactive Dyes used in Labeling DNA

- FAM (Blue)
- JOE (Green)
- TAMRA (Yellow)
- ROX (Red)

The succinimidyl ester reacts rapidly with amine linkers on DNA bases.

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.

Fluorescent Emission Spectra for ABI Dyes

- 5-FAM
- JOE
- NED
- ROX

NED is a brighter dye than TAMRA.
Please Note!

- There are no filters in a 310
- It's just the choice of pixels in the CCD detector
- All the light from the grating is collected
- You just turn some pixels on and some off

Comments on Matrices/Spectral Calibration (Multi-Component Analysis)

- Make sure that the right filter set and matrix are applied when collecting data
- You can always apply another matrix to a sample collected on the ABI 310 but it must be run with the right filter set (matrix must be run first with ABI 3100)
- It is important to update matrices on a regular basis (depending on use) due to differences in laser power over time
- A good indication of when to run a new matrix is the observation of pull-up between dye colors when peaks are smaller than ~4,000 RFUs

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!
Consumables for ABI 310/3100

What we use at NIST

- A.C.E.™ Sequencing Buffer 10X (Amresco)
  - $155/L = $0.0155/mL 1X buffer (costs 20 times less!)
  - http://www.amresco-inc.com

- 3700 POP-6 Polymer (Applied Biosystems)
  - $530 / 200 mL = $2.65/mL (costs 20 times less!)

What ABI protocols suggest

- 10X Genetic Analyzer Buffer with EDTA
  - $78/25 mL = $0.312/mL 1X buffer (ABI)

- 3100 POP-4 Polymer
  - $365 / 7 mL = $52/mL

Overall Thoughts on the ABI 310/3100/3130

- Settling on a common instrument platform has been good for the forensic DNA community in terms of data consistency (this is also true with the use of common STR kits)

- I am concerned that the community is very dependent primarily on one company…

- I really like using the instrument and can usually get nice data from it

- Like any instrument, it has its quirks…

Conclusions

DNA typing by capillary electrophoresis involves:

1) The use of entangled polymer buffers

2) Injection by sample stacking

3) Multichannel laser induced fluorescence

4) Internal and external calibration