Capillary Electrophoresis in DNA Analysis

Higher Throughput Approaches

NEAFS Workshop
Mystic, CT
September 29-30, 2004
Dr. John M. Butler
Dr. Bruce R. McCord

Outline for Workshop

• Introductions
• STR Analysis
• Introduction to CE and ABI 310
• Data Interpretation
• Additional Topics – Real-time PCR and miniSTRs
• Higher Throughput Approaches
• Troubleshooting the ABI 310 (Participant Roundtable)
• Additional Topics – Y-STRs, validation, accuracy
• Review and Test

STR Typing Technologies

Gels
Capillary Electrophoresis
Capillary Arrays

Microchip CE
Mass Spectrometry
Hybridization Arrays

Ways to Increase Sample Throughput

• Run more gels (FMBIO approach)
• Increase speed of single sample analysis (microchip CE systems)
• Multiplex fluorescent dyes of different colors (higher level PCR multiplexes)
• Parallel separations using capillary arrays
• New Detection Technologies (MALDI-TOF mass spectrometry)

Methods for Parallel Sample Processing

Multiplex by Size
Multiplex by Dye Color
Multiplex by Number of Capillaries

ABI 3100: 16 capillaries
ABI 3730: 96 capillaries
ABI 3100 Avant: 4 capillaries

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Capillary Array Electrophoresis

- Higher sample throughput
- Commercial 96 capillary systems were used to sequence the human genome
  - ABI 3700
  - MegaBACE
- Engineering and hardware challenges
- Software challenges

16 Capillary 3100

6 foot Table on wheels

High-Throughput STR Typing on the ABI 3100 (16-capillary array)

256 data points in 45 minutes with STR 16plex and 16 capillaries

Increasing Sample Throughput with Parallel Processing

 ABI 3100
16-capillary array

 ABI 310
single capillary

Subtle differences in matrix formation and sizing algorithms – NOT directly equivalent to 310

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

Tubing where bubbles hide

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Two 96 well plates on the autosampler
At 45-60 minutes per run two plates represent 12 runs or ~10-12 hours for 192 samples

Rubber septa wear. They must be replaced when the edges are ragged.

16 Capillary Array
Capillaries are inside of the cathodes (-)

Capillaries in buffer tank
Running and storage position
Spatial Calibration

Performed after:
- Installing or replacing a capillary array
- Removal of the array from the detection block,
  (Due to the design, to remove the upper polymer
  block for cleaning you must remove the Array
  from the detection window)

Information Provided:
- Position of the fluorescence from each capillary on
  the CCD

Spatial Results

Good Results

Bad results
- Try again

Maintenance of ABI 3100

- 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!

Spectral Calibration

- Performed:
  - New dye set on the instrument
  - After Laser or CCD camera has been realigned
  - You begin to see a decrease in the spectral
    separation (pull-up, pull-down).
- You must have a valid separation matrix on
  the instrument prior to running samples.

Use of the Correct Matrix is Critical

This figure shows the same sample, amplified with the Reliagene Y-Plex™ II kit, injected
into both the 310 and the 3100. Note that the DYS390 allele, which is labeled with TAMRA
as the yellow dye, does not show up in the 3100 result. The matrix used in this case
contained NED rather than TAMRA. Thus, if the matrix for the particular dye combination
is not established properly, peaks can disappear.
Powerplex 16 data

Time for a new matrix

Defining the Matrix on the ABI 3100

Matrices Created on NIST ABI 3100 with Various Dye Combinations

<table>
<thead>
<tr>
<th>Color</th>
<th>3100 Filters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue</td>
<td>FL dR110 dR110 5FAM 5FAM 5FAM</td>
</tr>
<tr>
<td>Red</td>
<td>dROX dROX ROX ROX ROX</td>
</tr>
<tr>
<td>Green</td>
<td>JOE JOE JOE VIC VIC VIC</td>
</tr>
<tr>
<td>Yellow</td>
<td>TMR TMR NED NED NED</td>
</tr>
<tr>
<td>Orange</td>
<td>CXR CXR LIZ LIZ LIZ</td>
</tr>
</tbody>
</table>

Different ABI 3100 matrix sets used at NIST in order to address a variety of applications and dye combinations.
Other Applications with ABI 3100 and POP6 besides STR Typing

mtDNA Sequencing (HV1)

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

Parameters in Run Modules

Default injection changes between 3100 data collection versions:
Version 1.0.1 = 10s @ 3kV
Version 1.1 = 22s @ 1kV

Consumables

- 10X Genetic Analyzer Buffer with EDTA
  - $75/25 mL = $0.30/mL 1X buffer (ABI)
  - Or A.C.E.™ Sequencing Buffer 10X
    - $155/L = $0.016/mL 1X buffer (Amresco)
- 3100 POP-4 Polymer $365 / 7 mL
- 3100 POP-6 Polymer $365 / 7 mL
- 3700 POP-6 Polymer $465 / 230 mL
  - What we have been using, runs take longer but you also get better resolution.

Data from ABI 3100 During the Run

Matrix is applied during the data collection so if there is a problem, the sample must be REINJECTED after a new matrix is applied rather than applying a new matrix to any raw data as can be done on the ABI 310…

Consumables

- ABI Optical Reaction Plates
  - $2,200 / 500 plates = $4.40 / plate
  - Phenix (mps-3590)
    - Plates $291/100 plates = $2.91 / plate
- Hi – Di Formamide
  - $28 / 25 mL
- 36 cm 3100 Capillary Array (100 runs) $695
  - 281 runs and still going (replace by resolution not # of injections)
- 36 cm 3100 Avant Capillary Array (150 runs) $560

Microchip CE Systems

What is under development for STR typing?
Attorney General John D. Ashcroft, holding a slide for DNA, hailed the technology as a tool in solving crimes. With him is Kellie Greene, whose attacker was found by DNA testing.

CE Microchips

- Channels are etched in glass microscope slides to make miniature CE columns
- More rapid separations are possible due to the shorter separation length
- Possible to etch many channels CAE microchips

Rapid Microchip CE Separation

Whithead Institute


PowerPlex™ 1.1

Allelic ladders mixed with samples for genotyping purposes

Typing 96 STR Samples in < 8 Minutes

Slide from Rich Mathies (UC-Berkeley)

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
PowerPlex 16 Allelic Ladders and Internal Lane Standard

- Color separation without the use of a matrix
- Separation based upon 4 PMT

Slide from Rich Mathies (UC-Berkeley)

Portable Genetic Diagnostics Device

- Integrated system includes:
  - glass CE microchannels
  - PCR chamber
  - heater
  - temperature sensor
  - microfabricated electrodes
  - microfabricated valves

From Richard Mathies presentation at 14th International Symposium on Human Identification, Oct 2003

Sex Determination PCR from human buccal cells

- Multiplex PCR directly from human buccal cells without DNA extraction
- Resistant PCR protocol activates polymerase and stops cells
- 20 cycles PCR after hotstart
- Short "clean-up" injection removes dyes, followed by diagnostic injection
- Female genotypes result in a single 162-bp product
- Mini-geneotype results in a 187-bp product (S) and a 260-bp product (Y)

Lagally et al., Lab-on-a-Chip, 1, 102 (2001)

15 minutes for PCR amplification and detection

From Richard Mathies presentation at 14th International Symposium on Human Identification, Oct 2003

Time-of-Flight Mass Spectrometry

Why it will not become widely used...

Virginia DNA Testing of Felon Arrestees

As of January 1, 2003, any individual arrested for a violent felony crime (Code of Virginia § 19.2-310.2:1) must provide a buccal sample for DNA analysis, with the resultant profile incorporated into the Virginia DNA Data Bank (Code of Virginia § 19.2-310.5).

Since January 2003
- Buccal swab collected upon arrest
- DNA sample processed within 72 hours
- DNA profile searched against state database (national database does not currently allow searches for individuals prior to conviction)
- If a match results, then arrestee is detained and later prosecuted
- From Jan 2003 – Dec 2003, VA processed 7,836 arrestee samples (not all analyzed) and scored 63 hits against their state database (Profiles in DNA, 2004, 7(1):3-5)

As of January 1, 2003, any individual arrested for a violent felony crime (Code of Virginia § 19.2-310.2:1) must provide a buccal sample for DNA analysis, with the resultant profile incorporated into the Virginia DNA Data Bank (Code of Virginia § 19.2-310.5).

Recent NIJ Publication

Final Report for NIJ Grant 97-LB-VX-0003 (work done at GeneTrace Systems Inc.)
- Describes new primer sets that are close to the STR repeat regions
- Many of these primers are being used in miniplex STR assays under development
- Y SNP multiplex primer sets are described
- 10plex mtSNP assay for HV1 and HV2 detailed

http://www.ojp.usdoj.gov/nij/pubs-sum/188292.htm

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC238995/
TH01 Alleles: CE vs. Mass Spec

- ABI 310 Result
  - 9.3 allele: 1071 sec
  - 10 allele: 1073 sec

- Mass Spec Result
  - 9.3 allele: 203.3 µsec
  - 10 allele: 204.5 µsec

104 bp Reduction in Allele Size with Equivalent Genotypes

Presented at ISFG 1999 Meeting

Redesigned primers for mass spec work

Timing for Data Collection

- Laser pulse (10 nsec)
- Wait (500 nsec)
- Extract DNA ions
- Turn off voltages
- Collect spectrum for ~300,000 nsec

REPEAT process 100+ times

All this occurs in less than 5 seconds per sample

Sum multiple spectra into final sample spectrum

Data processing and genotype determination

Time-of-Flight Mass Spectrometry (TOF-MS)

- DNA Reaction Products (Size separated and drifting to the detector)
- Pulsed Laser Beam
- High-Density Sample Array
- Ion Extractor
- Drift Region Electric-Field Free
- Detector
- Acceleration Region (20 kV)

Demonstrated Throughput at GeneTrace Systems

- 384 samples processed routinely in ~45 min (best was 96 samples in 2 min)
- ~4,000 samples in 11 hours on single mass spec and 3 robots
- Averaged around 2,000 samples daily at GTS per instrument (Jan-Aug 1999)
- Most samples run as singleplex reactions but demonstrated 10-plex SNP assay and 3-plex STR assay

http://www.atp.nist.gov/atp/success/genet.htm

Improvements in Information Throughput with Multiplexed Markers and Multiple Capillaries

<table>
<thead>
<tr>
<th>#Markers Multiplexed</th>
<th>Single capillary (ABI310)</th>
<th>16 capillary array (ABI3100)</th>
<th>96 capillary array (ABI3700)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Each run: 30 min</td>
<td>Each run: 45 min</td>
<td>Run: 2 h 46 min</td>
</tr>
<tr>
<td>1</td>
<td>1800 s (per capillary)</td>
<td>2700 s (per capillary)</td>
<td>9960 s (per capillary)</td>
</tr>
<tr>
<td>8</td>
<td>225 s (30 min)</td>
<td>21 s (2.8 min)</td>
<td>104 s (1.7 min)</td>
</tr>
<tr>
<td>16</td>
<td>113 s</td>
<td>10.5 s</td>
<td>6.5 s</td>
</tr>
</tbody>
</table>

Time required to obtain each genotype...


Technology Implementation Takes Time

First Rapid STR Typing with Capillary Electrophoresis

- FBI did not start running casework samples using STRs and CE until January 1999

FBI did not start running casework samples using STRs and CE until January 1999

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Where is the Future Going?...
Miniaturization and Portability

Palm Pilot
(handheld computer)

NanoChip™ from Nanogen
(Miniature Bioassay Device)

http://www.nanogen.com/products/nanochip_cart.asp