Topics and Techniques for Forensic DNA Analysis

Capillary Electrophoresis

(and microchip CE)

Fundamentals

Houston DNA
Training Workshop

Houston, TX
April 3-4, 2007

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National Institute of Standards and Technology

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NIST and NIJ Disclaimer

Funding: Interagency Agreement 2003-IJ-R-029 between the National Institute of Justice and NIST Office of Law Enforcement Standards

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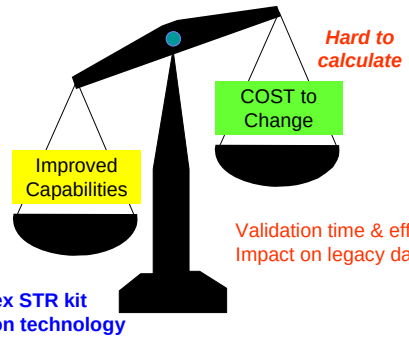
Our publications and presentations are made available at:
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

Stages of Technology for Forensic DNA Typing

- Idea
- Demonstration of feasibility
- Research and development
- Commercialization
- Validation by forensic labs
- Routine use by the community

TIME MONEY

Decision to Switch/Upgrade to New Technology



New multiplex STR kit
New detection technology
New DNA markers

Decisions about Changing Technologies

- Cost to change
- Comfort and experience levels
  - court approved methods must be used in forensic labs
- Capabilities…Enhancements
  - Are they really needed?
  - Will legacy data be impacted?

Where Is the Future Going for DNA Technology That Can Be Applied to Forensic DNA Typing?

Constant state of evolution (like computers)

- Higher levels of multiplexes
- More rapid DNA separations
- Better data analysis software
- New DNA Markers

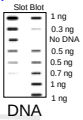
Steps Involved
- Collection
- Specimen Storage
- Extraction
- Quantitation
- Multiplex PCR
- STR Typing
- Interpretation of Results
- Database Storage & Searching
- Calculation of Match Probability

Steps in DNA Analysis

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

Blood Stain Buccal swab
Sample Collection & Storage

DNA Extraction



DNA Quantitation

Biology

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

Technology

DNA Database Search

Male: 13,14-15,16-12,13-10,13-15,16
Interpretation of Results

Presentation Outline

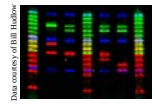
- History and background on CE
- Separation
- Injection and sample preparation
- Detection
- Microchip CE: similarities and differences

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

STR Typing Technologies

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

Gels

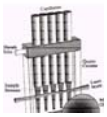


J. Forensic Sci. (1998) 43: 1168-1180

Capillary Electrophoresis

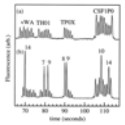
Electrophoresis. (1998) 19: 86-93

Capillary Arrays



Nucleic Acids Res. (1999) 27: e36

Microchip CE

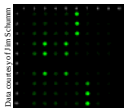


PNAS (1997) 94: 10273-10278

Mass Spectrometry

Int. J. Legal Med. (1998) 112: 45-49

Hybridization Arrays



Nucleic Acids Res. (2000) 28: e17

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

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

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

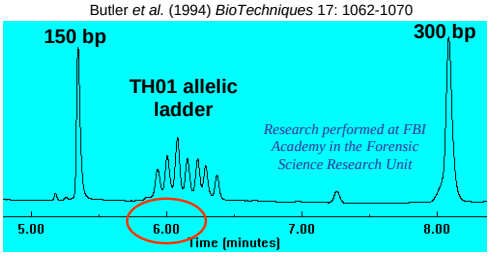
Early Work with CE and STRs

- 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**
- Rich Mathies' group (1995)
  - 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**

First Rapid STR Typing with Capillary Electrophoresis

Single color detection with dual internal size standards

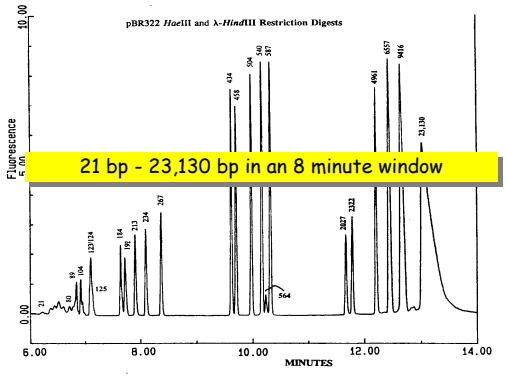
Butler *et al.* (1994) *BioTechniques* 17: 1062-1070



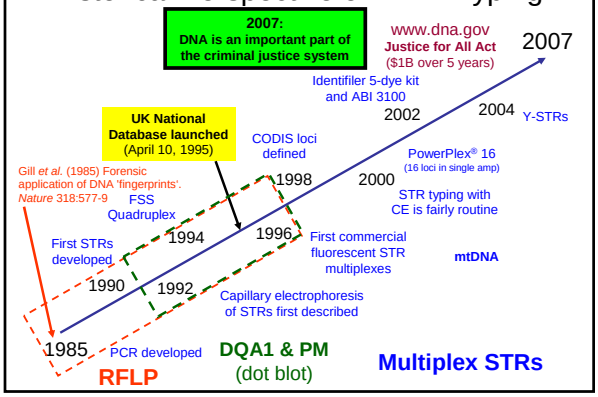
Performed in December 1993

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

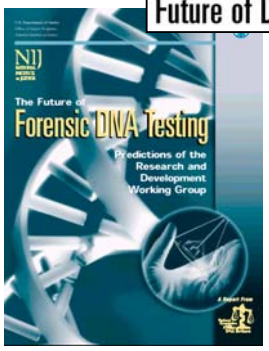
Results from 1995 Butler Ph.D. Dissertation



Historical Perspective on DNA Typing



National Commission on the Future of DNA Evidence



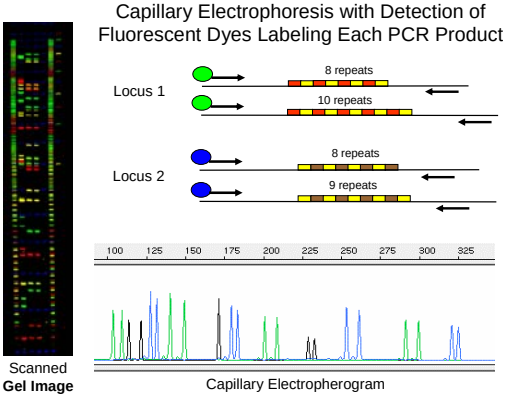
http://www.ojp.usdoj.gov/nij/pubs-sum/183697.htm

- Report published in Nov 2000
- Asked to estimate where DNA testing would be 2, 5, and 10 years into the future

Conclusions

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

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

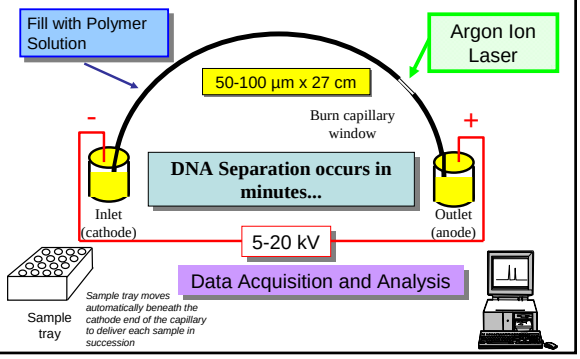


Why Use CE for DNA Analysis?

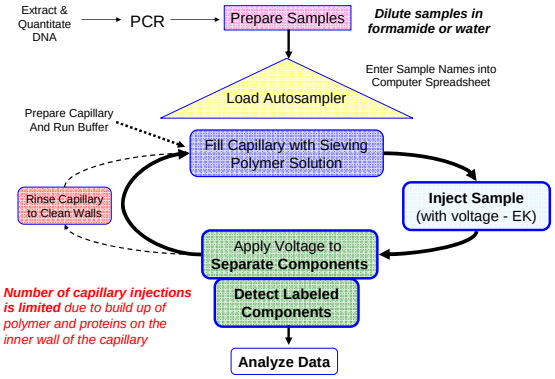
1. **Injection, separation, and detection are automated.**
2. **Rapid separations are possible**
3. **Excellent sensitivity and resolution**
4. **The time at which any band elutes is precisely determined**
5. **Peak information is automatically stored for easy retrieval**

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

Capillary Electrophoresis (CE)



Flowchart of CE Sample Analysis



Typical Instruments Used for STR Typing

GeneAmp 9700

Thermal Cycler for PCR Amplification

Capillary electrophoresis instruments for separating and sizing PCR products

single capillary

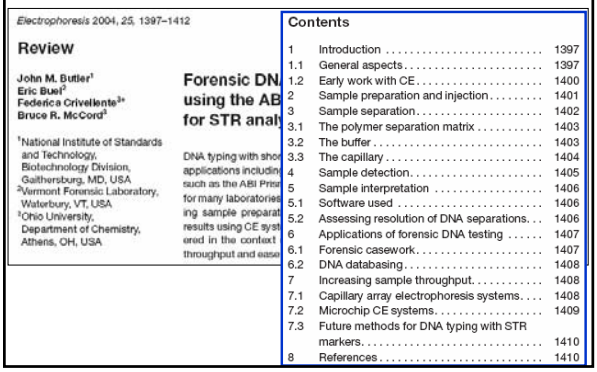
ABI 310

16-capillary array

ABI 3100

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 preparat
results using CE syst
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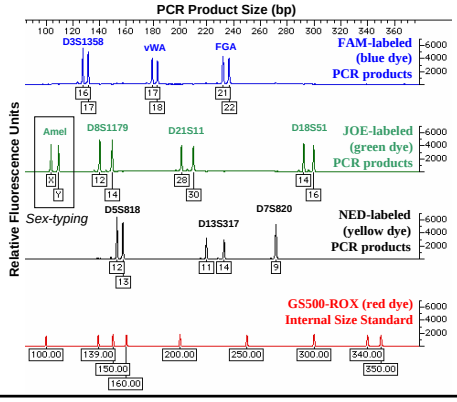
throughput and ease

Contents

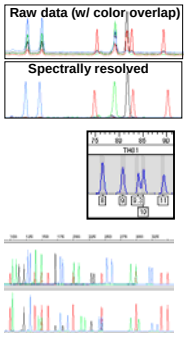
Genotype Results with Profiler Plus™ kit



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



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 °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…

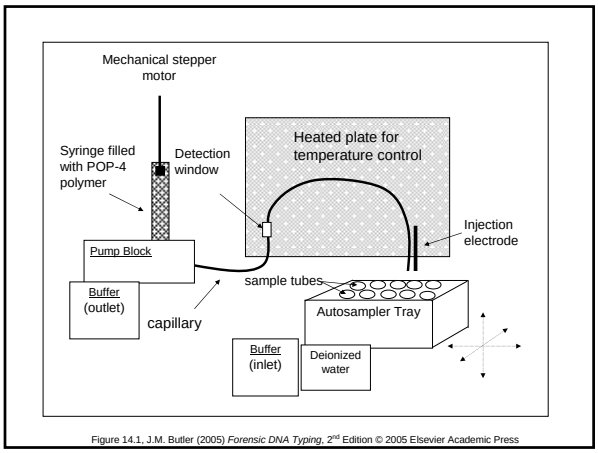
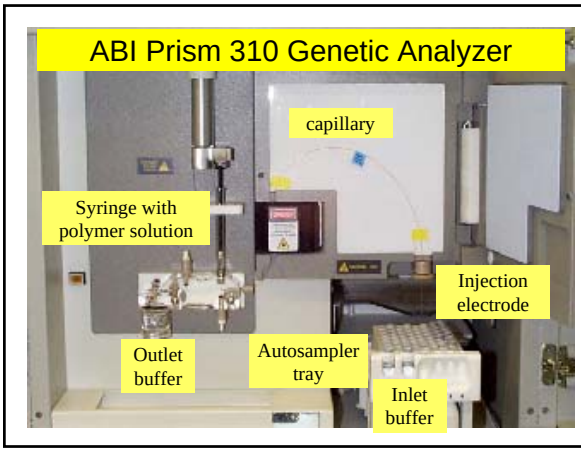
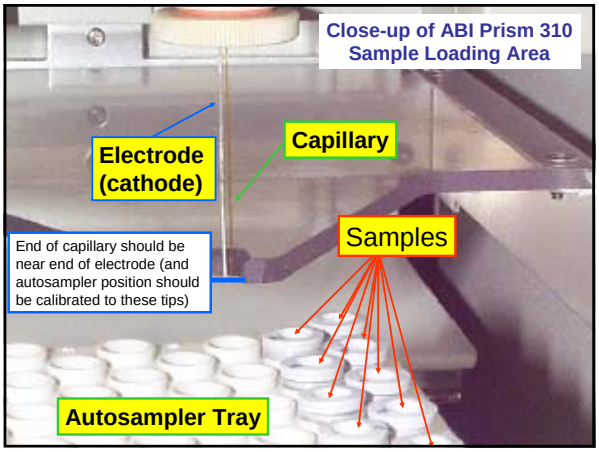
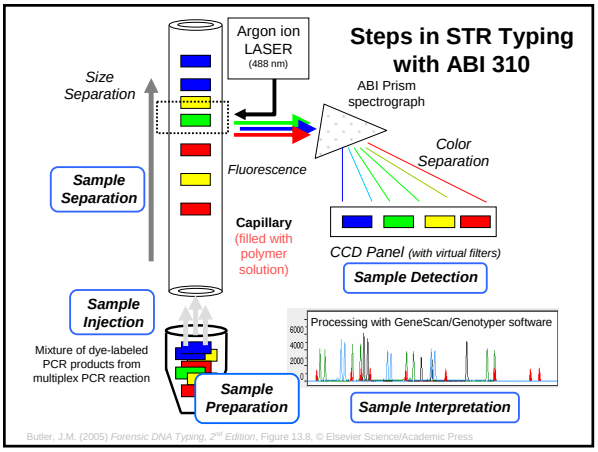


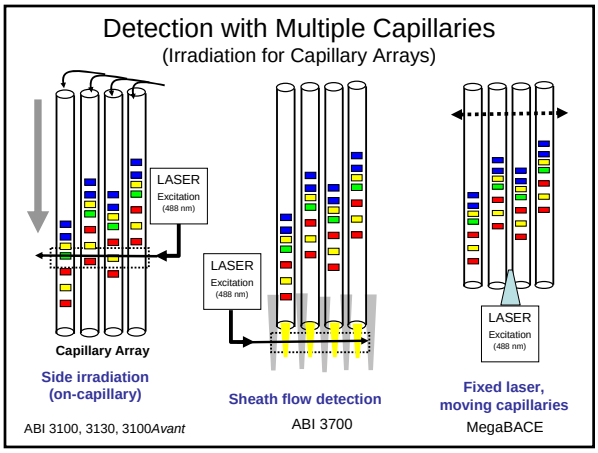
Figure 14.1, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press







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



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

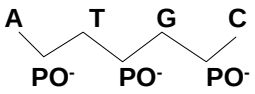
Ohm's Law

- 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!

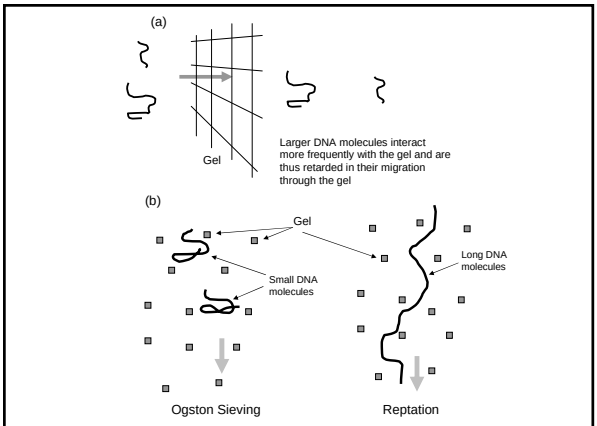


Figure 12.4, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

Separation Issues

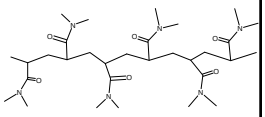
- Electrophoresis buffer –
  - Urea for denaturing and viscosity
  - Buffer for consistent pH
  - Pyrolidinone for denaturing DNA
  - EDTA for stability and chelating metals
- Polymer solution -- POP-4 (but others work also)
- Capillary wall coating -- dynamic coating with polymer
  - Wall charges are masked by methyl acrylamide
- Run temperature -- 60 ºC helps reduce secondary structure on DNA and improves precision. (Temperature control affects DNA sizing)

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

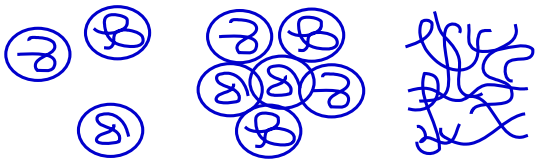
- Polymers are not cross-linked (above entanglement threshold)
- "Gel" is not attached to the capillary wall
- Pumpable -- can be replaced after each run
- Polymer length and concentration determine the separation characteristics
- Examples:
  - 1% HEC (hydroxyethyl cellulose)
  - 4% polyvinyl pyrolidinone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



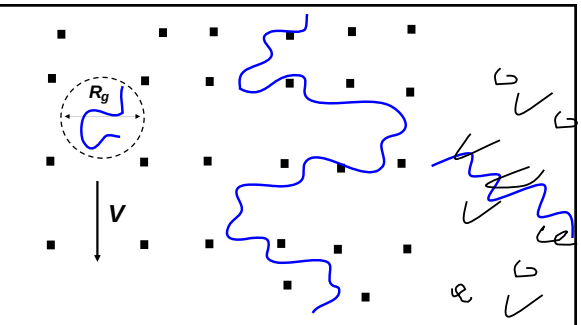
POP4 Polymer

Polydimethyl acrylamide

Transient Pores Are Formed Above the Entanglement Threshold.



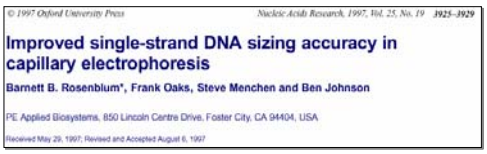
$C < C^*$ $C = C^*$ $C > C^*$



Ogston Sieving **Reptation** **Entanglement**

$\mu \sim \mu_0 e^{-NC}$ $\mu \sim 1/N$ $\mu \sim f(1/CN)$

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

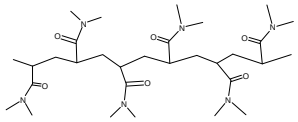
Received May 29, 1997; Revised and Accepted August 6, 1997

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

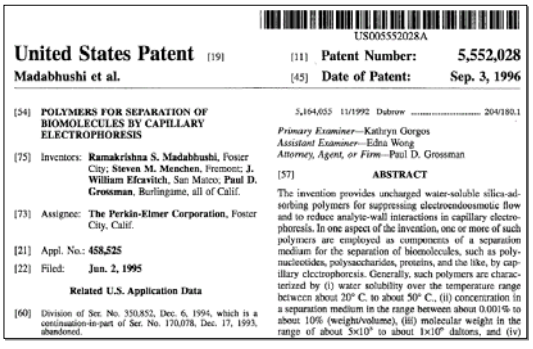
POP-4 (4% poly-dimethylacrylamide, 8 M urea, 5% 2-pyrrolidinone)

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*



US Patent Covering POP-4



US005552028A

United States Patent [19]

Madabhushi et al.

[11] **Patent Number: 5,552,028**

[45] **Date of Patent: Sep. 3, 1996**

[54] **POLYMERS FOR SEPARATION OF BIOMOLECULES BY CAPILLARY ELECTROPHORESIS**

[75] Inventors: **Ramakrishna S. Madabhushi**, Foster City; **Steven M. Menchen**, Fremont; **J. William Efcavitch**, San Mateo; **Paul D. Grossman**, Burlingame, all of Calif.

[73] Assignee: **The Perkin-Elmer Corporation**, Foster City, Calif.

[21] Appl. No.: **458,525**

[22] Filed: **Jun. 2, 1995**

Related U.S. Application Data

[60] Division of Ser. No. 350,852, Dec. 6, 1994, which is a continuation-in-part of Ser. No. 170,078, Dec. 17, 1993, abandoned.

5,164,055 11/1992 Dubrow 204/180.1

Primary Examiner—Kathryn Gorgos
Assistant Examiner—Edna Wong
Attorney, Agent, or Firm—Paul D. Grossman

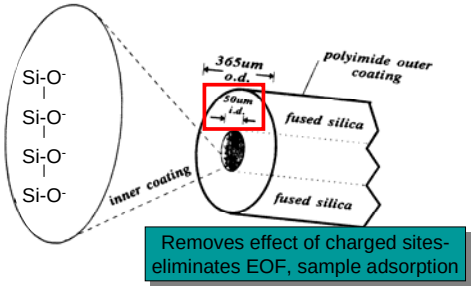
[57] **ABSTRACT**

The invention provides uncharged water-soluble silica-adsorbing polymers for suppressing electroendoosmotic flow and to reduce analyte-wall interactions in capillary electrophoresis. In one aspect of the invention, one or more of such polymers are employed as components of a separation medium for the separation of biomolecules, such as polynucleotides, polysaccharides, proteins, and the like, by capillary electrophoresis. Generally, such polymers are characterized by (i) water solubility over the temperature range between about 20° C. to about 50° C., (ii) concentration in a separation medium in the range between about 0.001% to about 10% (weight/volume), (iii) molecular weight in the range of about 5x10³ to about 1x10⁶ daltons, and (iv)

Why TAPS instead of Tris-borate (TBE) buffer?

- TBE is temperature/pH sensitive
  - as temperature increases, the pH decreases (0.02 pH units with every 1 °C); this is the principle by which TaqGold activation works
- At lower pH, fluorescence emission of dyes decreases
  - see Singer and Johnson (1997) *Proceedings of the Eighth International Symposium on Human Identification*, pp. 70-77
- Thus when running at 60 °C on the ABI 310, if Tris-borate was used, fluorescent intensity of PCR products would be lower

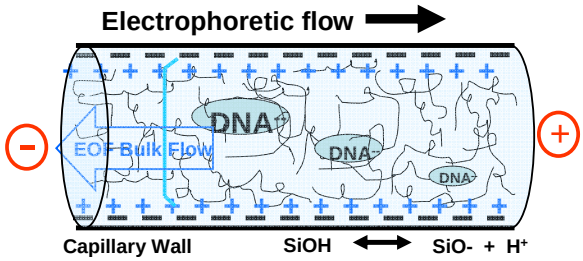
Capillary Coating



Removes effect of charged sites-eliminates EOF, sample adsorption

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

Capillary Wall Coatings Impact DNA Separations



Electroosmotic flow (EOF)

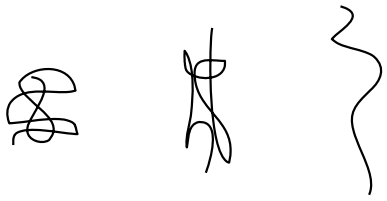
Solvated ions drag solution towards cathode in a flat flow profile

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

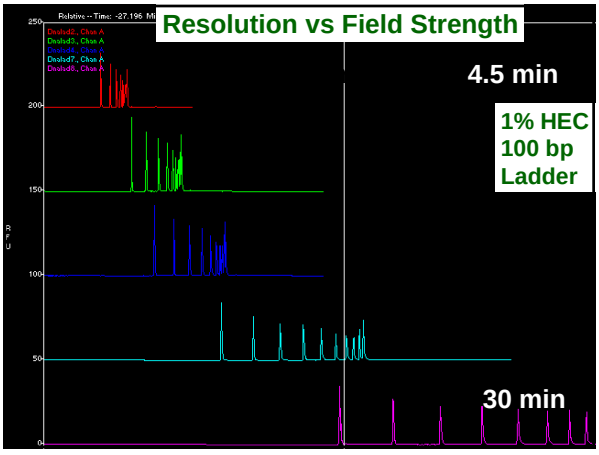
The electric field strength can influence the shape of the DNA molecule.



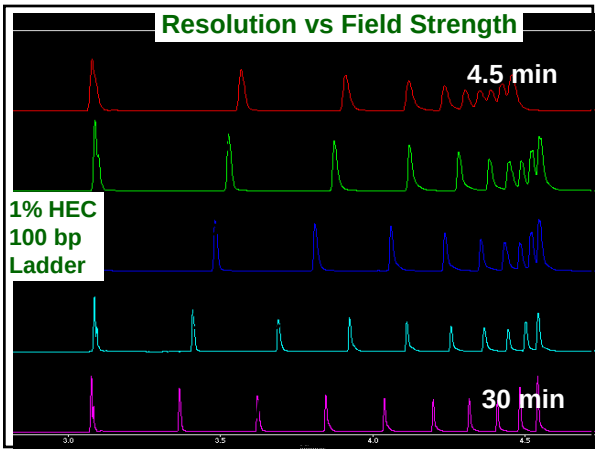
low *moderate* *high*

Optimal separations usually 180-200 V/cm

Resolution vs Field Strength

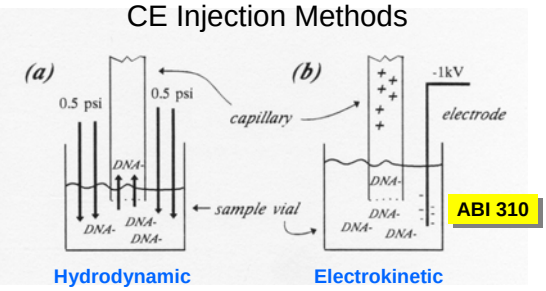


Resolution vs Field Strength



Injection

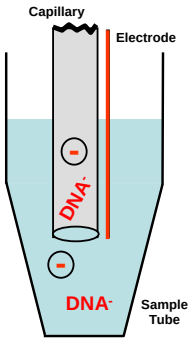
CE Injection Methods



Ulfelder K. J.; McCord, B. R. (1996) Capillary Electrophoresis of DNA, In *Handbook of Capillary Electrophoresis* (Landers, J., ed.), CRC Press: NY, pp. 347-378.

Butler, J.M. (1997) Effects of sample matrix and injection on DNA separations. *Analysis of Nucleic Acids by Capillary Electrophoresis* (Heller, C., ed.), Vieweg: Germany, Chapter 5, pp. 125-134

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

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

$[DNA_{inj}]$ 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

$[DNA_{sample}]$ is the concentration of DNA in the sample

λ_{buffer} is the buffer conductivity

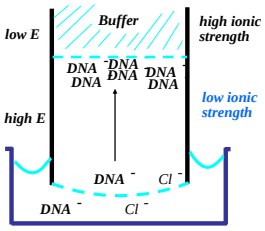
λ_{sample} is the sample conductivity

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

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 = μ_{ep} = velocity/ 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 ºC with thermocycler
4. Cool to 4 ºC in thermocycler or ice bath
5. Sample will remain denatured for at least 3 days

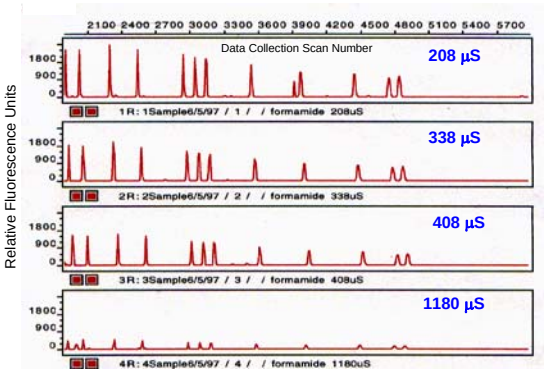


Figure 14.3, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

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
  - Biega and Duceman (1999) *J. Forensic Sci.* 44: 1029-1031
  - *Crivellente, Journal of Capillary Electrophoresis* **2002,** *7 (3-4),* 73-80.
  - 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 3730/3730*xl* 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 6
- Guidelines for Use on Page 6

Three Loading Solutions Tested

Loading Solution Background

Applied Biosystems presently recommends the use of Hi-Di™ Formamide as the sample-loading solution for all Applied Biosystems DNA sequencers to ensure sample preservation and resistance to evaporation. However, many users of the 3730 choose either deionized water or dilute EDTA solutions. These choices are driven largely by cost and safety/hazardous material considerations.

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)

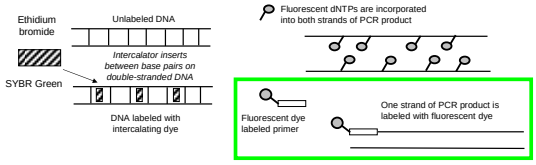
Filters determine which wavelengths of light are collected onto the CCD camera

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

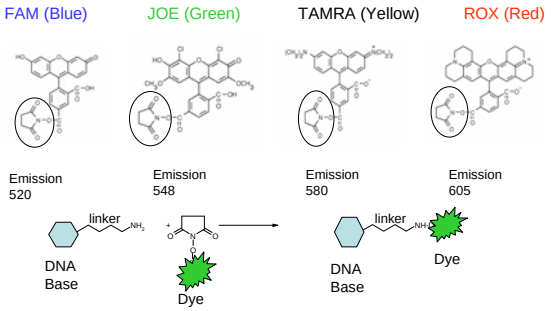


Butler, J.M. (2001) *Forensic DNA Typing*, Figure 10.2, ©Academic Press

Fluorescent Labeling of PCR Products

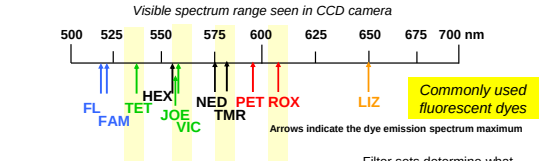
- Dyes are attached to one primer in a pair used to amplify a STR marker
- Dyes are coupled to oligonucleotides (primers) through NHS-esters and amine linkages on the 5’end of the primer: Dye-$(CH_2)_6$-primer
- Dye-labeled oligonucleotides are incorporated during multiplex PCR amplification giving a specific color “tag” to each PCR product
- PCR products are distinguished using CCD imaging on the 310

Amine Reactive Dyes used in Labeling DNA



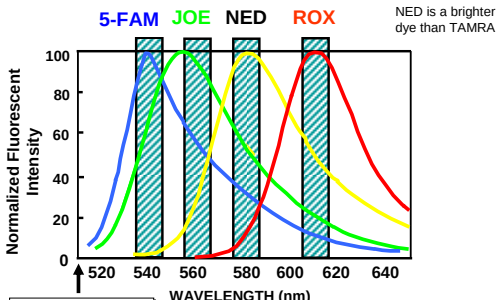
The succinimidyl ester reacts rapidly with amine linkers on DNA bases

Virtual Filters Used in ABI 310



	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

Fluorescent Emission Spectra for ABI Dyes



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

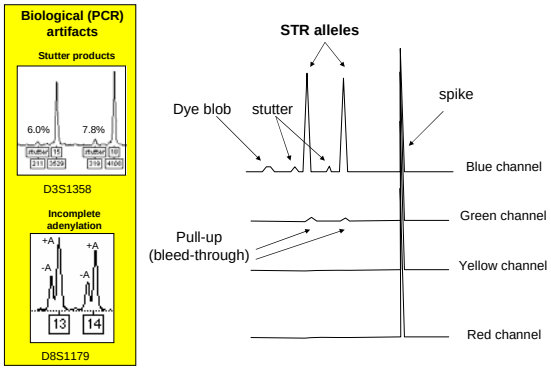
Please Note!

- There are no filters in a 310
- Its 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

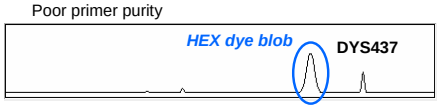
Deciphering Artifacts from the True Alleles



Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 15.4, © Elsevier Science/Academic Press

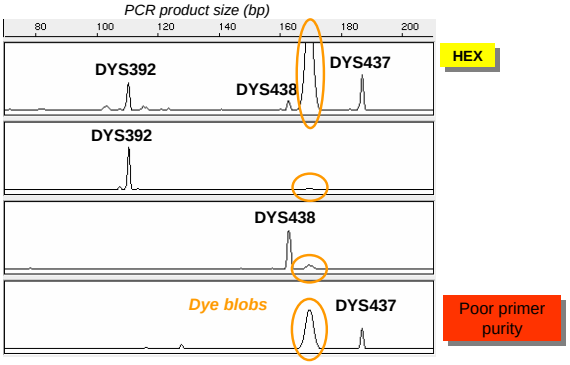
Dye Blobs ("Artifacts")

- Free dye (not coupled to primer) can be injected into the CE capillary and interfere with detection of true STR alleles
- **Dye blobs are wider and usually of less intensity** than true STR alleles (amount depends on the purity of the primers used)
- Dye blobs usually appear at an apparent size that is unique for each dye (e.g., FAM ~120 bp, PET ~100 bp)

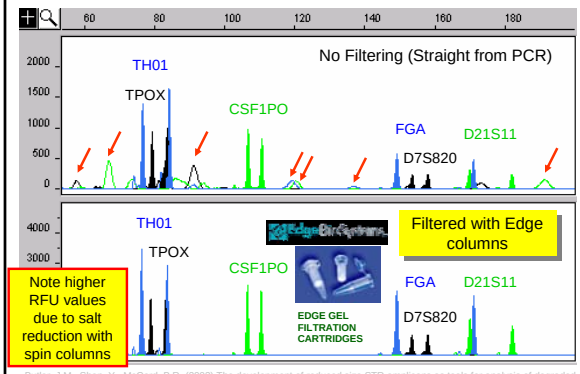


Dye Blob Problems with Some PCR Primers

Individual Y-STR Locus Amplifications



Removal of Dye Artifacts Following PCR Amplification



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.

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

Practical Aspects of ABI 310/3100 Use

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

Jan 2007 – upgraded to 3130xl with data collection v3.0

- 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

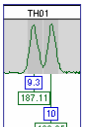
- Data collection software, version 1.0.1
- **POP-6** with 36 cm capillary array

- 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

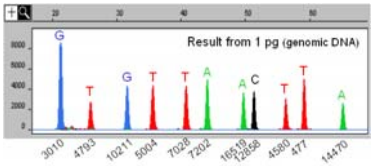
We can routinely get more than 400 runs per capillary array by not changing the polymer between applications

NIST ABI 3100 Analysis Using POP-6 Polymer

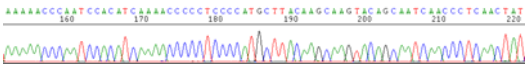
High Resolution STR Typing



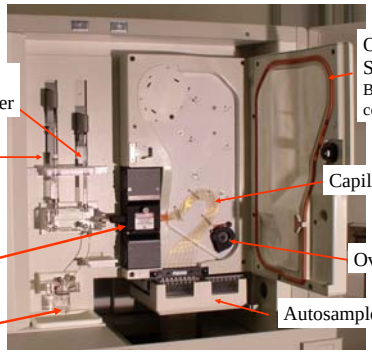
SNaPshot SNP Typing
(Coding Region mtSNP 11plex minisequencing assay)



mtDNA Sequencing (HV1)

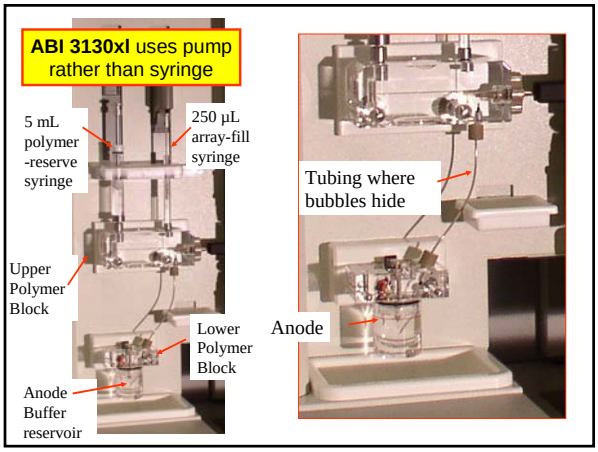


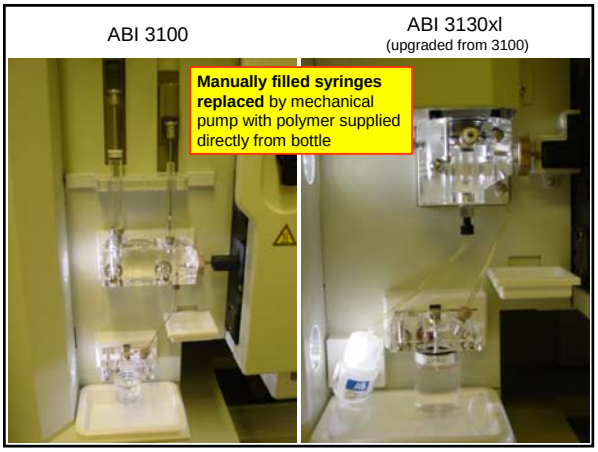
Inside the 3100

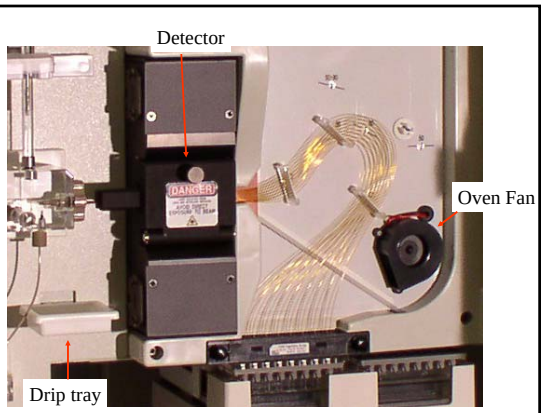


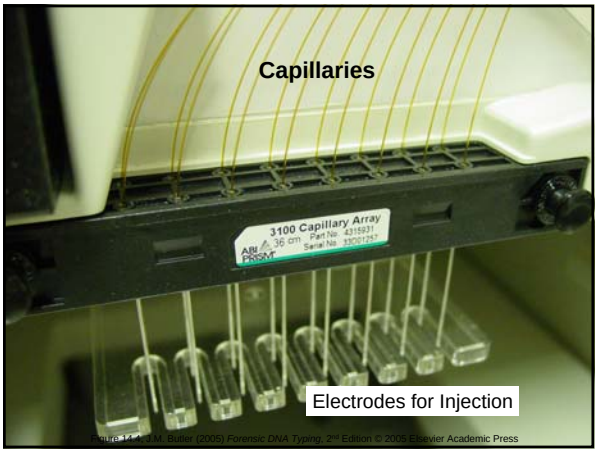
ABI 3100 and 3130xl Differences

- 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)

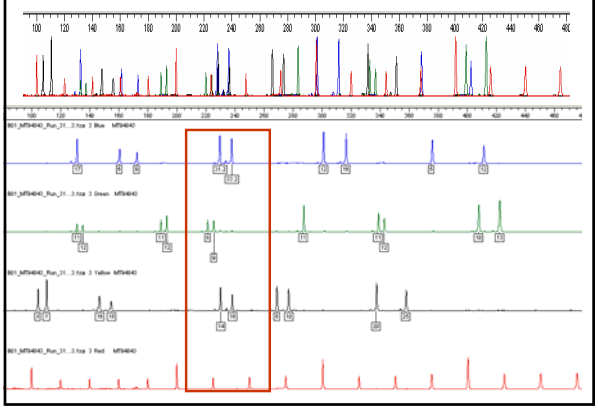


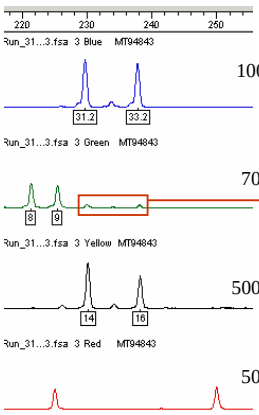






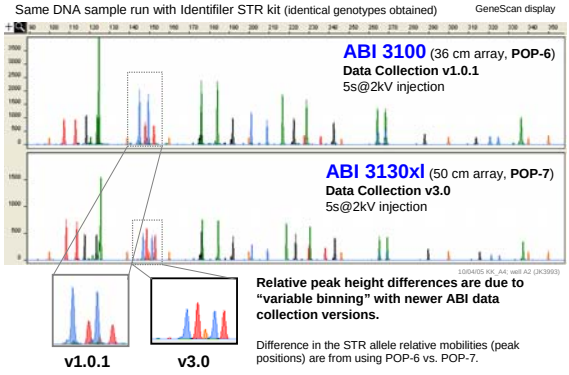
Powerplex 16 data





Comparison of ABI 3100 Data Collection Versions

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



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.

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

2004 prices

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!

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…

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)

Microchip CE Systems

What is under development for STR typing?

What's All the Hype Over Microchip CE Systems?

http://www.washingtonpost.com/wp-dyn/articles/A12570-2003Mar11.html
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.

S. Hjertén comments in a 2003 interview

Recently you have been working with chip based techniques. Do you think Lab on a Chip research is a 'fad' or is here to stay?

Generally one can state that any method has its advantages and disadvantages: the chip technique is no exception. No doubt, it has its niche, but I think there is some work still to be done. To be used successfully for quantitative analyses one must find simple methods to eliminate adsorption onto the walls of the channels. This is not a simple problem, especially when the sample is protein-based and the chip is made from plastic, the most widely used material. **"Small is beautiful", but not always: when the sample amounts are sufficiently large more robust conventional methods may be preferred.**

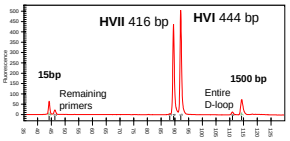
***Analyst* (2003) 128: 1307-1309**

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 (but usually lower resolution)
- Possible to etch many channels CAE microchips

- Sample injection differences with µCE
- Bending channels to get more length slows separation time and introduces possibility of band broadening
- Ratio of injection plug width to separation channel length influences resolution seen

Use of Agilent 2100 Bioanalyzer (µCE)



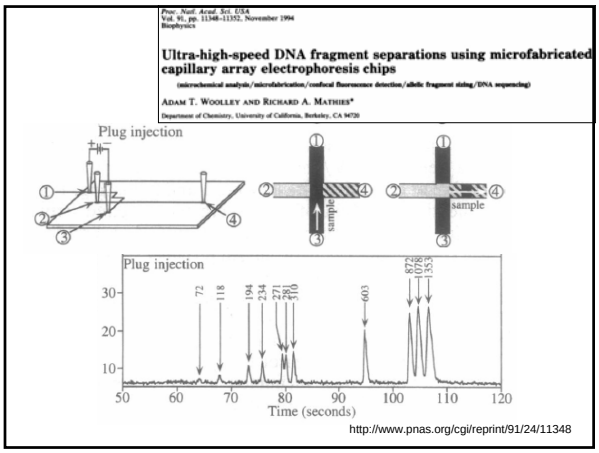
Analysis of mtDNA HVI/HVII PCR Products

Separations to 1500 bp are complete in ~2 minutes (120 seconds)

Agilent 2100 Bioanalyzer sized and quantified HVI/HVII products

12 samples can be run in ~45 minutes

Only single color so tested samples must have non-overlapping PCR product sizes
Only single channel so samples must be run sequentially
Poor resolution due to short channel length (optimized for speed not resolution)
Failure to fill channel with polymer means no result



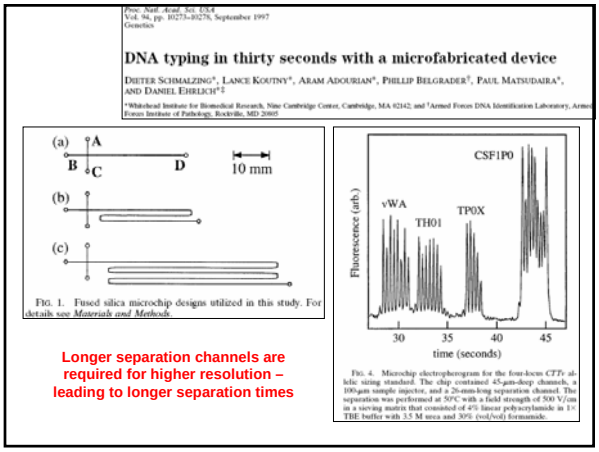
Injection onto a Microchip CE Channel

A sample injection bias can also exist with µCE systems (smaller fragments inject preferentially)

A narrow injection plug means equivalent resolution can be achieved in shorter distances (faster speeds)

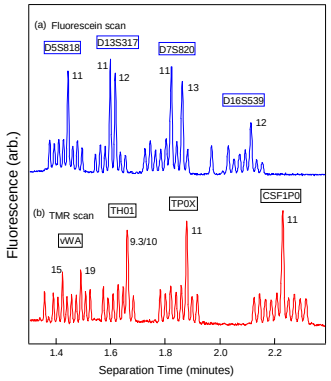
Detection must be more sensitive due to lower amounts of material being loaded

Geometry of the channels defines plug size (**typically ~100 pL**)



Longer separation channels are required for higher resolution – leading to longer separation times

Rapid Microchip CE Separation of STR Alleles



Dan Ehrlich's group
Whitehead Institute

Anal. Biochem. (1999) 270: 148-152

PowerPlex™ 1.1

Allelic ladders mixed with samples for genotyping purposes

Figure 17.1, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

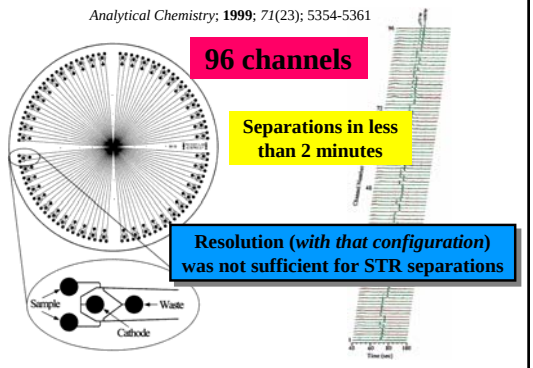
Radial Capillary Array Electrophoresis Microplate

Analytical Chemistry; **1999**; *71*(23); 5354-5361

96 channels

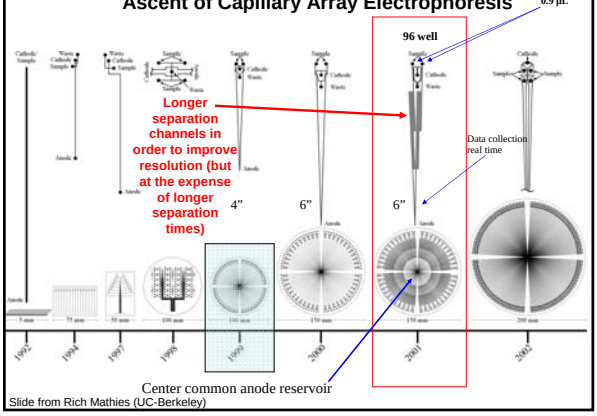
Separations in less than 2 minutes

Resolution (*with that configuration*) was not sufficient for STR separations



Rich Mathies Group (UC-Berkeley)

Ascent of Capillary Array Electrophoresis



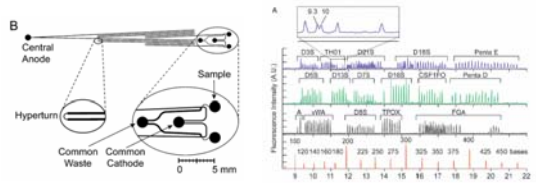
Slide from Rich Mathies (UC-Berkeley)

Separation Channels on These uCE Chips Are a Similar Length to Capillaries and Therefore Produce Similar Separation Times

J Forensic Sci, July 2006, Vol. 51, No. 4
doi:10.1111/j.1556-4029.2006.00153.x
Available online at: www.blackwell-synergy.com

Stephanie H.I. Yeung,[1] B.S.; Susan A. Greenspoon,[2] Ph.D.; Amy McGuckian,[3] M.S.; Cecelia A. Crouse,[3] Ph.D.; Charles A. Emrich,[4] B.S.; Jeffrey Ban,[2] M.S.; and Richard A. Mathies,[5] Ph.D.

Rapid and High-Throughput Forensic Short Tandem Repeat Typing Using a 96-Lane Microfabricated Capillary Array Electrophoresis Microdevice*



µCE Injection Plug Width Influences Resolution and Separation Time

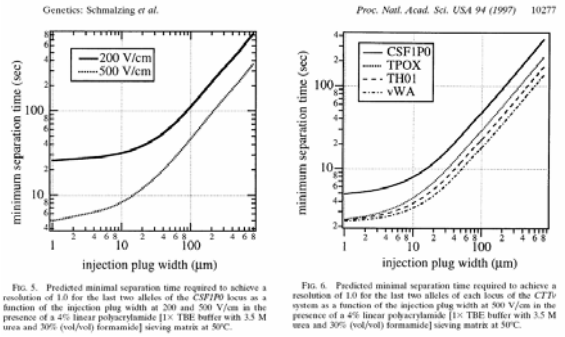
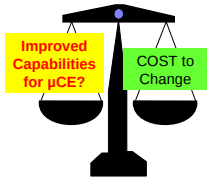


FIG. 5. Predicted minimal separation time required to achieve a resolution of 1.0 for the last two alleles of the *CSF1P0* locus as a function of the injection plug width at 200 and 500 V/cm in the presence of a 4% linear polyacrylamide [1× TBE buffer with 3.5 M urea and 30% (vol/vol) formamide] sieving matrix at 50°C.

FIG. 6. Predicted minimal separation time required to achieve a resolution of 1.0 for the last two alleles of each locus of the *CT7-* system as a function of the injection plug width at 500 V/cm in the presence of a 4% linear polyacrylamide [1× TBE buffer with 3.5 M urea and 30% (vol/vol) formamide] sieving matrix at 50°C.

My Thoughts on µCE Work

- Progress is being made but still has not shown significant enough advances to justify change from the already well-established CE and CE array systems
- There are fundamental barriers to improving separation speed and detection sensitivity (that have not been overcome in >10 years of research effort)…sometimes I feel like the "wheel" is being regularly re-invented…
- A greater challenge exists for the consistent filling of small channels with sieving polymer and therefore µCE systems are not always as robust (e.g., work every time)



Smaller is not always better…

Acknowledgments

NIST Human Identity Project Team

Leading the Way in Forensic DNA…

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Bruce McCord (Florida International University) for many of the slides

Thank you for your attention…

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

Questions?

See also http://www.dna.gov/research/nist
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john.butler@nist.gov