Advanced Topics in Forensic DNA Analysis

ABI 310/3100/3130

Capillary Electrophoresis

Fundamentals

New Jersey State Police
Training Workshop

Hamilton, NJ
December 5-6, 2006

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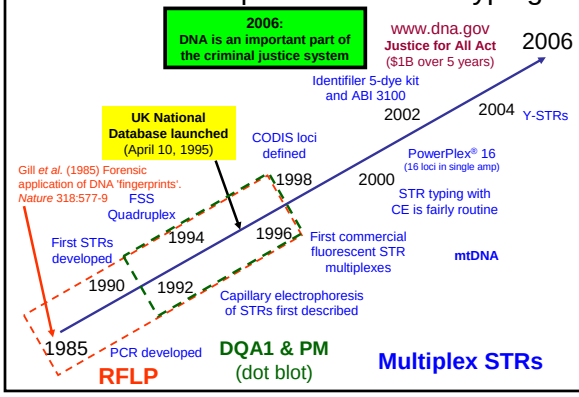
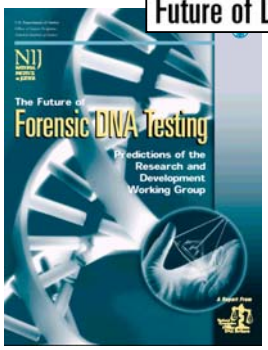
Presentation Outline

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

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

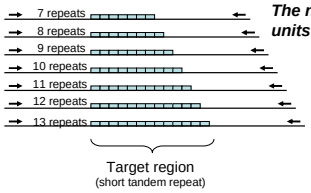
STR typing is here to stay for a few years because of DNA databases that have grown to contain millions of profiles

Short Tandem Repeat (STR) Markers

An accordion-like DNA sequence that occurs between genes

TCCCAAGCTCTTCCTCTTCCCTAGATCAATACAGACAGAAGACA
GGTG**GATAGATAGATAGATAGATAGATAGATAGATAGATAGA**
TAGATAGATATCATTGAAAGACAAAACAGAGATGGATGATAGAT
ACATGCTTACAGATGCACAC

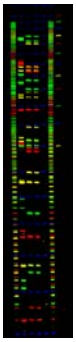
= 12 GATA repeats ("12" is all that is reported)



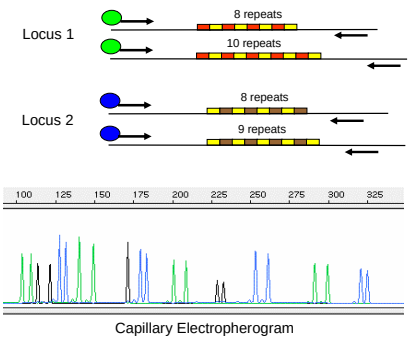
The number of consecutive repeat units can vary between people

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



Scanned Gel Image



Capillary Electropherogram

Types of STR Repeat Units

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

- **Di**nucleotide (CA)(CA)(CA)(CA)
- **Tri**nucleotide (GCC)(GCC)(GCC)
- **Tetra**nucleotide (AATG)(AATG)(AATG)
- **Penta**nucleotide (AGAAA)(AGAAA)
- **Hexa**nucleotide (AGTACA)(AGTACA)

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

Categories for STR Markers

Category	Example Repeat Structure	13 CODIS Loci
Simple repeats – contain units of identical length and sequence	(GATA)(GATA)(GATA)	TPOX, CSF1PO, D5S818, D13S317, D16S539
Simple repeats with non-consensus alleles (e.g., TH01 9.3)	(GATA)(GAT-)(GATA)	TH01, D18S51, D7S820
Compound repeats – comprise two or more adjacent simple repeats	(GATA)(GATA)(GACA)	VWA, FGA, D3S1358, D8S1179
Complex repeats – contain several repeat blocks of variable unit length	(GATA)(GACA)(CA)(CATA)	D21S11

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

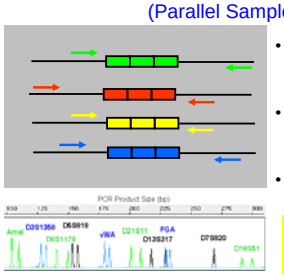
How many STRs in the human genome?

- 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 micro-satellite 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 (Ellegren H. Microsatellites: simple sequences with complex evolution. Nature Rev Genet 2004;5:435-445).
- STR sequences account for approximately 3% of the total human genome (Lander et al. Initial sequencing and analysis of the human genome. Nature 2001;409:860-921).

Butler, J.M. (2006) Genetics and genomics of core STR loci used in human identity testing. *J. Forensic Sci.* 51(2): 253-265

Multiplex PCR

(Parallel Sample Processing)

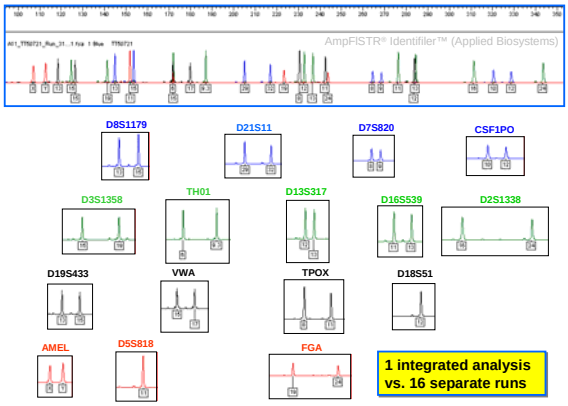


- **Compatible primers are the key to successful multiplex PCR**
- **STR kits are commercially available**
- **15 or more STR loci can be simultaneously amplified**

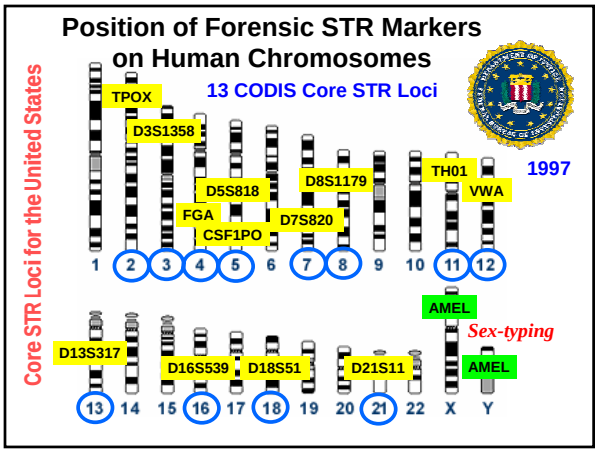
Challenges to Multiplexing
- primer design to find compatible primers (no program exists)
- reaction optimization is highly empirical often taking months

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)

Information is tied together with multiplex PCR and data analysis



Position of Forensic STR Markers on Human Chromosomes

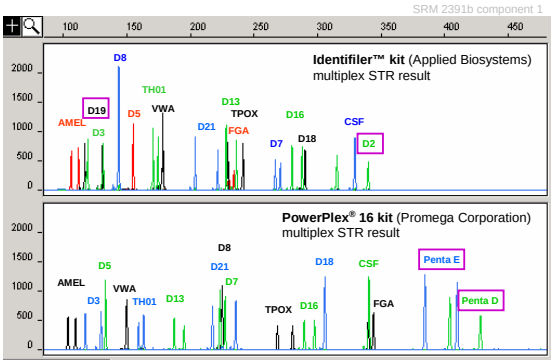


Characteristics of Core STR Loci

Locus	Chromosomal Location	Physical Position (May 2004; NCBI build 35)
TPOX	2p25.3 thyroid peroxidase, 10th intron	Chr 2; 1.472 Mb
D3S1358	3p21.31	Chr 3; 45.557 Mb
FGA	4q31.3 alpha fibrinogen, 3rd intron	Chr 4; 155.866 Mb
D5S818	5q23.2	Chr 5; 123.139 Mb
CSF1PO	5q33.1 c-fms proto-oncogene, 6th intron	Chr 5; 149.436 Mb
D7S820	7q21.11	Chr 7; 83.433 Mb
D8S1179	8q24.13	Chr 8; 125.976 Mb
TH01	11p15.5 tyrosine hydroxylase, 1st intron	Chr 11; 2.149 Mb
VWA	12p13.31 von Willebrand Factor, 40th intron	Chr 12; 5.963 Mb
D13S317	13q31.1	Chr 13; 81.620 Mb
D16S539	16q24.1	Chr. 16; 84.944 Mb
D18S51	18q21.33	Chr 18; 59.100 Mb
D21S11	21q21.1	Chr 21; 19.476 Mb

Butler, J.M. (2006) Genetics and genomics of core STR loci used in human identity testing. *J. Forensic Sci.* 51(2): 253-265

Commercial STR 16plex Kits



From Butler, J.M. (2005) Constructing STR multiplex assays. *Methods in Molecular Biology: Forensic DNA Typing Protocols* (Carracedo, A., ed.), Humana Press: Totowa, New Jersey, *297: 53-66.*

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**

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:

Gaines et al. *J Forensic Sci* 2002; 47(6):1224-1237. Reduced volume PCR amplification reactions using the AmpFISTR Profiler Plus kit.
Leclair et al. *J Forensic Sci* 2003; 48(5):1001-1013. STR DNA typing: increased sensitivity and efficient sample consumption using reduced PCR reaction volumes.
Fregeau et al. *J Forensic Sci* 2003; 48(5):1014-1034. AmpFISTR profiler Plus short tandem repeat DNA analysis of casework samples, mixture samples, and nonhuman DNA samples amplified under reduced PCR volume conditions (25 microL).

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.

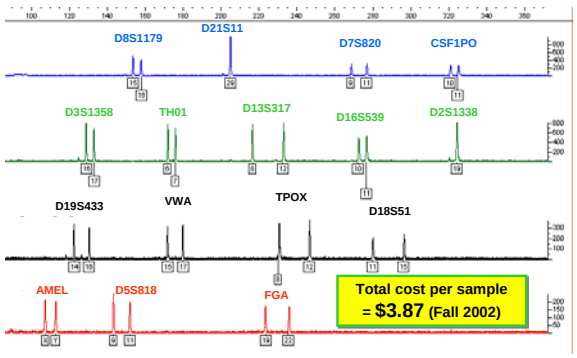
POP™-6 (3700 POP6) rather than POP™-4 was utilized for higher resolution separations.

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

Butler JM, Schoske R, Vallone PM, Redman JW, Kline MC. Allele frequencies for 15 autosomal STR loci on U.S. Caucasian, African American, and Hispanic populations. *J Forensic Sci* 2003; 48(4):908-911.

Identifiler 5 µL PCR

(lower 3100 injection; 5s@2kV instead of 10s@3kV)



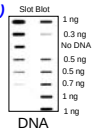
Steps in DNA Analysis

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

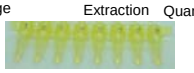
- Collection
- Specimen Storage
- Extraction
- Quantitation
- Genotyping
- Interpretation of Results
- Database
  Storage & Searching

Sample Collection & Storage

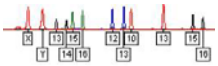
DNA Extraction



DNA Quantitation



Multiplex PCR
(**Amplification of STR Loci**)



STR Typing (**DNA separation**)

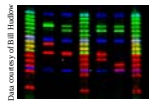
Male: 13,14-15,16-12,13-10,13-15,16
Interpretation of Results

DNA Database

STR Typing Technologies

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

Gels

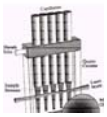


J. Forensic Sci. (1998) 43: 1168-1180

Capillary Electrophoresis

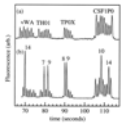
Electrophoresis. (1998) 19: 86-93

Capillary Arrays



Nucleic Acids Res. (1999) 27: e36

Microchip CE

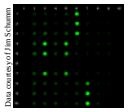


PNAS (1997) 94: 10273-10278

Mass Spectrometry

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

Hybridization Arrays



Nucleic Acids Res. (2000) 28: e17

Typical Instruments Used for STR Typing

GeneAmp 9700

Thermal Cycler for PCR Amplification

Capillary electrophoresis instruments for separating and sizing PCR products

single capillary

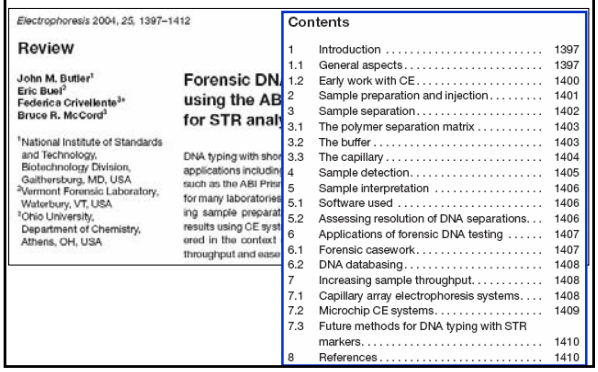
ABI 310

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

John M. Butler[1]
Eric Buel[2]
Federica Crivellente[3]*
Bruce R. McCord[3]

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[3]Ohio University, Department of Chemistry, Athens, OH, USA

Forensic DN
using the AB
for STR anal

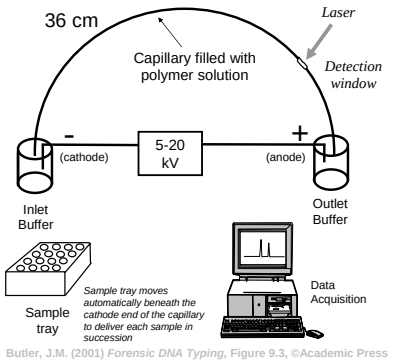
DNA typing with shor
applications including
such as the ABI Prism
for many laboratories
ing sample preparat
results using CE syst
ered in the context
throughput and ease

Contents

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**

Components of a Capillary Electrophoresis System



Butler, J.M. (2001) *Forensic DNA Typing*, Figure 9.3, ©Academic Press

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

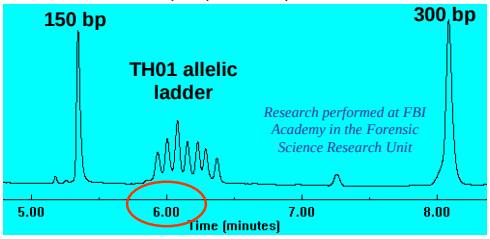
Early Work with CE and STRs

- 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



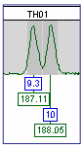
Performed in December 1993

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

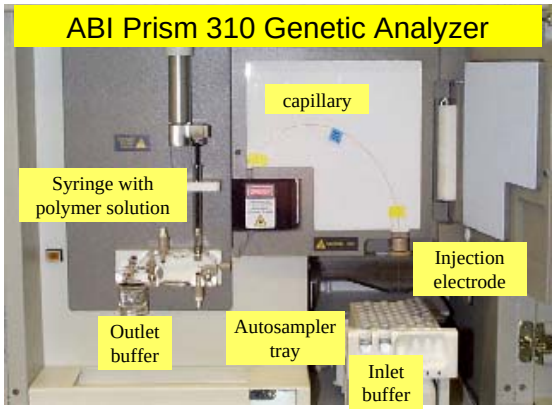
Requirements for Reliable STR Typing

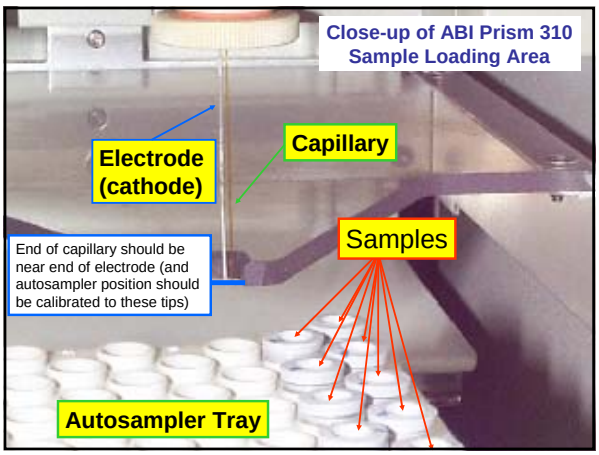
Butler *et al.* (2004) Electrophoresis 25: 1397-1412

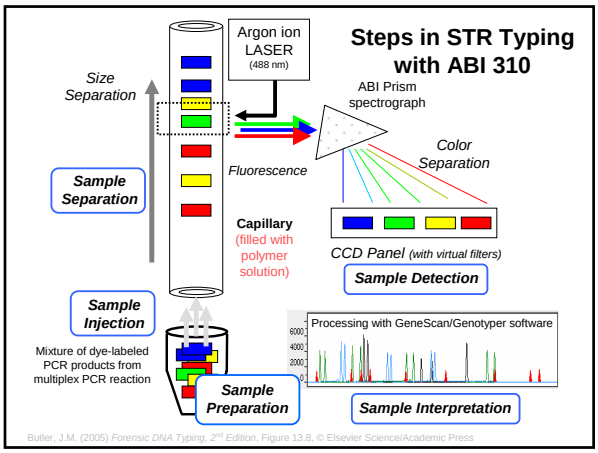
- Reliable sizing over a 75-500 bp size region
- High run-to-run precision between processed samples to permit comparison of allelic ladders to sequentially processed STR samples
- Effective color separations of different dye sets used to avoid bleed through between 4 or 5 different colors
- Resolution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

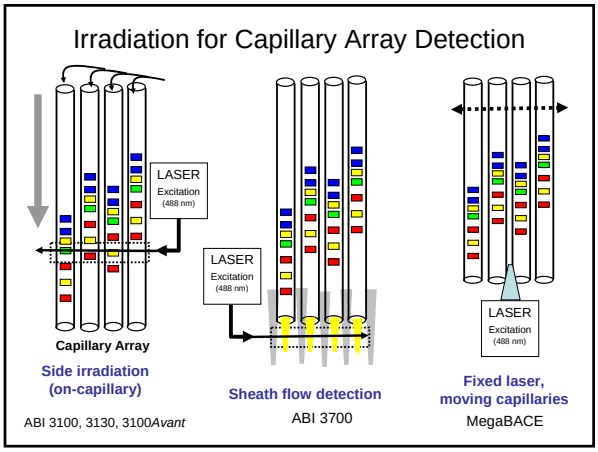


ABI Prism 310 Genetic Analyzer









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

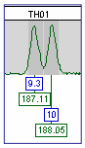
- Data collection software, version 1.0.1
- **POP-6** with 36 cm capillary array

- 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

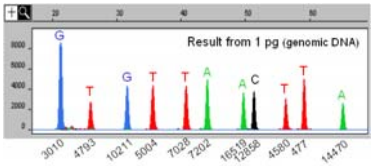
We can routinely get more than 400 runs per capillary array by not changing the polymer between applications

NIST ABI 3100 Analysis Using POP-6 Polymer

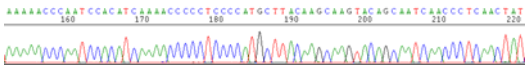
High Resolution STR Typing



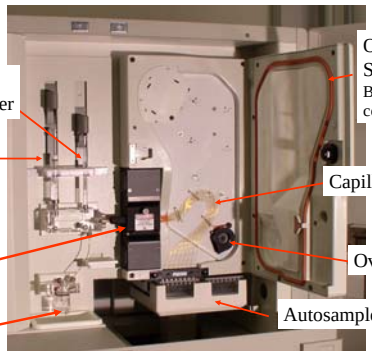
SNaPshot SNP Typing
(Coding Region mtSNP 11plex minisequencing assay)



mtDNA Sequencing (HV1)

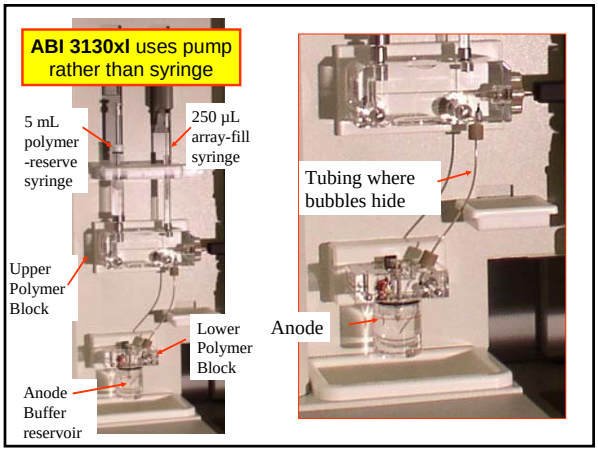


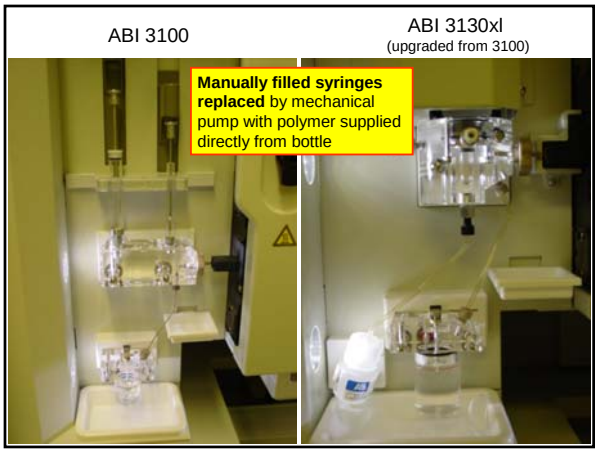
Inside the 3100



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)



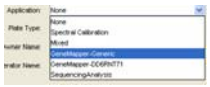


Benefits of the 3130xl Upgrade

(Compared to the original 3100, Data Collection 1.0.1)

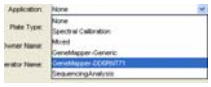
- Takes much less time to change the polymer
- User-friendly wizards to install capillary arrays and change polymer
- **Can easily duplicate plate templates**
- Creation of results group to determine the format of how the data is saved
- Can easily import data, analysis methods, bins and panels, and 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

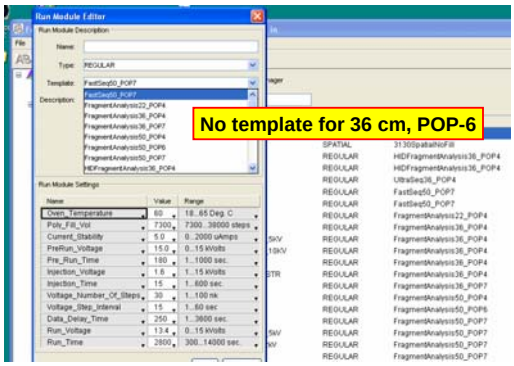
GeneScan/Genotyper



GeneMapper ID only

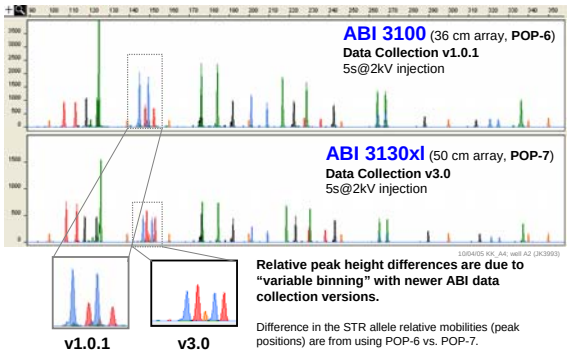
We always choose the "GeneMapper – Generic" setting as the **Application** in our plate templates so we can use both types of data analysis software - allows more flexibility

Pre-Set Templates cannot be changed



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.

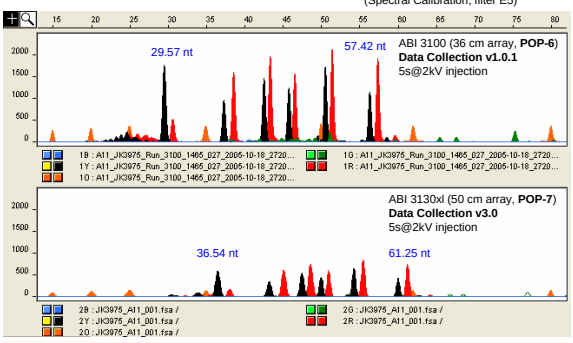
Difference in the STR allele relative mobilities (peak positions) are from using POP-6 vs. POP-7.

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

Comparison of SNaPshot Data

(Spectral Calibration, filter E5)



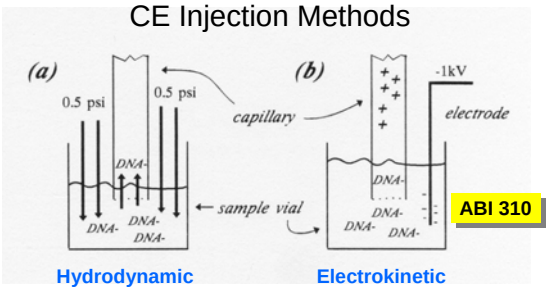
Due to drastic size differences with POP-7, we decided to continue using POP-6 for SNaPshot assays so we wouldn't have to rewrite the Genotyper macros

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, pyrolidinone
- Detection
  - fluorescent dyes with excitation and emission traits
  - CCD with defined virtual filters produced by assigning certain pixels

Injection

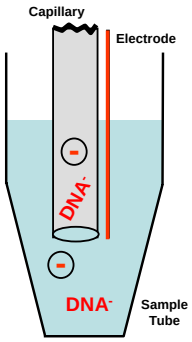
CE Injection Methods



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

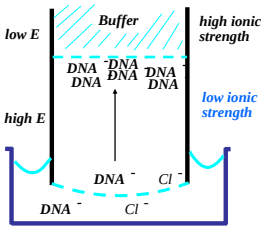
λ_{sample} is the sample conductivity

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

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

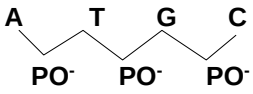
Applied Biosystems presently recommends the use of Hi-Di™ Formamide as the sample-loading solution for all Applied Biosystems DNA sequencers to ensure sample preservation and resistance to evaporation. However, many users of the 3730 choose either deionized water or dilute EDTA solutions. These choices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

Separation

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

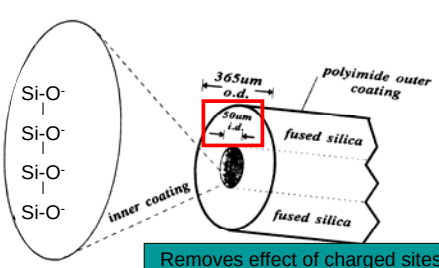


As size increases so does charge!

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
  - Pyrolidinone for denaturing DNA
  - EDTA for stability and chelating metals
- Polymer solution -- POP-4 (but others work also)
- Run temperature -- 60 ºC helps reduce secondary structure on DNA and improves precision. (Temperature control affects DNA sizing)

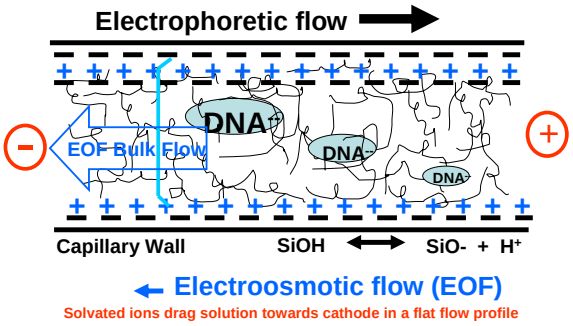
Capillary Coating



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

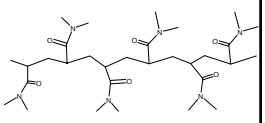


DNA Separations in Entangled Polymer Sieving Solutions

- 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

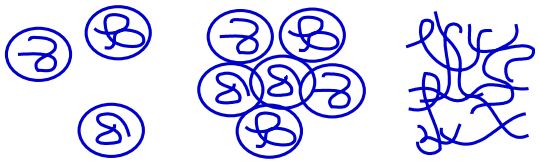
- Polymers are not cross-linked (above entanglement threshold)
- "Gel" is not attached to the capillary wall
- Pumpable -- can be replaced after each run
- Polymer length and concentration determine the separation characteristics
- Examples:
  - 1% HEC (hydroxyethyl cellulose)
  - 4% polyvinyl pyrolidinone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



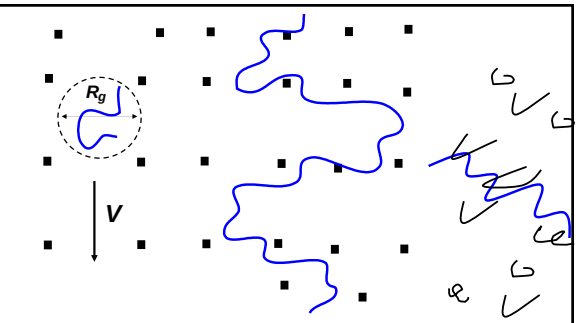
POP4 Polymer

Polydimethyl acrylamide

Transient Pores Are Formed Above the Entanglement Threshold.



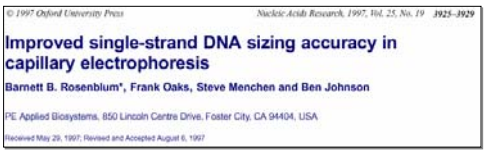
C* < *C* ***C* = *C**** ***C* > *C****



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

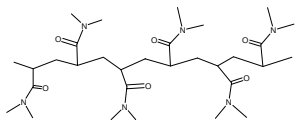
Received May 29, 1997; Revised and Accepted August 6, 1997

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*



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)

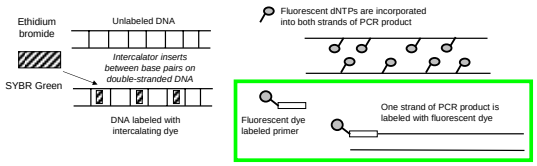
Filters determine which wavelengths of light are collected onto the CCD camera

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

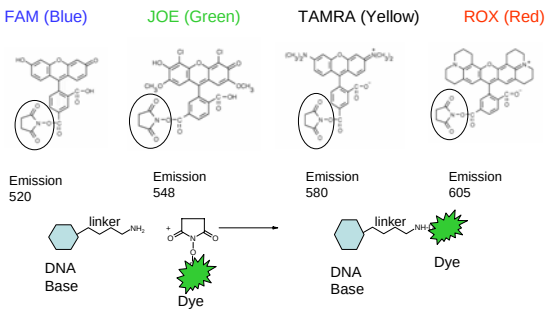


Butler, J.M. (2001) *Forensic DNA Typing*, Figure 10.2, ©Academic Press

Fluorescent Labeling of PCR Products

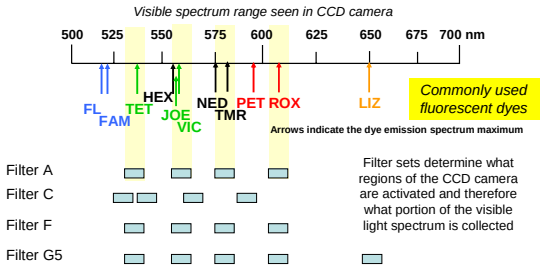
- Dyes are attached to one primer in a pair used to amplify a STR marker
- Dyes are coupled to oligonucleotides (primers) through NHS-esters and amine linkages on the 5'end of the primer: Dye-$(CH_2)_6$-primer
- Dye-labeled oligonucleotides are incorporated during multiplex PCR amplification giving a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

Amine Reactive Dyes used in Labeling DNA



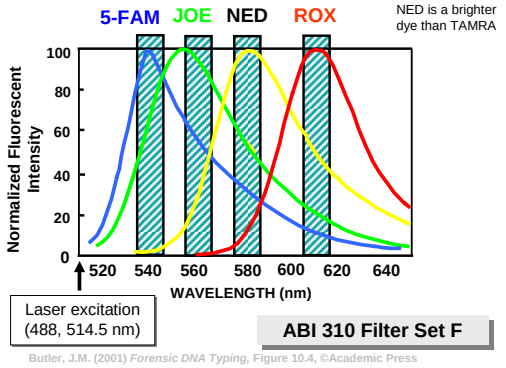
The succinimidyl ester reacts rapidly with amine linkers on DNA bases

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

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

2004 prices

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