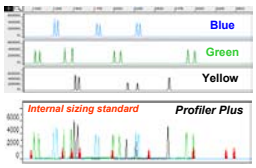


Capillary Electrophoresis Instrumentation and Available STR Kits

John M. Butler, Ph.D.

Canadian Forensic DNA Technology Workshop
Toronto, Ontario
June 8, 2005

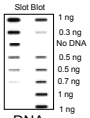
Steps in DNA Analysis

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

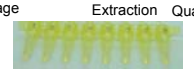
- Collection
- Specimen Storage
- Extraction
- Quantitation
- Genotyping
- Interpretation of Results
- Database
  Storage & Searching

Blood Stain Buccal swab
Sample Collection & Storage

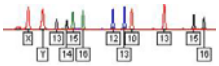
DNA Extraction



DNA Quantitation



Multiplex PCR
(Amplification of STR Loci)



STR Typing (DNA separation)

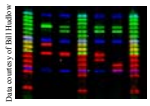
Male: 13,14-15,16-12,13-10,13-15,16
Interpretation of Results

DNA Database

STR Typing Technologies

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

Gels

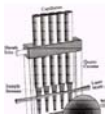


J. Forensic Sci. (1998) 43: 1168-1180

Capillary Electrophoresis

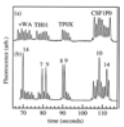
Electrophoresis. (1998) 19: 86-93

Capillary Arrays



Nucleic Acids Res. (1999) 27: e36

Microchip CE

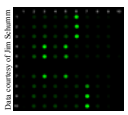


PNAS (1997) 94: 10273-10278

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

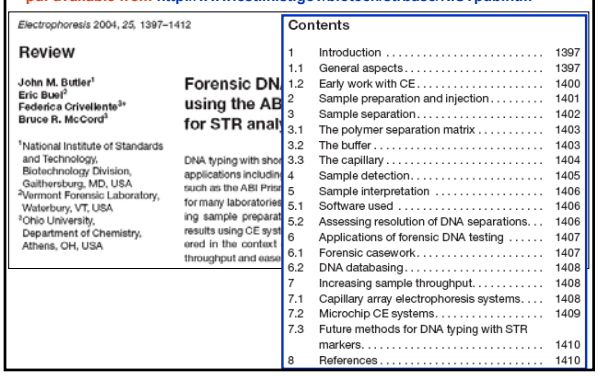
ABI 310

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]

[1]National Institute of Standards and Technology, Biotechnology Division, Gaithersburg, MD, USA
[2]Vermont Forensic Laboratory, Waterbury, VT, USA
[3]Ohio University, Department of Chemistry, Athens, OH, USA

Forensic DN
using the AB
for STR anal

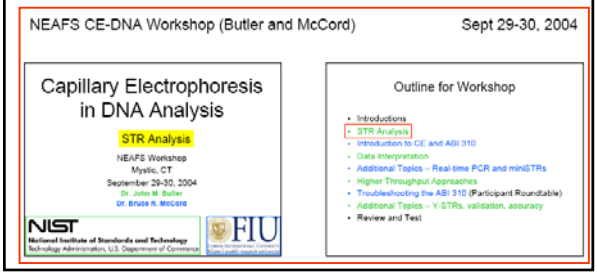
DNA typing with shor
applications includin
such as the ABI Prism
for many laboratories
ing sample preparat
results using CE syst
ered in the context
throughput and ease

Contents

NEAFS Workshop Slide Handouts

Handouts available as downloadable pdf files from
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm#NEAFSworkshop

2 day workshop with **>500 slides** describing STRs and CE (ABI 310 and ABI 3100)



NEAFS CE-DNA Workshop (Butler and McCord) Sept 29-30, 2004

Capillary Electrophoresis in DNA Analysis

STR Analysis

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

NIST National Institute of Standards and Technology
FIU

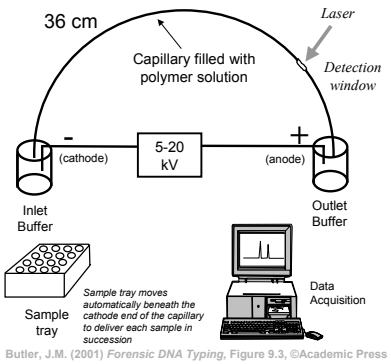
Outline for Workshop

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

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

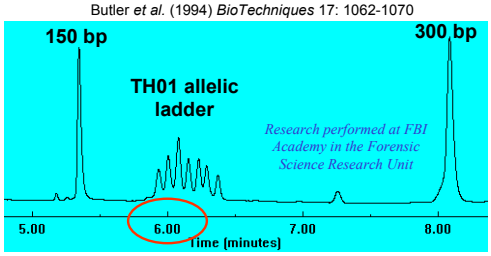
Early Work with CE and STRs

- 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



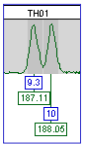
Performed in December 1993

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

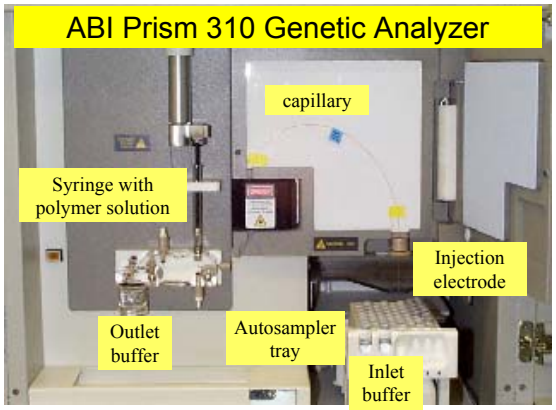
Requirements for Reliable STR Typing

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

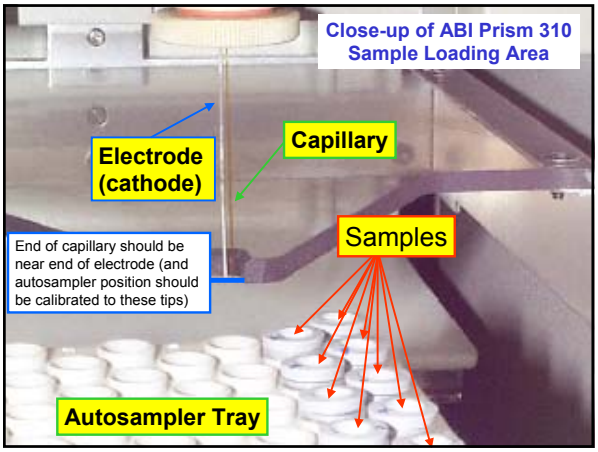
- Reliable sizing over a 75-500 bp size region
- High run-to-run precision between processed samples to permit comparison of allelic ladders to sequentially processed STR samples
- Effective color separations of different dye sets used to avoid bleed through between 4 or 5 different colors
- Resolution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



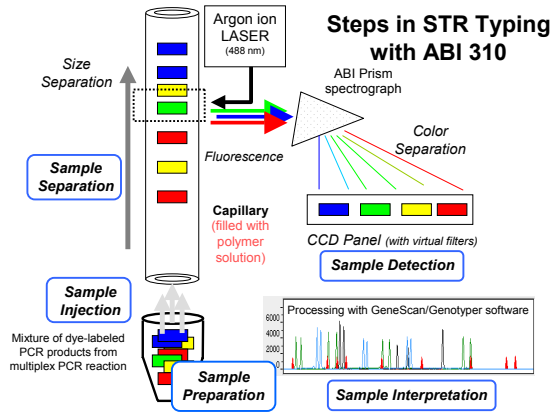
ABI Prism 310 Genetic Analyzer



Close-up of ABI Prism 310 Sample Loading Area



Steps in STR Typing with ABI 310



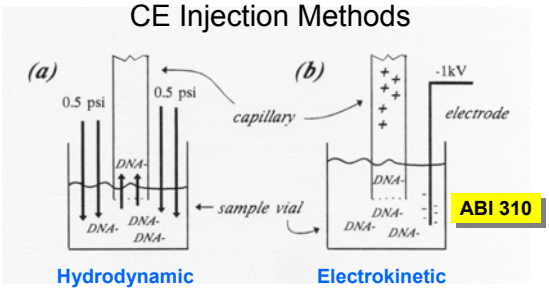
Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 13.8, © Elsevier Science/Academic Press

Process Involved in 310 Analysis

- Injection
  - electrokinetic injection process (formamide, water)
  - importance of sample stacking
- Separation
  - Capillary – 50um fused silica, 43 cm
  - 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

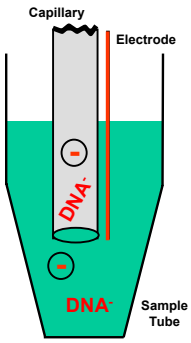
CE Injection Methods



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

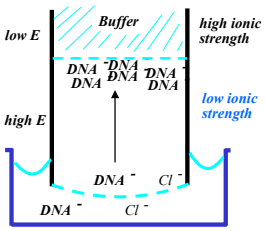
λ_{sample} is the sample conductivity

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



Typical Sample Preparation for ssDNA

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

Comments on Sample Preparation

- Use high quality formamide (<100 µS/cm)!
  - ABI sells Hi-Di formamide
  - regular formamide can be made more pure with ion exchange resin
- Deionized water vs. formamide
  - 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...

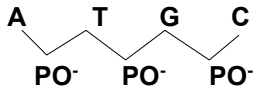
Separation

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

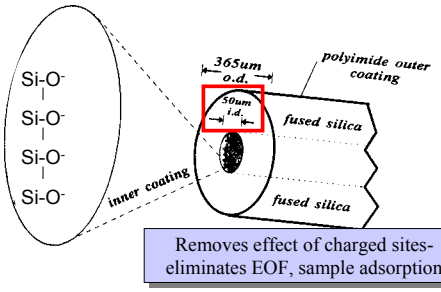


As size increases so does charge!

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 °C helps reduce secondary structure on DNA and improves precision. (Temperature control affects DNA sizing)

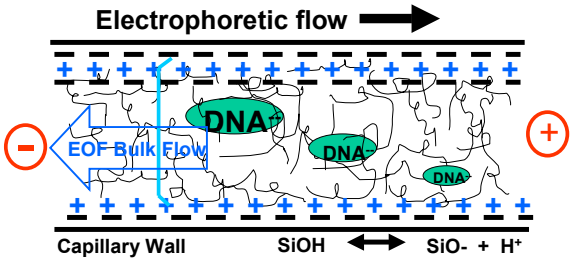
Capillary Coating



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



Electroosmotic flow (EOF)

Solvated ions drag solution towards cathode in a flat flow profile

DNA Separations in Entangled Polymer Sieving Solutions

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

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

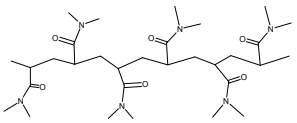
Received May 29, 1997; Revised and Accepted August 6, 1997

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)

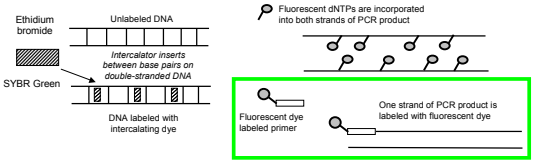
Filters determine which wavelengths of light are collected onto the CCD camera

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

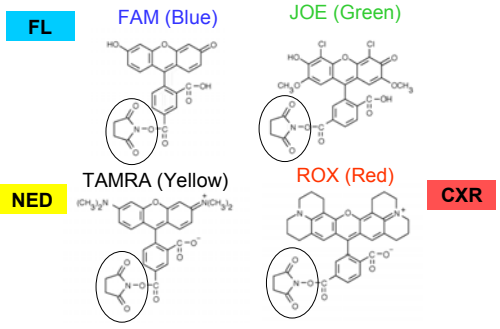


Butler, J.M. (2001) *Forensic DNA Typing*, Figure 10.2, ©Academic Press

Fluorescent Labeling of PCR Products

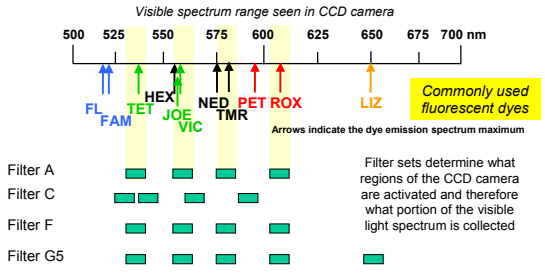
- Dyes are attached to one primer in a pair used to amplify a STR marker
- Dyes are coupled to oligonucleotides (primers) through NHS-esters and amine linkages on the 5'end of the primer: Dye-$(CH_2)_6$-primer
- Dye-labeled oligonucleotides are incorporated during multiplex PCR amplification giving a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

Fluorescent Dyes Used in 4-Color Detection



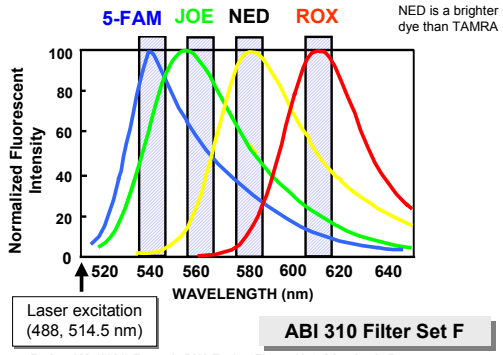
Butler, J.M. (2001) *Forensic DNA Typing*, Figure 10.3, ©Academic Press

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

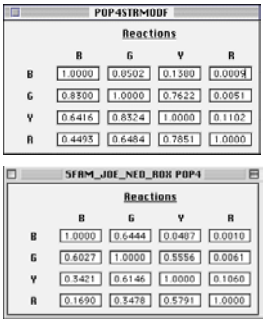
Please Note!

- 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
(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

Same Dye Set and Filter F with Different ABI 310s



Instrument lasers make a big difference

Injection List in Data Collection Software

- Lists samples to be analyzed (repeats can be easily performed)
- Sets virtual filter on CCD camera
- Sets electrophoresis time and voltage
- Sets injection time and voltage
- Sets run temperature
- If desired, sample analysis can be set up for automatic matrix color separation and sizing with internal standards using defined analysis parameters

Steps Performed in Standard Module

See J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition; Chapter 14

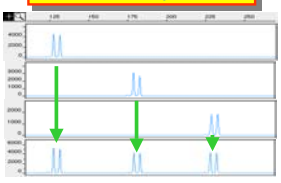
- **Capillary fill** – polymer solution is forced into the capillary by applying a force to the syringe
- **Pre-electrophoresis –** the separation voltage is raised to 10,000 volts and run for 5 minutes;
- **Water wash of capillary –** capillary is dipped several times in deionized water to remove buffer 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

Ways to Increase Sample Throughput

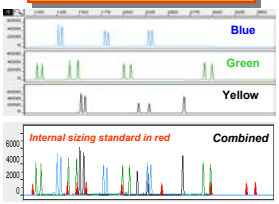
- Run more gels (FMBIO approach)
- Increase speed of single sample analysis (microchip CE systems)
- Multiplex fluorescent dyes of different colors (higher level PCR multiplexes)
- **Parallel separations using capillary arrays** (e.g., ABI 3100 or 3130)
- New detection technologies (MALDI-TOF mass spectrometry)

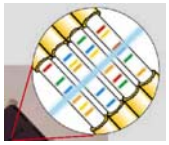
Methods for Parallel Sample Processing

Multiplex by Size



Multiplex by Dye Color

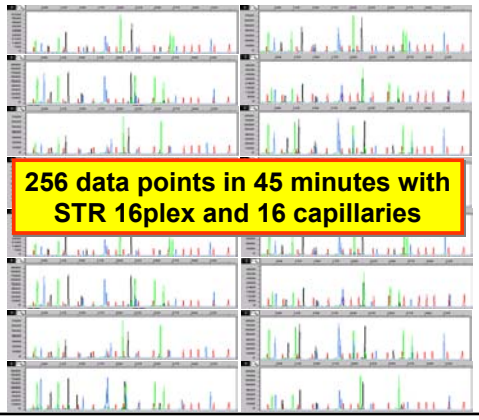




Multiplex by Number of Capillaries

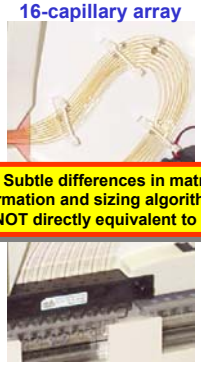
ABI 3100: 16 capillaries
ABI 3730: 96 capillaries
ABI 3100 *Avant*: 4 capillaries

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



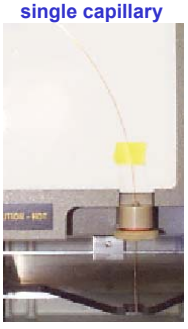
Increasing Sample Throughput with Parallel Processing

ABI 3100
16-capillary array

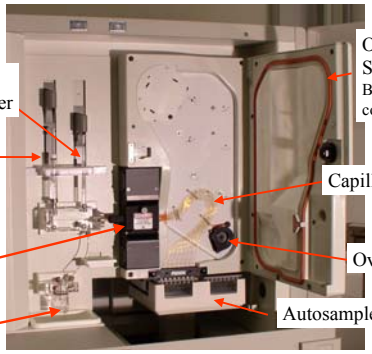


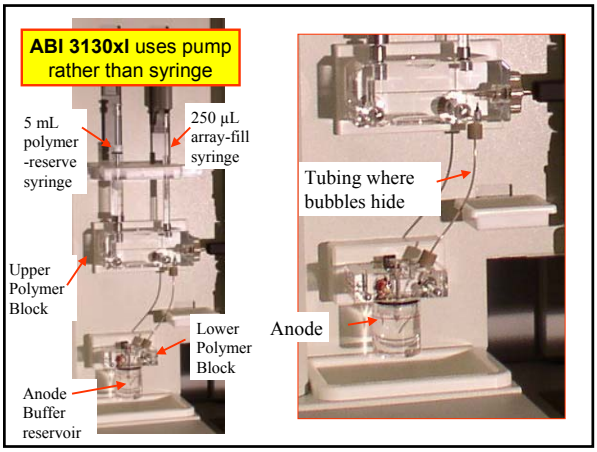
Subtle differences in matrix formation and sizing algorithms – NOT directly equivalent to 310

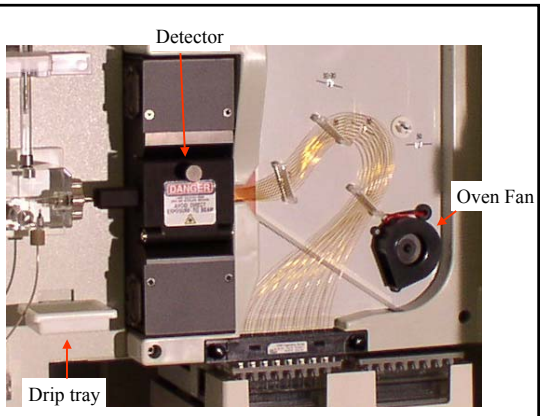
ABI 310
single capillary



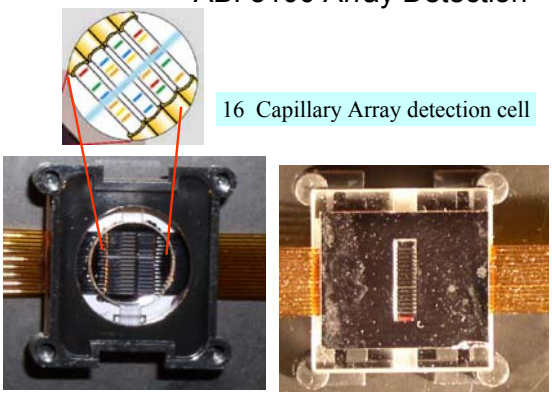
Inside the 3100





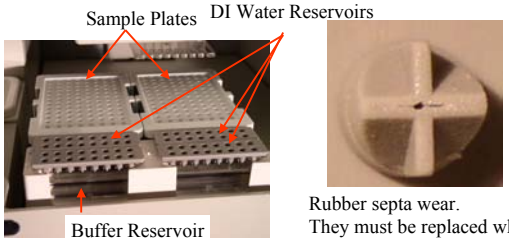


ABI 3100 Array Detection



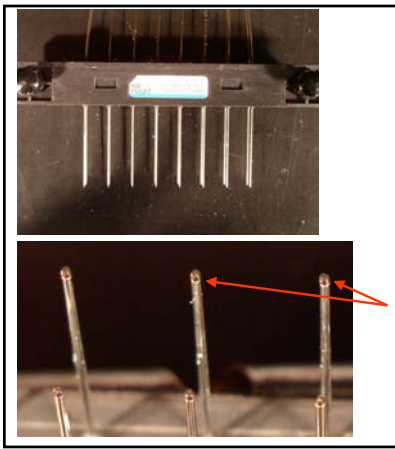
Two 96 well plates on the autosampler

At 45-60 minutes per run two plates represent 12 runs or ~10-12 hours for 192 samples



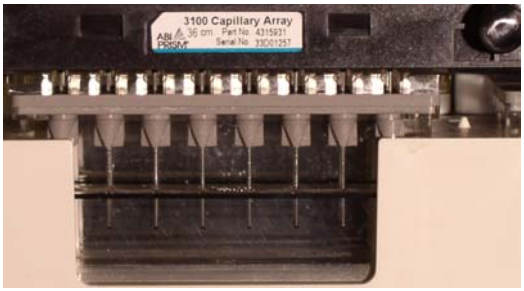
Rubber septa wear.
They must be replaced when the edges are ragged..

16 Capillary Array

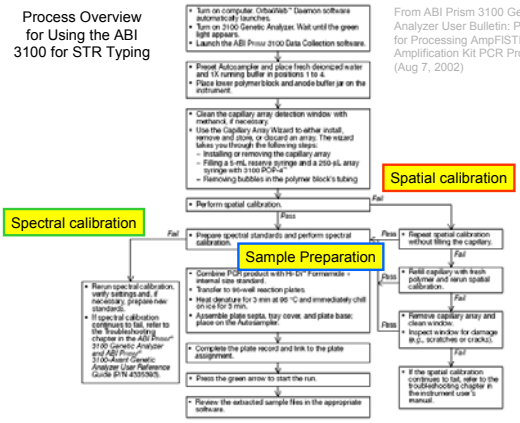


Capillaries in buffer tank

Running and storage position



Process Overview for Using the ABI 3100 for STR Typing



From ABI Prism 3100 Genetic Analyzer User Bulletin: Protocols for Processing AmpFISTR PCR Amplification Kit PCR Products (Aug 7, 2002)

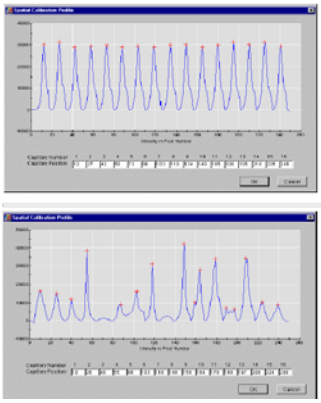
Spatial Calibration

Performed after:

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

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

Spatial Results



Good Results

Bad results
Try again

Maintenance of ABI 3100

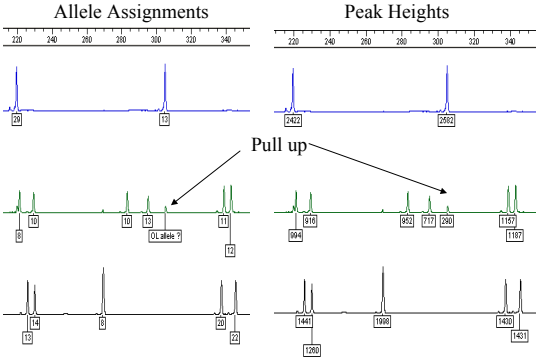
- Syringe – leaks cause capillary to not fill properly
- Capillary storage & wash – **it dries, it dies!**
- Pump block – cleaning helps insure good fill
- Change the running buffer regularly

YOU MUST BE CLEAN AROUND A CE!

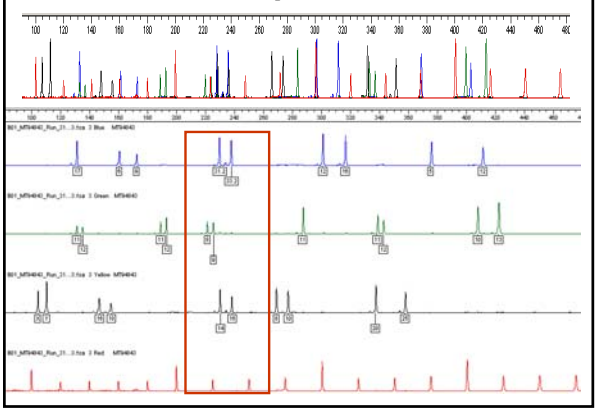
Spectral Calibration

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

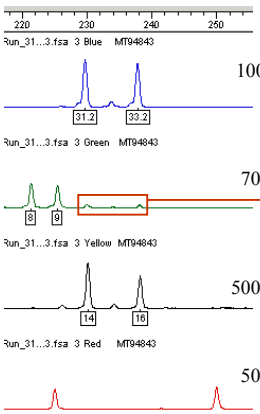
Pull-up issue



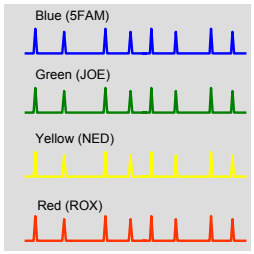
Powerplex 16 data



Time for a new matrix

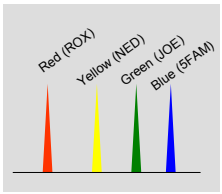


(A) ABI 310 Matrix Samples



Separate samples run for each dye color
Each sample contains multiple peaks
All peaks labeled with the same dye color

(B) ABI 3100 Matrix (Spectral Calibration) Sample

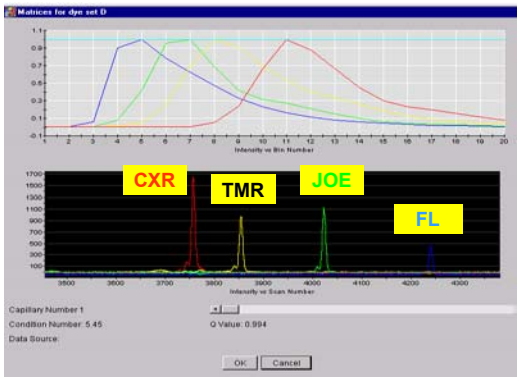


Single sample run containing all dye colors
Only one peak per dye color
Injected into each capillary of the array

A separate spectral calibration file is created for each capillary

Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 14.5, © Elsevier Science/Academic Press

Defining the Matrix on the ABI 3100



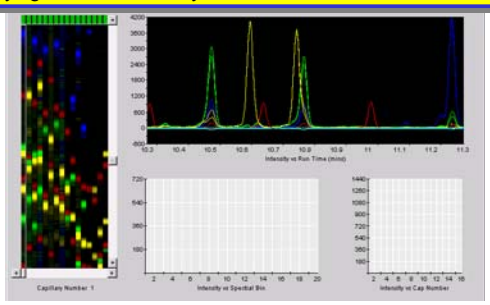
Matrices Created on NIST ABI 3100 with Various Dye Combinations

		3100 Filters				
Color	D	E	E5	F	G5	Z
Blue	FL	dR110	dR110	5FAM	6FAM	6FAM
Green	JOE	dR6G	dR6G	JOE	VIC	VIC
Yellow	TMR	dTAMRA	dTAMRA	NED	NED	NED
Red	CXR	dROX	dROX	ROX	PET	ROX
Orange			LIZ		LIZ	
	PowerPlex 16	Big Dye Sequencing	SNaPshot	Profiler Plus, COfiler, SGM Plus	Identifiler, Y STR 20plex	Y STR 16plex

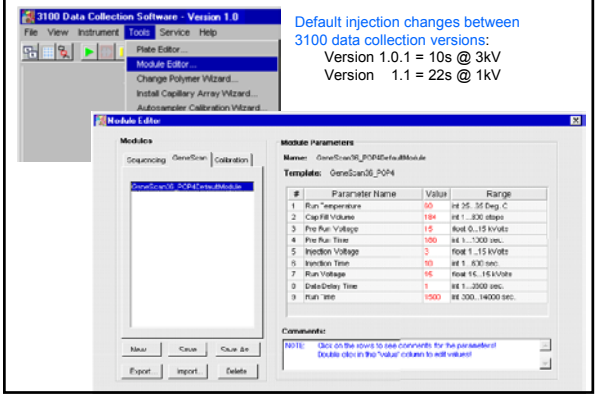
Different ABI 3100 matrix sets used at NIST in order to address a variety of applications and dye combinations.

Data from ABI 3100 During the Run

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



Parameters in Run Modules



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

Consumables for ABI 310/3100

What we use at NIST

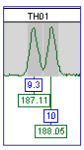
- A.C.E.™ Sequencing Buffer 10X (Amresco)
  - $155/L = $0.0155/mL 1X buffer **(costs 20 times less!)**
  - http://www.amresco-inc.com
- 3700 **POP-6 Polymer** (Applied Biosystems)
  - $530 / 200 mL = $2.65/mL **(costs 20 times less!)**

What ABI protocols suggest

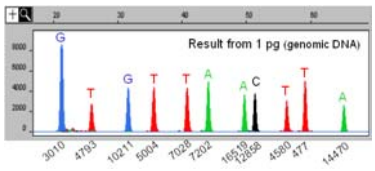
- 10X Genetic Analyzer Buffer with EDTA
  - $78/25 mL = $0.312/mL 1X buffer (ABI)
- 3100 POP-4 Polymer
  - $365 / 7 mL = $52/mL

NIST ABI 3100 Analysis Using POP6 Polymer

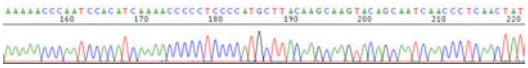
High Resolution STR Typing



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



mtDNA Sequencing (HV1)



ABI 310 Reagents and Operating Costs

ABI 310 Reagent Costs		*for 500 samples*		factor for 500	Total Cost
	Part Number	Quantity Provided	Cost	*1000 runs with P+C*	
Capillaries	402839	5/pk (47cm x 50 um uncoated)	$294	2	$588
POP-4 polymer	402838	5 mL	$196	2	$392
Buffer, Genetic Analyzer 10X	402824	25 mL	$78	1	$78
Sample tubes (0.5 mL)	401957	500/pk	$52	2	$104
Septa for tubes	401956	500/pk	$163	2	$326
Formamide, Hi-Di	4311320	25 mL (for ~1000-1500 samples)	$29	1	$29
GS500-ROX size standard	401734	800 tests/pk	$260	1.25	$325
Matrix standards	4312131	5FAM, JOE, NED, ROX	$70	1	$70
PCR tubes, strips	N801-0580	1000/pk	$76	1	$76
PCR tube caps	N801-0535	1000/pk	$60	1	$60
Pipet tips		~$0.10/tip x 550 tips	$55	2	$110
Profiler Plus STR kit	4303326	100 tests/kit	$2,018.94	5	$10,095
COfiler STR kit	4305246	100 tests/kit	$1,816.54	5	$9,083
Syringe, Kloehn 1.0 mL	4304471	each	$82	1	$82
Genetic Analyzer vials, 4 mL	401955	50/pk	$62	1	$62
48-tube sample tray kit	402867	each	$230	1	$230

**following manufacturer's protocols (based on 500 samples total)*

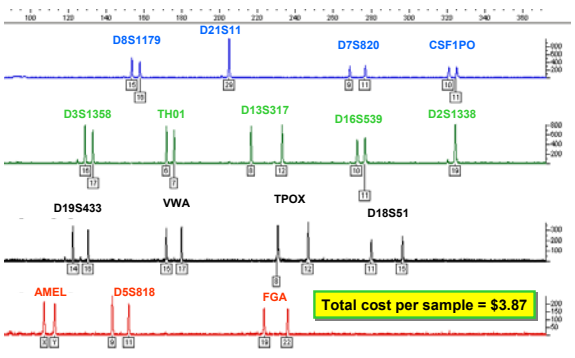
Total per Sample Cost to Obtain Result on 13 CODIS core loci (with Profiler Plus and COfiler STR kits): $43.42

(materials other than STR kits = $5.06)

10 µL PCR (1/5 vol) = $12.73

Identifiler 5 uL PCR

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



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).

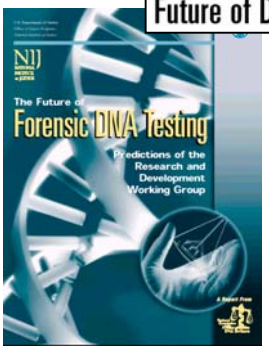
Butler JM, Schoske R, Vallone PM, Redman JW, Kline MC. Allele frequencies for 15 autosomal STR loci on U.S. Caucasian, African American, and Hispanic populations. *J Forensic Sci* 2003; 48(4):908-911.

Overall Thoughts on the ABI 310/3100

- 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…

Available STR Kits

National Commission on the Future of DNA Evidence



•Report published in Nov 2000

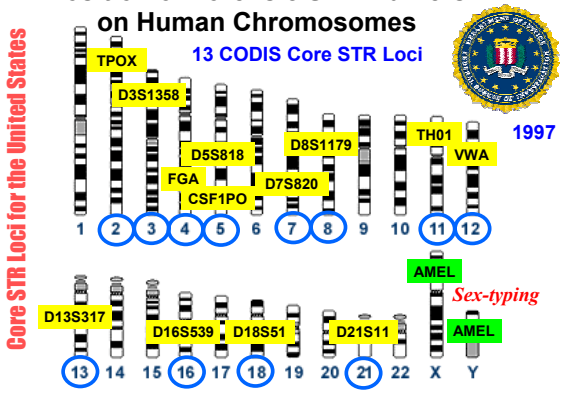
•Asked to estimate where DNA testing would be 2, 5, and 10 years into the future

Conclusions

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

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

Position of Forensic STR Markers on Human Chromosomes



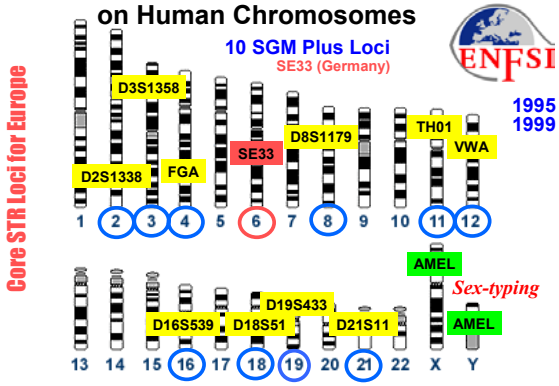
Position of Each CODIS STR Locus in Human Genome

Locus Name	Chromosomal Location	Physical Position*
CSF1PO	5q33.1 c-fms proto-oncogene, 6th intron	Chr 5 149.484 Mb
FGA	4q31.3 alpha fibrinogen, 3rd intron	Chr 4 156.086 Mb
TH01	11p15.5 tyrosine hydroxylase, 1st intron	Chr 11 2.156 Mb
TPOX	2p25.3 thyroid peroxidase, 10th intron	Chr 2 1.436 Mb
VWA	12p13.31 von Willebrand Factor, 40th intron	Chr 12 19.826 Mb
D3S1358	3p21.31	Chr 3 45.543 Mb
D5S818	5q23.2	Chr 5 123.187 Mb
D7S820	7q21.11	Chr 7 83.401 Mb
D8S1179	8q24.13	Chr 8 125.863 Mb
D13S317	13q31.1	Chr 13 80.52 Mb
D16S539	16q24.1	Chr 16 86.168 Mb
D18S51	18q21.33	Chr 18 59.098 Mb
D21S11	21q21.1	Chr 21 19.476 Mb

Review article on core STR loci genetics and genomics to be published this fall

From Table 5.2, *Forensic DNA Typing*, 2nd Edition, p. 96 (J.M. Butler, 2005)

Position of Forensic STR Markers on Human Chromosomes



Commercial STR Kits

Kit Contents:

Allelic Ladders for Genotyping

PCR Component Mix

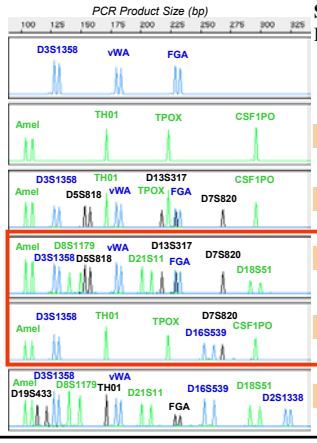
Primer Mix

Positive Control DNA Sample

Cost to User: $15-30 per DNA sample tested

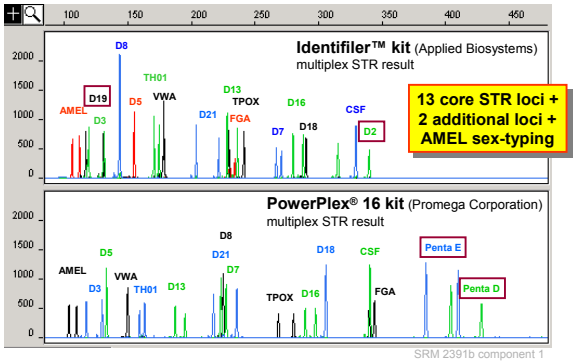
Currently 2 Suppliers: Applied Biosystems and Promega Corporation

Same DNA Sample Run with Each of the ABI STR Kits



	Power of Discrimination
Blue	1:5000
Green I	1:410
Profiler™	$1:3.6 \times 10^{9}$
Profiler Plus™	$1:9.6 \times 10^{10}$
COfiler™	$1:8.4 \times 10^{5}$
SGM Plus™	$1:3.3 \times 10^{12}$

Commercial STR 16plex Kits



Promega Corporation Autosomal STR Kits

Kit Name	STR Loci Included	Random Match Probability with Author's Profile*
PowerPlex 1.2	CSF1PO, TPOX, TH01, VWA, D16S539, D13S317, D7S820, D5S818	7.4×10^{-10}
PowerPlex ES	FGA, TH01, VWA, D3S1358, D8S1179, D18S51, D21S11, SE33, amelogenin	1.3×10^{-10}
PowerPlex 16	CSF1PO, FGA, TPOX, TH01, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, Penta D, Penta E, amelogenin	1.2×10^{-18}

*Allele frequencies used for random match probability calculations (to unrelated individuals) from U.S. Caucasian population data associated with Butler et al. (2003) J Forensic Sci 48:908-11 , Reid et al. (2003) J Forensic Sci 48:1422-3 , and Levadokou et al. (2001) J Forensic Sci 46:736-61. Subpopulation structure adjustments (theta corrections) were not made with these calculations (i.e., only p^2 and 2 pq were used).

Applied Biosystems Autosomal STR Kits

Kit Name	STR Loci Included	Random Match Probability with Author's Profile*
AmpFlSTR Blue	D3S1358, VWA, FGA	1.0×10^{-3}
AmpFlSTR Green I	Amelogenin, TH01, TPOX, CSF1PO	7.8×10^{-4}
AmpFlSTR COfiler	D3S1358, D16S539, Amelogenin, TH01, TPOX, CSF1PO, D7S820	2.0×10^{-7}
AmpFlSTR Profiler Plus	D3S1358, VWA, FGA, Amelogenin, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820	2.4×10^{-11}
AmpFlSTR Profiler Plus ID	D3S1358, VWA, FGA, Amelogenin, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820 *(extra unlabeled D8-R primer)*	2.4×10^{-11}
AmpFlSTR Profiler	D3S1358, VWA, FGA, Amelogenin, TH01, TPOX, CSF1PO, D5S818, D13S317, D7S820	9.0×10^{-11}
AmpFlSTR SGM Plus	D3S1358, VWA, D16S539, D2S1338, Amelogenin, D8S1179, D21S11, D18S51, D19S433, TH01, FGA	4.5×10^{-13}
AmpFlSTR SEfiler	FGA, TH01, VWA, D3S1358, D8S1179, D16S539, D18S51, D21S11, D2S1338, D19S433, SE33, amelogenin	5.1×10^{-15}
AmpFlSTR Identifiler	CSF1PO, FGA, TPOX, TH01, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, D2S1338, D19S433, amelogenin	7.2×10^{-19}

Value of STR Kits

Advantages

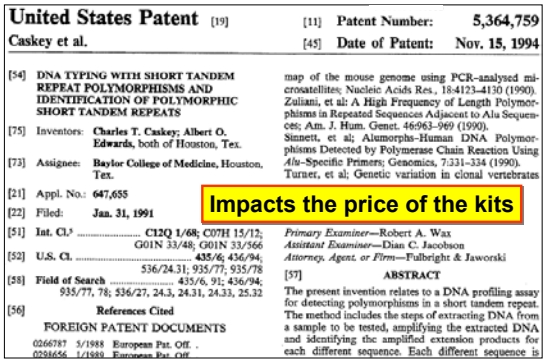
- Quality control of materials is in the hands of the manufacturer
- Improves consistency in results across laboratories – same allelic ladders used
- Common loci and PCR conditions used – aids DNA databasing efforts
- Simpler for the user to obtain results

Disadvantages

- Contents may not be completely known to the user (e.g., primer sequences)
- Higher cost to obtain results

FSS: 5X higher cost with SGM Plus kit

Core Patent Licensed to Promega Corporation and Applied Biosystems Inc



United States Patent [19]
Caskey et al.

[11] Patent Number: 5,364,759
[45] Date of Patent: Nov. 15, 1994

[54] DNA TYPING WITH SHORT TANDEM REPEAT POLYMORPHISMS AND IDENTIFICATION OF POLYMORPHIC SHORT TANDEM REPEATS

[75] Inventors: Charles T. Caskey; Albert O. Edwards, both of Houston, Tex.

[73] Assignee: Baylor College of Medicine, Houston, Tex.

[21] Appl. No.: 647,655

[22] Filed: Jan. 31, 1991

[51] Int. Cl.[5] C12Q 1/68; C07H 15/12; G01N 33/48; G01N 33/566

[52] U.S. Cl. 435/6; 436/94; 536/24.31; 935/77; 935/78

[58] Field of Search 435/6, 91; 436/94; 935/77, 78; 536/27, 24.3, 24.31, 24.33, 25.32

[56] References Cited
FOREIGN PATENT DOCUMENTS
0266787 5/1988 European Pat. Off. .
0298656 1/1989 European Pat. Off.

map of the mouse genome using PCR-analysed microsatellites; Nucleic Acids Res., 18:4123–4130 (1990).
Zuliani, et al; A High Frequency of Length Polymorphisms in Repeated Sequences Adjacent to Alu Sequences; Am. J. Hum. Genet. 46:963–969 (1990).
Sinnett, et al; Alumorphs-Human DNA Polymorphisms Detected by Polymerase Chain Reaction Using *Alu*-Specific Primers; Genomics, 7:331–334 (1990).
Turner, et al; Genetic variation in clonal vertebrates

Impacts the price of the kits

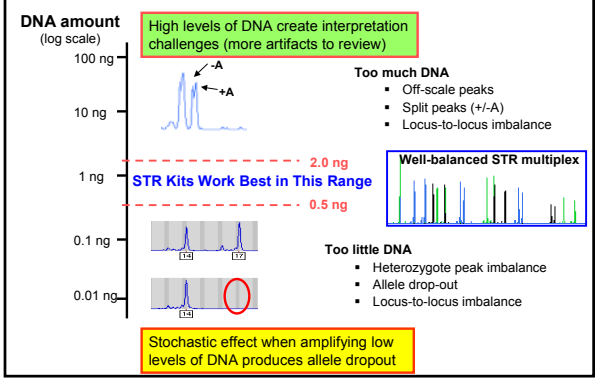
Primary Examiner—Robert A. Wax
Assistant Examiner—Dian C. Jacobson
Attorney, Agent, or Firm—Fulbright & Jaworski

[57] ABSTRACT

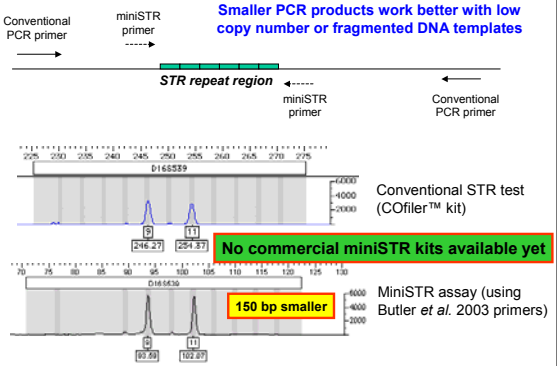
The present invention relates to a DNA profiling assay for detecting polymorphisms in a short tandem repeat. The method includes the steps of extracting DNA from a sample to be tested, amplifying the extracted DNA and identifying the amplified extension products for each different sequence. Each different sequence is

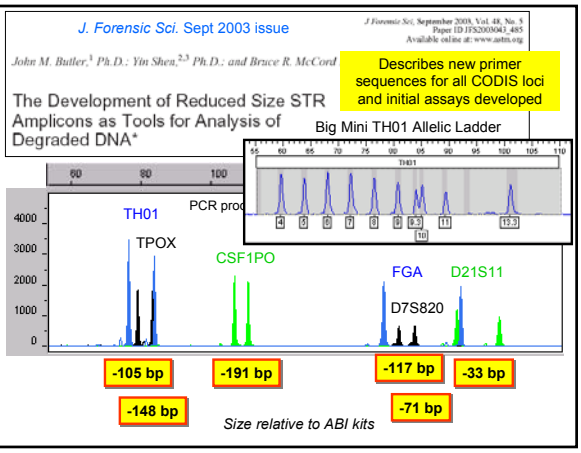
Importance of DNA Quantitation

(prior to multiplex PCR)



miniSTRs: new tool for degraded DNA





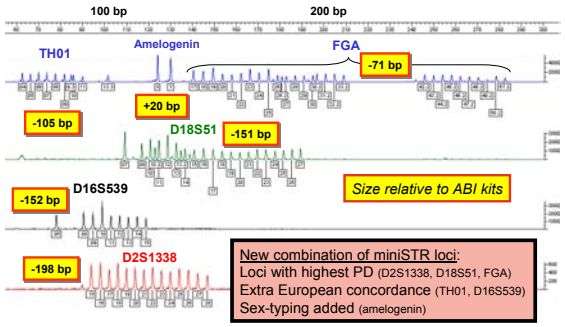
Recent Publications on miniSTRs

- 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.
- Chung, D.T., Drabek, J., Opel, K.L., Butler, J.M., McCord, B.R. (2004) A study on the effects of degradation and template concentration on the efficiency of the STR miniplex primer sets. *J. Forensic Sci.* 49(4): 733-740.
- Drabek, J., Chung, D.T., Butler, J.M., McCord, B.R. (2004) Concordance study between miniplex STR assays and a commercial STR typing kit, *J. Forensic Sci.* 49(4): 859-860.
- Coble, M.D. and Butler, J.M. (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA., *J. Forensic Sci.*, 50: 43-53.

http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm
http://www.cstl.nist.gov/biotech/strbase/miniSTR/timeline.htm

New miniSGM miniplex assay

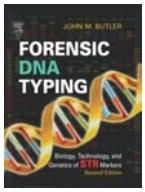
Provided to EDNAP/ENFSI group for degraded DNA study (Fall 2004)



Retains same miniSTR primers from Butler et al. (2003) *J. Forensic Sci* 48(5): 1054-1064

Additional Resources

- *Forensic DNA Typing: Biology,Technology, and Genetics of STR Markers (2nd Edition)* by John M. Butler, Elsevier Academic Press, 2005
- Butler, J.M., *et al.* (2004) Forensic DNA typing by capillary electrophoresis using the ABI Prism 310 and 3100 genetic analyzers for STR analysis. *Electrophoresis* 25: 1397-1412.
- NIST website: **http://www.cstl.nist.gov/biotech/strbase**



Content of STRBase Website

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

- …/str_fact.htm STR Fact Sheets on Core Loci
- …/multiplx.htm Multiplex STR Kit Information
- …/y_strs.htm Y-Chromosome Information
- …/var_tab.htm Variant Alleles Reported
- …/mutation.htm Mutation Rates for Common STRs
- …/str_ref.htm Reference List with ~2,300 Papers
- …/training.htm Downloadable PowerPoints for Training
- …/validation.htm Validation Information
- …/miniSTR.htm miniSTR Information
- …/address.htm Addresses for Scientists
- …/NISTpub.htm Publications & Presentations from NIST

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THANK YOU FOR YOUR ATTENTION

Questions!

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