Capillary Electrophoresis in DNA Analysis

Higher Throughput Approaches

NEAFS Workshop
Mystic, CT
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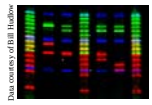
Outline for Workshop

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

STR Typing Technologies

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

Gels

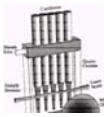


J. Forensic Sci. (1998) 43: 1168-1180

Capillary Electrophoresis

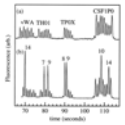
Electrophoresis. (1998) 19: 86-93

Capillary Arrays



Nucleic Acids Res. (1999) 27: e36

Microchip CE

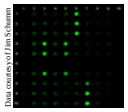


PNAS (1997) 94: 10273-10278

Mass Spectrometry

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

Hybridization Arrays



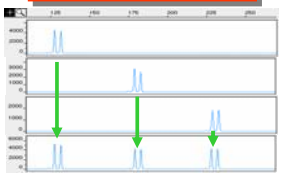
Nucleic Acids Res. (2000) 28: e17

Ways to Increase Sample Throughput

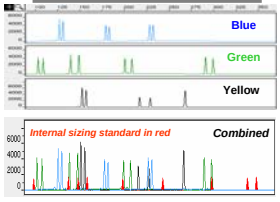
- Run more gels (FMBIO approach)
- Increase speed of single sample analysis (microchip CE systems)
- Multiplex fluorescent dyes of different colors (higher level PCR multiplexes)
- Parallel separations using capillary arrays
- New Detection Technologies (MALDI-TOF mass spectrometry)

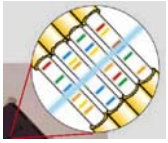
Methods for Parallel Sample Processing

Multiplex by Size



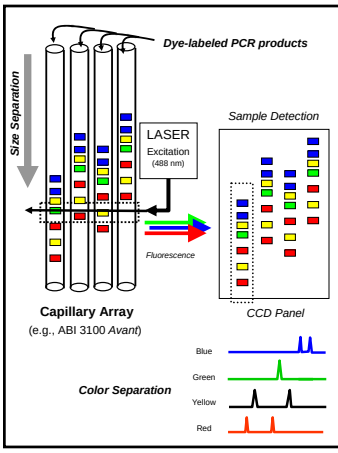
Multiplex by Dye Color





Multiplex by Number of Capillaries

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



From Butler, J.M. and Vallone, P.M. (2004) High-throughput genetic analysis through multiplexed PCR and multicapillary electrophoresis. *PCR Technologies: Current Innovations* (2nd edition), Weissensteiner, T., Griffin, H.G., Griffin, A. (Eds.), CRC Press: Boca Raton, Chapter 11, pp 111-120.

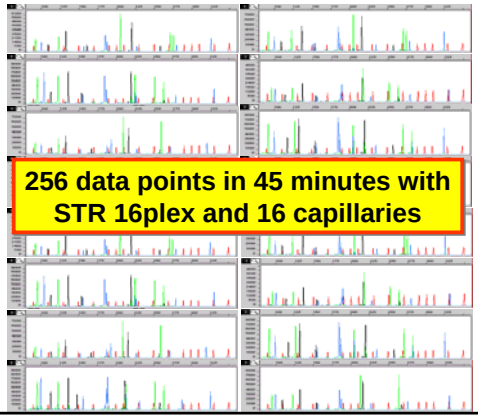
Capillary Array Electrophoresis

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

16 Capillary 3100

6 foot Table on wheels

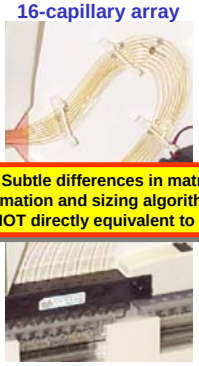
High-Throughput STR Typing on the ABI 3100 (16-capillary array)



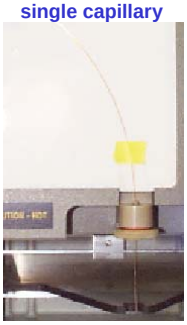
256 data points in 45 minutes with STR 16plex and 16 capillaries

Increasing Sample Throughput with Parallel Processing

ABI 3100 16-capillary array

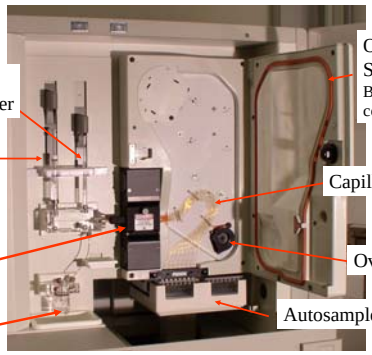


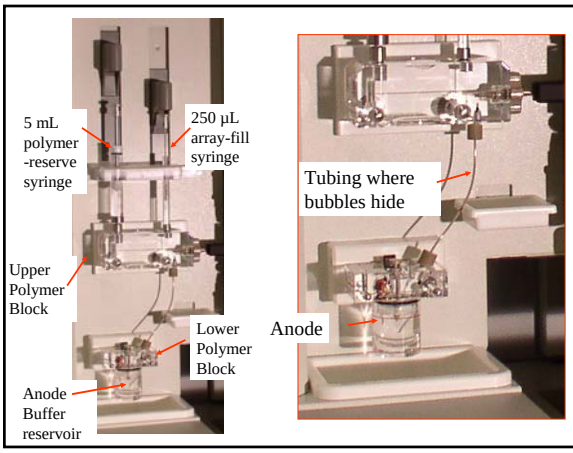
ABI 310 single capillary

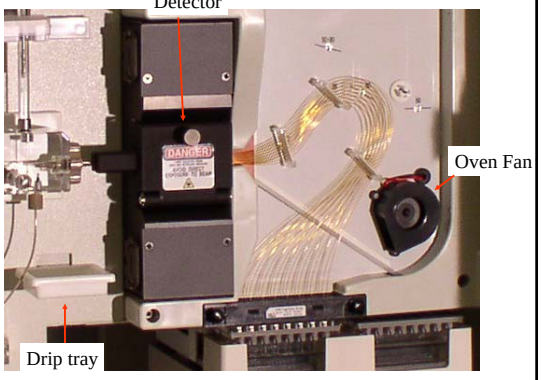


Subtle differences in matrix formation and sizing algorithms – NOT directly equivalent to 310

Inside the 3100

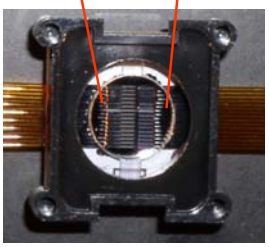


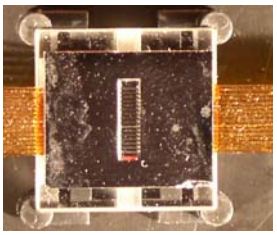




ABI 3100 Array Detection

16 Capillary Array detection cell

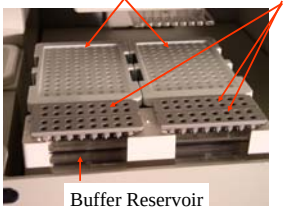




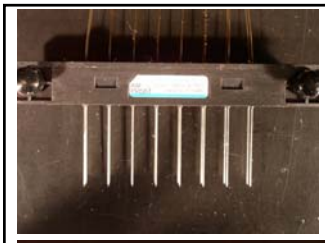
Two 96 well plates on the autosampler

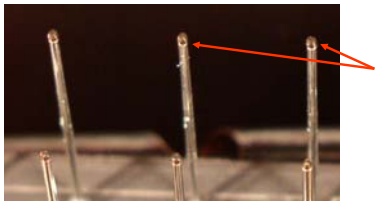
At 45-60 minutes per run two plates represent 12 runs or ~10-12 hours for 192 samples

Sample Plates DI Water Reservoirs



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



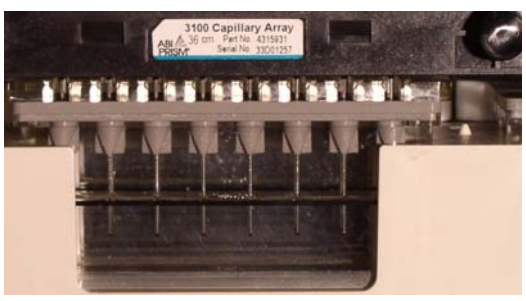


16 Capillary Array

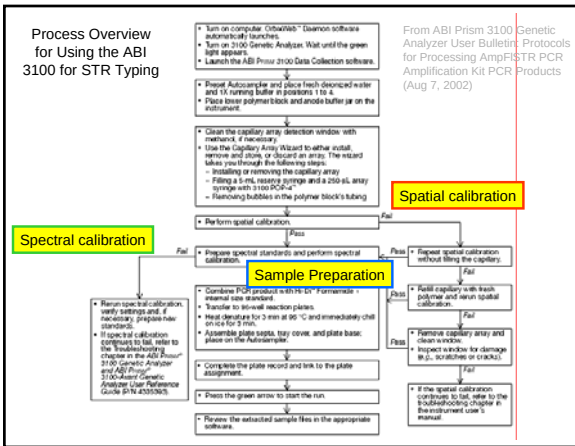
Capillaries are inside of the cathodes (-)

Capillaries in buffer tank

Running and storage position



Process Overview for Using the ABI 3100 for STR Typing



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

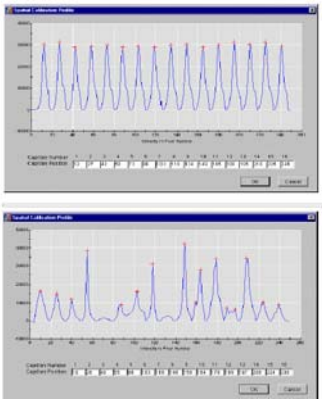
Spatial Calibration

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

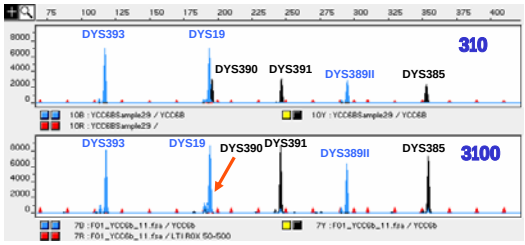
- Syringe – leaks cause capillary to not fill properly
- Capillary storage & wash – **it dries, it dies!**
- Pump block – cleaning helps insure good fill
- Change the running buffer regularly

YOU MUST BE CLEAN AROUND A CE!

Spectral Calibration

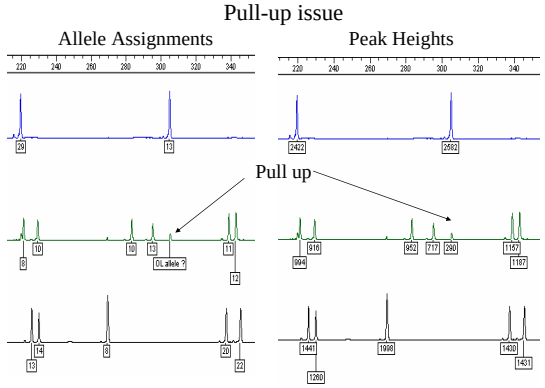
- Performed:
  - New dye set on the instrument
  - After Laser or CCD camera has been realigned
  - You begin to see a decrease in the spectral separation (pull-up, pull-down).
- You must have a valid separation matrix on the instrument prior to running samples.

Use of the Correct Matrix is Critical

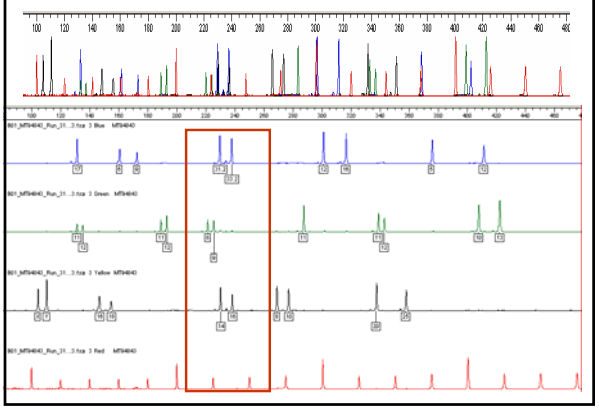


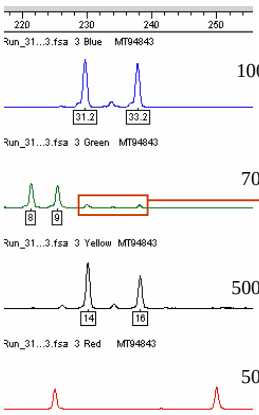
This figure shows the same sample, amplified with the Reliagene Y-Plex™ 6 kit, injected into both the 310 and the 3100. Note that the DYS390 allele, which is labeled with TAMRA as the yellow dye, does not show up in the 3100 result. The matrix used in this case contained NED rather than TAMRA. Thus, if the matrix for the particular dye combination is not established properly, peaks can disappear.

Pull-up issue

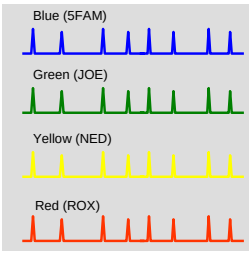


Powerplex 16 data



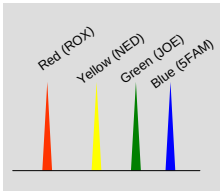


(A) **ABI 310 Matrix Samples**



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

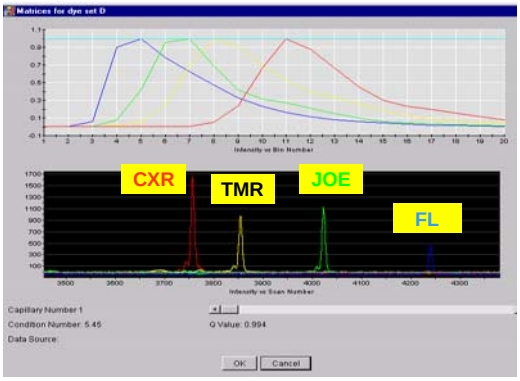
(B) **ABI 3100 Matrix (Spectral Calibration) Sample**



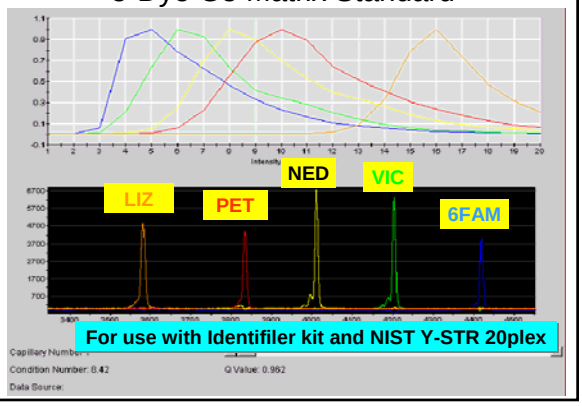
Single sample run containing all dye colors
Only one peak per dye color
Injected into each capillary of the array

A separate spectral calibration file is created for each capillary

Defining the Matrix on the ABI 3100



5-Dye G5 Matrix Standard



For use with Identifiler kit and NIST Y-STR 20plex

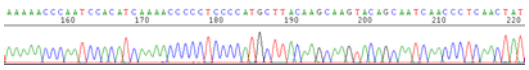
Matrices Created on NIST ABI 3100 with Various Dye Combinations

		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

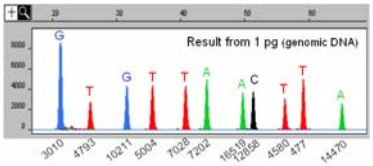
Different ABI 3100 matrix sets used at NIST in order to address a variety of applications and dye combinations.

Other Applications with ABI 3100 and POP6 besides STR Typing

mtDNA Sequencing (HV1)

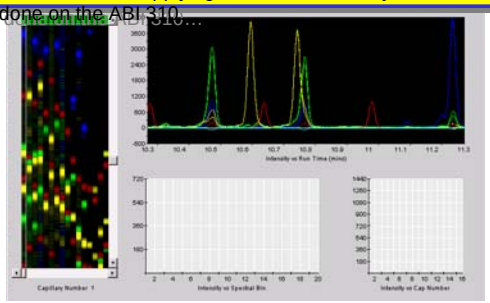


SNaPshot SNP Typing
(Coding Region mtSNP 11plex minisequencing assay)

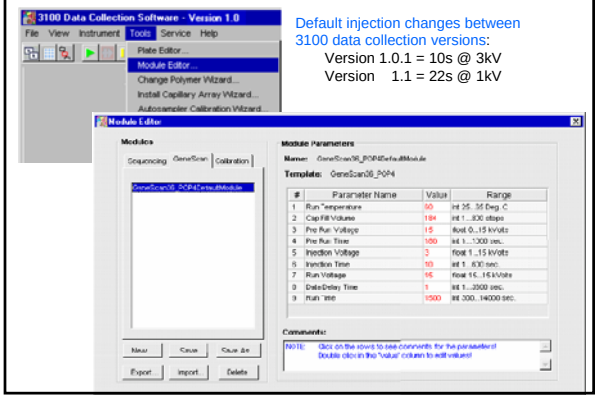


Data from ABI 3100 During the Run

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

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

Consumables

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

Microchip CE Systems

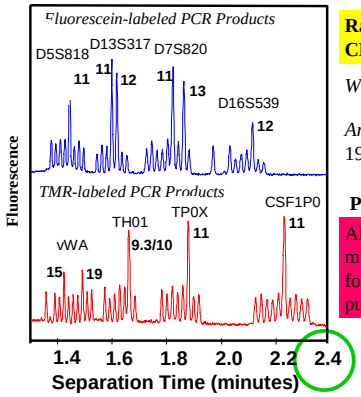
What is under development for STR typing?

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.

CE Microchips

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



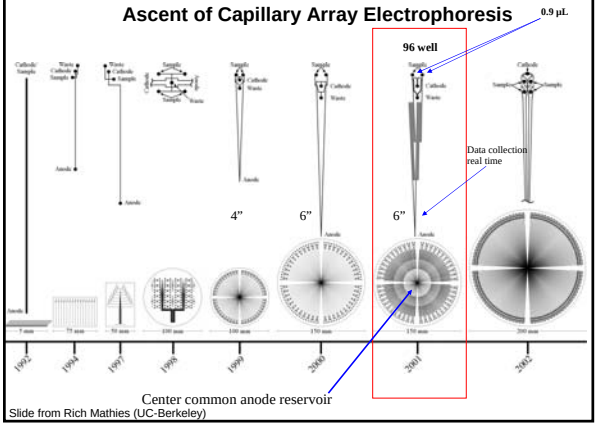
Rapid Microchip CE Separation

Whitehead Institute

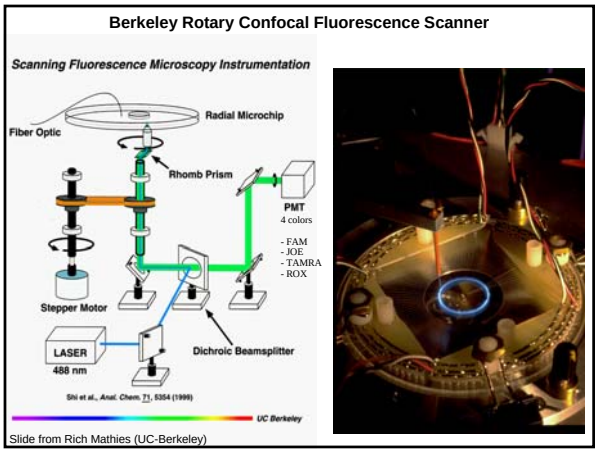
Anal. Biochem. 1999, *270*, 148-152

PowerPlex™ 1.1

Allelic ladders mixed with samples for genotyping purposes



Slide from Rich Mathies (UC-Berkeley)



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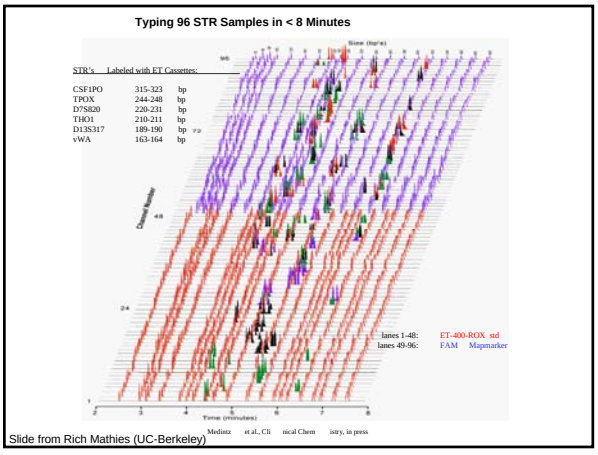
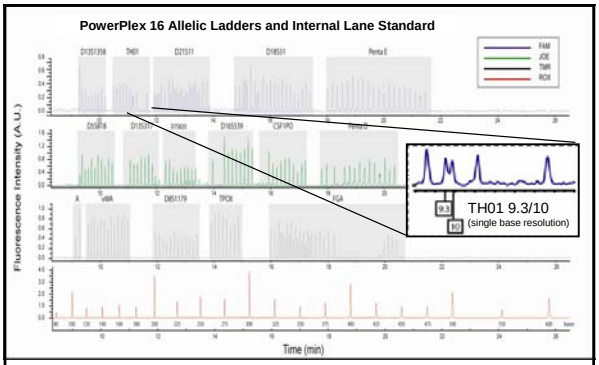


Slide from Rich Mathies (UC-Berkeley)

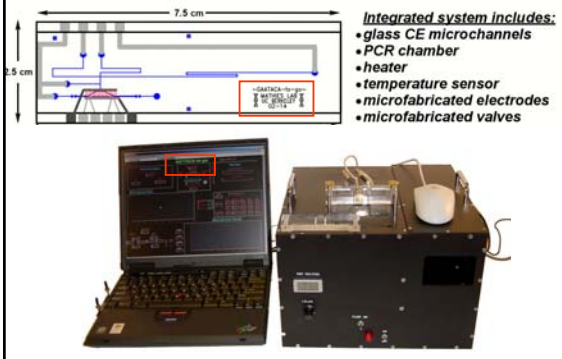
PowerPlex 16 Allelic Ladders and Internal Lane Standard



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

Slide from Rich Mathies (UC-Berkeley)

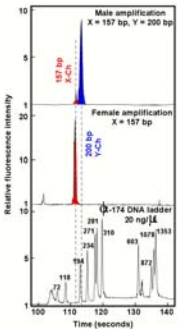
Portable Genetic Diagnostics Device



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

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

Sex Determination PCR from human buccal cells



Multiplex PCR *directly* from human buccal cells without DNA extraction

Hotstart PCR protocol activates polymerase and lyses cells

20 cycles PCR after hotstart

Short "clean-up" injection removes debris, followed by diagnostic injection

Female genotype results in a single 157-bp product

Male genotype results in a 157-bp product (X) and a 200-bp product (Y)

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

15 minutes for PCR amplification and detection

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

Virginia DNA Testing of Felon Arrestees

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

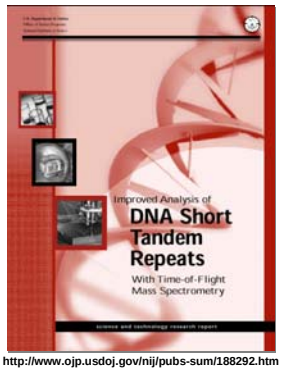
Since January 2003

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

Time-of-Flight Mass Spectrometry

Why it will not become widely used…

Recent NIJ Publication

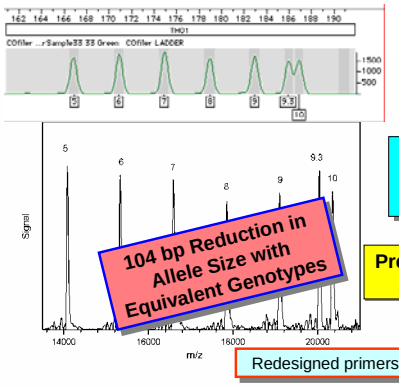


http://www.ojp.usdoj.gov/nij/pubs-sum/188292.htm

Final Report for NIJ Grant 97-LB-VX-0003 (work done at GeneTrace Systems Inc.)

- Describes new primer sets that are close to the STR repeat regions
- Many of these primers are being used in miniplex STR assays under development
- Y SNP multiplex primer sets are described
- 10plex mtSNP assay for HV1 and HV2 detailed

TH01 Alleles: CE vs. Mass Spec



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

Mass Spec Result
9.3 allele: 203.3 μsec
10 allele: 204.8 μsec

Presented at ISFG 1999 Meeting

Redesigned primers for mass spec work

Timing for Data Collection

Laser pulse (10 nsec) → Wait (500 nsec) → Turn on voltages for ion optics (+20 kV) → Extract DNA ions → Collect spectrum for ~300,000 nsec → Turn off voltages

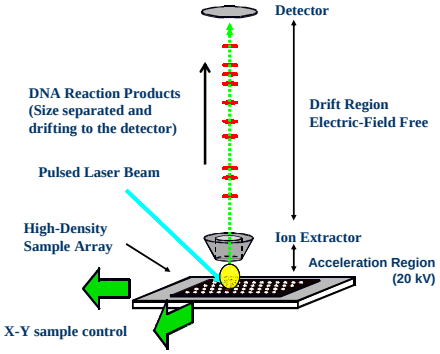
REPEAT process 100+ times

All this occurs in less than 5 seconds per sample

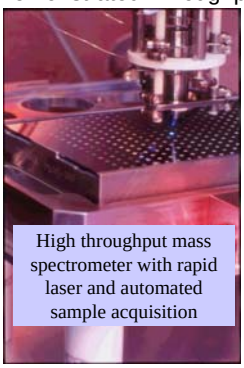
Sum multiple spectra into final sample spectrum

Data processing and genotype determination

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



Demonstrated Throughput at GeneTrace Systems



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

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

Improvements in Information Throughput with Multiplexed Markers and Multiple Capillaries

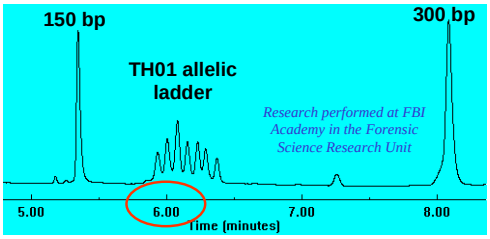
Time required to obtain each genotype…

	Single capillary (ABI 310)	16 capillary array (ABI 3100)	96 capillary array (ABI 3700)
	Each run: 30 min	**Each run: 45 min**	**Run: 2 h 46 min**
#Markers Multiplexed	1800 s (per capillary)	2700 s (per capillary)	9960 s (per capillary)
1	1800 s (30 min)	169 s (2.8 min)	104 s (1.7 min)
8	225 s	21 s	13 s
16	113 s	10.5 s	**6.5 s**

From Butler, J.M. and Vallone, P.M. (2004) High-throughput genetic analysis through multiplexed PCR and multicapillary electrophoresis. *PCR Technologies: Current Innovations* (2nd edition), Weissensteiner, T., Griffin, H.G., Griffin, A. (Eds.), CRC Press: Boca Raton, Chapter 11, pp 111-120.

Technology Implementation Takes Time

First Rapid STR Typing with Capillary Electrophoresis



Performed in December 1993

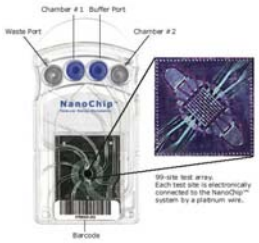
FBI did not start running casework samples using STRs and CE until January 1999

Where is the Future Going?…

Miniaturization and Portability

Palm Pilot
(handheld computer)

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



NanoChip™ from Nanogen
(Miniature Bioassay Device)