Capillary Electrophoresis in DNA Analysis

Stats and Higher Throughput Approaches

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Outline for Workshop

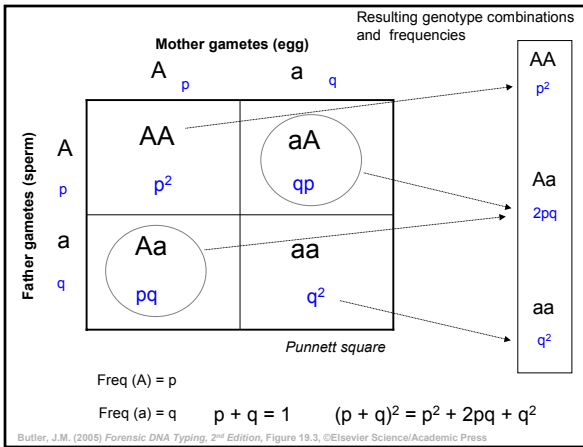
- Introductions
- STR Analysis
- Introduction to CE and ABI 310
- Validation and Interlaboratory Studies
- DNA Quantitation by Real-time PCR and miniSTRs
- Stats and Higher Throughput Approaches
- Y-Chromosome Analysis
- Troubleshooting the ABI 310
- Review and Test

Statistics

How Statistical Calculations are Made

- Generate data with set(s) of samples from desired population group(s)
  - Generally only 100-150 samples are needed to obtain reliable allele frequency estimates
- Determine allele frequencies at each locus
  - Count number of each allele seen
- Allele frequency information is used to estimate the rarity of a particular DNA profile
  - Homozygotes (p^2), Heterozygotes (2pq)
  - Product rule used (multiply locus frequency estimates)

For more information, see Chapters 20 and 21 in *Forensic DNA Typing*, 2nd Edition



Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 19.3, ©Elsevier Science/Academic Press

Assumptions with Hardy-Weinberg Equilibrium

The Assumption	The Reason
Large population	Lots of possible allele combinations
No natural selection	No restriction on mating so all alleles have equal chance of becoming part of next generation
No mutation	No new alleles being introduced
No immigration/emigration	No new alleles being introduced or leaving
Random mating	Any allele combination is possible

None of these assumptions are really true…

Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Table 20.6, ©Elsevier Science/Academic Press

Individual Genotypes Are Summarized and Converted into Allele Frequencies

Genotype Array	8	9	10	11	12	13	14	15		Allele Count	Observed Frequency
	8,8	8,9	8,10	8,11	8,12	8,13	8,14	8,15			
8	9	9	1	17	13	10	0	0	8	68	0.11258
		9,9	9,10	9,11	9,12	9,13	9,14	9,15			
9		1	2	15	10	4	3	0	9	45	0.07450
			10,10	10,11	10,12	10,13	10,14	10,15			
10			2	12	6	3	2	1	10	31	0.05132
				11,11	11,12	11,13	11,14	11,15			
11				37	54	21	12	0	11	205	0.33940
					12,12	12,13	12,14	12,15			
12					21	18	7	0	12	150	0.24834
						13,13	13,14	13,15			
13						7	5	0	13	75	0.12417
							14,14	14,15			
14							0	0	14	29	0.04801
								15,15			
15								0	15	1	0.00166
										604	

The **11,12** genotype was seen **54 times** in 302 samples (604 examined chromosomes)

Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Table 20.2, ©Elsevier Science/Academic Press

Allele Frequency Tables

Allele frequencies denoted with an asterisk (*) are **below the 5/2N minimum allele threshold** recommended by the National Research Council report (NRCII) *The Evaluation of Forensic DNA Evidence* published in 1996.

D3S1358

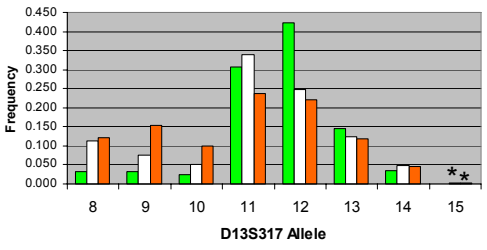
Allele	Caucasian (Butler *et al.* (2003) *JFS* 48(4):908-911) N= 302	Caucasian (Einum *et al.* (2004) *JFS* 49(6)) N= 7,636	Allele	African American N=258	African American N= 7,602
11	0.0017*	0.0009	11	--	0.0003*
12	0.0017*	0.0007	12	--	0.0045
13	--	0.0031	13	0.0019*	0.0077
14	0.1027	0.1240	14	0.0892	0.0905
15	**0.2616**	**0.2690**	15	0.3023	0.2920
15.2	--	--	15.2	0.0019*	0.0010
16	0.2533	0.2430	16	**0.3353**	**0.3300**
17	0.2152	0.2000	17	0.2054	0.2070
18	0.15232	0.1460	18	0.0601	0.0630
19	0.01160	0.0125	19	0.0039*	0.0048
20	0.0017*	0.0001*	20		

Most common allele

Figure 20.3

U.S. Population Samples

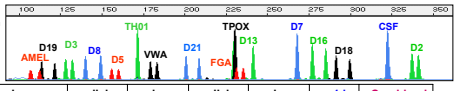
(Appendix II)



Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 20.3, ©Elsevier Science/Academic Press

DNA Profile Frequency with all 13 CODIS STR loci

AmpFISTR® Identifiler™ (Applied Biosystems)



What would be entered into a DNA database for searching: 16,17-17,18-21,22-12,14-28,30-14,16-12,13-11,14-9,9-9,11-6,6-8,8-10,10

Locus	allele	value	allele	value	1 in	Combined
D3S1358	16	*0.2533*	17	*0.2152*	9.17	9.17
VWA	17	*0.2815*	18	*0.2003*	8.87	81
FGA	21	*0.1854*	22	*0.2185*	12.35	1005
D8S1179	12	*0.1854*	14	*0.1656*	16.29	16,364
D21S11	28	*0.1589*	30	*0.2782*	11.31	185,073
D18S51	14	*0.1374*	16	*0.1391*	26.18	4,845,217
D5S818	12	*0.3841*	13	*0.1407*	9.25	44,818,259
D13S317	11	*0.3394*	14	*0.0480*	30.69	1.38 x 10^9
D7S820	9	*0.1772*			31.85	4.38 x 10^{10}
D16S539	9	*0.1126*	11	*0.3212*	13.8	6.05 x 10^{11}
THO1	6	*0.2318*			18.62	1.13 x 10^{13}
TPOX	8	*0.5348*			3.50	3.94 x 10^{13}
CSF1PO	10	*0.2169*			21.28	8.37 x 10^{14}

PRODUCT RULE

The Random Match Probability for this profile in the U.S. Caucasian population is **1 in 837 trillion (10^{12})**

The Same 13 Locus STR Profile in Different Populations

1 in 837 trillion

1 in 0.84 quadrillion (10^{15}) in U.S. Caucasian population (NIST)
1 in 2.46 quadrillion (10^{15}) in U.S. Caucasian population (FBI)*
1 in 1.86 quadrillion (10^{15}) in Canadian Caucasian population*

1 in 16.6 quadrillion (10^{15}) in African American population (NIST)
1 in 17.6 quadrillion (10^{15}) in African American population (FBI)*

1 in 18.0 quadrillion (10^{15}) in U.S. Hispanic population (NIST)

These values are **for unrelated individuals** assuming no population substructure (using only p^2 and 2 pq)

NIST study: Butler, J.M., *et al.* (2003) Allele frequencies for 15 autosomal STR loci on U.S. Caucasian, African American, and Hispanic populations. *J. Forensic Sci.* 48(4):908-911. (http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm)

*http://www.csfs.ca/pplus/profiler.htm

STR Cumulative Profile Frequency with Multiple Population Databases

STR Locus	Profile Computed	Number of Populations Used	Cumulative Profile Frequency Range (1 in ...)	Cumulative Profile Frequency against U.S. Caucasians (Appendix II)
D3S1358	16,17	166	5.24 to 62.6	9.19
VWA	17,18	166	37.6 to 1080	81.8
FGA	21,22	166	737 to 119 000	1010
D8S1179	12,14	166	8980 to 5 430 000	16 400
D21S11	28,30	166	165 000 to 248 000 000	186 000
D18S51	14,16	166	3.85×10^4 to 2.68×10^{10}	4.88×10^4
D5S818	12,13	166	2.28×10^3 to 4.22×10^{11}	4.51×10^3
D13S317	11,14	166	4.32×10^4 to 1.69×10^{13}	1.38×10^4
D7S820	9,9	166	1.17×10^{10} to 2.98×10^{14}	4.22×10^{10}
D16S539	9,11	97	4.06×10^{11} to 1.11×10^{18}	5.82×10^{11}
TH01	6,6	97	9.30×10^{12} to 1.45×10^{19}	1.05×10^{13}
TPOX	8,8	97	3.33×10^{13} to 1.54×10^{20}	3.63×10^{13}
CSF1PO	10,10	97	3.43×10^{14} to 2.65×10^{21}	7.43×10^{14}

10^{14} to 10^{21}

Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, D.N.A. Box 21.1, ©Elsevier Science/Academic Press

Example Calculations with Population Substructure Adjustments

Table 21.5
Example calculations with NRC II recommendations for population substructure adjustments (see Appendix VI). Scenarios with theta equal to 0.01 and 0.03 are examined.

From U.S. Caucasian (N = 302): Appendix II – sample in database						Under HWE		NRCII Recommendation 4.1			NRCII Recommendation 4.10	
	A1	A2	Allele 1 freq (p)	Allele 2 freq (q)		Calc freq		θ = 0.01	θ = 0.03		θ = 0.01	θ = 0.03
D13S317	11	14	0.33940	0.04801	2pq	0.0326	2pq	0.0326	0.0326	eq. 4.10b	0.0386	0.0504
TH01	6	6	0.23179	—	p^2	0.0537	$p^2 + p(1-p)\,\theta$	0.0555	0.0591	eq. 4.10a	0.0628	0.0821
D16S539	14	16	0.13742	0.12907	2pq	0.0382	2pq	0.0382	0.0382	eq. 4.10b	0.0419	0.0493
D21S11	28	30	0.15694	0.27815	2pq	0.0884	2pq	0.0884	0.0884	eq. 4.10b	0.0927	0.1011
D3S1358	16	17	0.25331	0.21523	2pq	0.1090	2pq	0.1090	0.1090	eq. 4.10b	0.1129	0.1206
D5S818	12	13	0.38411	0.14073	2pq	0.1081	2pq	0.1081	0.1081	eq. 4.10b	0.1131	0.1228
D7S820	9	9	0.17715	—	p^2	0.0314	$p^2 + p(1-p)\,\theta$	0.0328	0.0358	eq. 4.10a	0.0390	0.0556
D8S1179	12	14	0.18543	0.16556	2pq	0.0614	2pq	0.0614	0.0614	eq. 4.10b	0.0654	0.0733
CSF1PO	10	10	0.21689	—	p^2	0.0470	$p^2 + p(1-p)\,\theta$	0.0487	0.0521	eq. 4.10a	0.0558	0.0744
FGA	21	22	0.18543	0.21854	2pq	0.0810	2pq	0.0810	0.0810	eq. 4.10b	0.0851	0.0930
D16S539	9	11	0.11258	0.32119	2pq	0.0723	2pq	0.0723	0.0723	eq. 4.10b	0.0773	0.0871
TPOX	8	8	0.53477	—	p^2	0.2860	$p^2 + p(1-p)\,\theta$	0.2885	0.2934	eq. 4.10a	0.2983	0.3227
VWA	17	18	0.28146	0.20033	2pq	0.1128	2pq	0.1128	0.1128	eq. 4.10b	0.1167	0.1245
AMEL	X	Y										
						1.20E-15		1.35E-15	1.70E-15		3.92E-15	3.02E-14

Example Calculations with Corrections for Relatives

Table 21.6
Example calculations with corrections for relatives using the NRC II recommended formula.

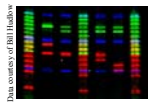
From U.S. Caucasian (N = 302): Appendix II – sample in database					Under HWE		NRCII Recommendation 4.4				
	A1	A2	Allele 1 freq (p)	Allele 2 freq (q)	Calc freq		F = 1/4 (parent)	F = 1/8 (half sib)	F = 1/16 (1st cousin)		Full sib
D13S317	11	14	0.33940	0.04801	2pq 0.0326	eq. 4.8b	0.1937	0.1131	0.0729	eq. 4.9b	0.3550
TH01	6	6	0.23179	—	p^2 0.0537	eq. 4.8a	0.2318	0.1428	0.0982	eq. 4.9a	0.3793
D16S539	9	11	0.11258	0.32119	2pq 0.0723	eq. 4.8b	0.2169	0.1446	0.1085	eq. 4.9b	0.3765
D18S51	14	16	0.13742	0.13907	2pq 0.0382	eq. 4.8b	0.1382	0.0862	0.0632	eq. 4.9b	0.3287
D21S11	28	30	0.15894	0.27815	2pq 0.0884	eq. 4.8b	0.2185	0.1535	0.1209	eq. 4.9b	0.3814
D3S1358	16	17	0.25331	0.21523	2pq 0.1090	eq. 4.8b	0.2343	0.1717	0.1403	eq. 4.9b	0.3944
D5S818	12	13	0.38411	0.14073	2pq 0.1081	eq. 4.8b	0.2624	0.1853	0.1467	eq. 4.9b	0.4082
D7S820	9	9	0.17715	—	p^2 0.0314	eq. 4.8a	0.1772	0.1043	0.0678	eq. 4.9a	0.3464
D8S1179	12	14	0.18543	0.14556	2pq 0.0614	eq. 4.8b	0.1755	0.1184	0.0899	eq. 4.9b	0.3531
CSF1PO	10	10	0.21689	—	p^2 0.0470	eq. 4.8a	0.2169	0.1320	0.0895	eq. 4.9a	0.3702
FGA	21	22	0.18543	0.21854	2pq 0.0810	eq. 4.8b	0.2020	0.1415	0.1113	eq. 4.9b	0.3713
TPOX	8	8	0.53477	—	p^2 0.2860	eq. 4.8a	0.5348	0.4104	0.3482	eq. 4.9a	0.5889
VWA	17	18	0.28146	0.20033	2pq 0.1128	eq. 4.8b	0.2409	0.1768	0.1448	eq. 4.9b	0.3986
AMEL	X	Y									
					1.20E-15		3.17E-09	1.68E-11	3.74E-13		4.04E-06 1 in 247 616

Capillary Arrays and Higher Throughput STR Typing

STR Typing Technologies

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

Gels



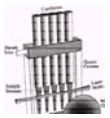
Data courtesy of Bill Hudlow

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

Capillary Electrophoresis

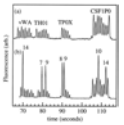
Electrophoresis. (1998) 19: 86-93

Capillary Arrays



Nucleic Acids Res. (1999) 27: e36

Microchip CE



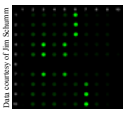
PNAS (1997) 94: 10273-10278

Mass Spectrometry

www.sequenom.com

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

Hybridization Arrays



Data courtesy of Jim Schumm

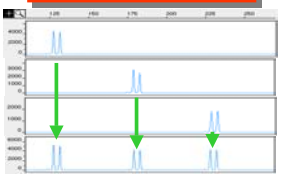
Nucleic Acids Res. (2000) 28: e17

Ways to Increase Sample Throughput

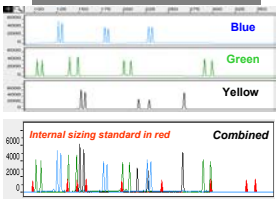
- Run more gels (FMBIO approach)
- Increase speed of single sample analysis (microchip CE systems)
- Multiplex fluorescent dyes of different colors (higher level PCR multiplexes)
- Parallel separations using capillary arrays
- New Detection Technologies (MALDI-TOF mass spectrometry)

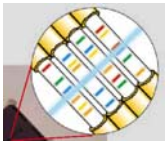
Methods for Parallel Sample Processing

Multiplex by Size



Multiplex by Dye Color



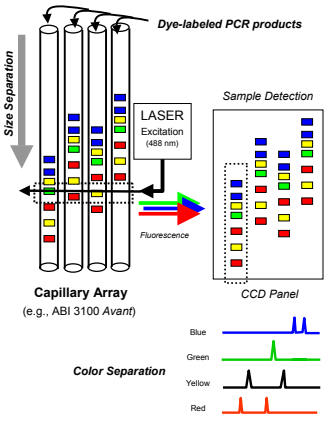


Multiplex by Number of Capillaries

ABI 3100/3130: 16 capillaries
ABI 3700/3730: 96 capillaries
ABI 3100 *Avant*: 4 capillaries

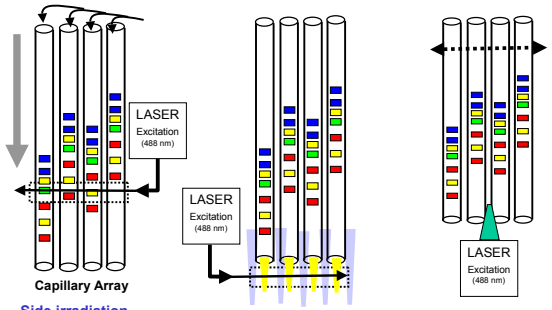
Capillary Array Electrophoresis

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



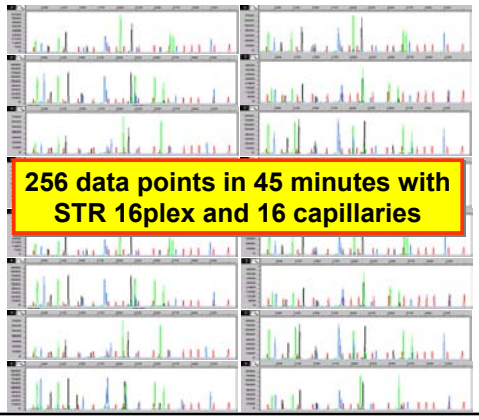
From Butler, J.M. and Vallone, P.M. (2004) High-throughput genetic analysis through multiplexed PCR and multicapillary electrophoresis. *PCR Technologies: Current Innovations* (2nd edition), Weissensteiner, T., Griffin, H.G., Griffin, A. (Eds.), CRC Press: Boca Raton, Chapter 11, pp 111-120.

Irradiation for Capillary Array Detection



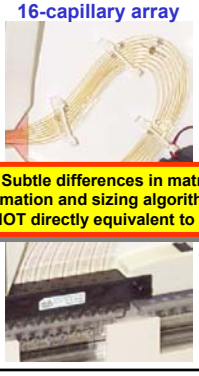
16 Capillary 3100

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

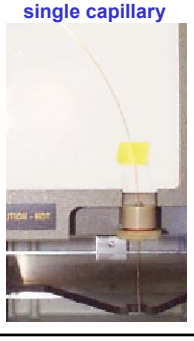


Increasing Sample Throughput with Parallel Processing

ABI 3100
16-capillary array

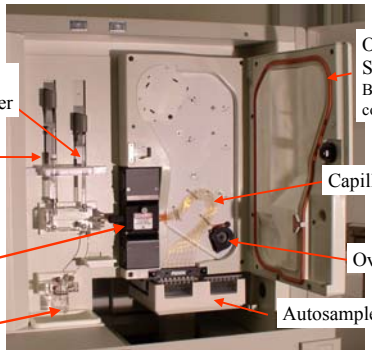


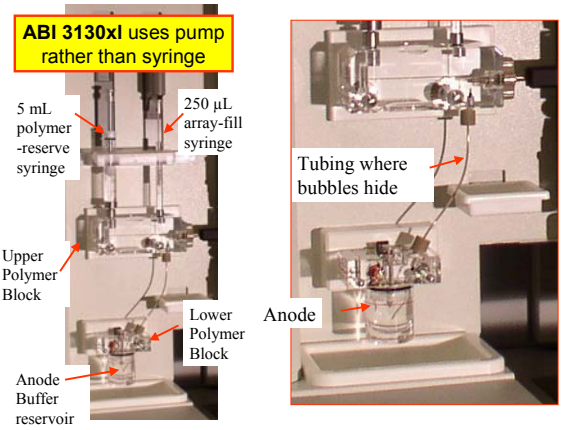
ABI 310
single capillary

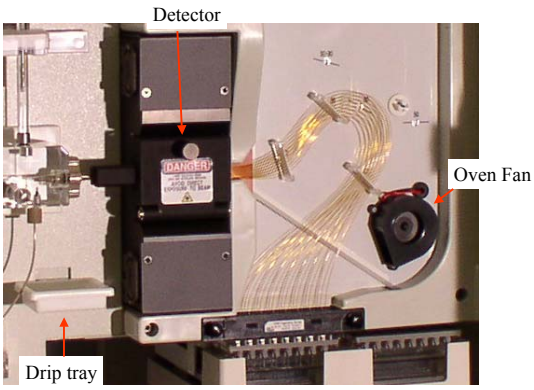


Subtle differences in matrix formation and sizing algorithms – NOT directly equivalent to 310

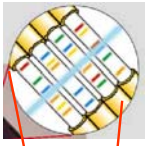
Inside the 3100



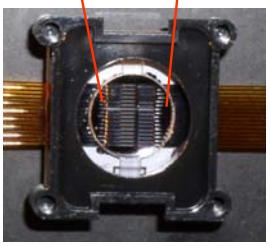


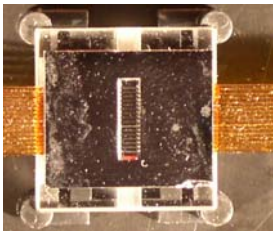


ABI 3100 Array Detection



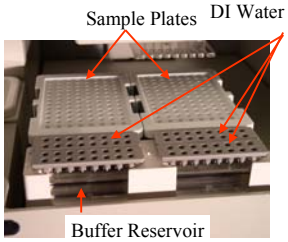
16 Capillary Array detection cell

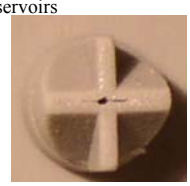




Two 96 well plates on the autosampler

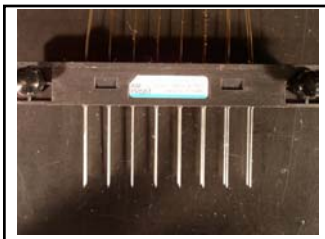
At 45-60 minutes per run two plates represent 12 runs or ~10-12 hours for 192 samples

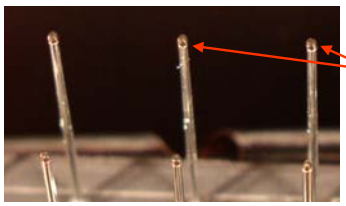




Rubber septa wear.
They must be replaced when the edges are ragged..

16 Capillary Array

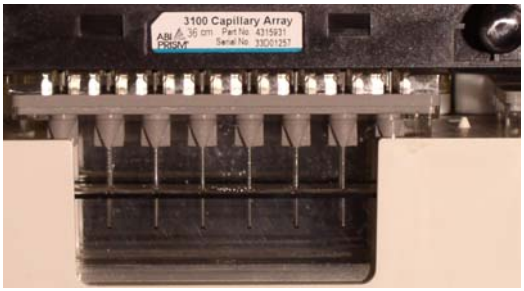




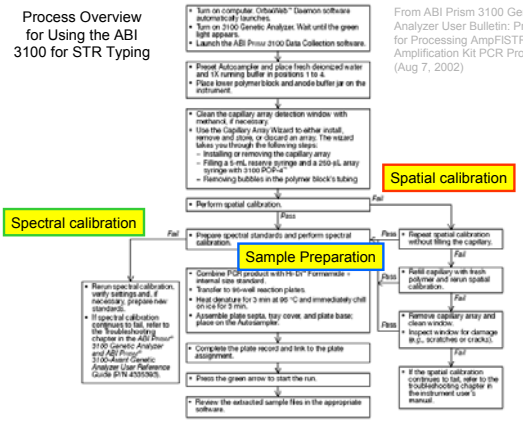
Capillaries are inside of the cathodes (-)

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 AmpFISTR PCR Amplification Kit PCR Products (Aug 7, 2002)

Spatial Calibration

Performed after:

Installing or replacing a capillary array

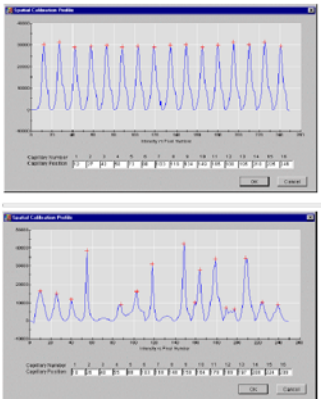
Removal of the array from the detection block,

(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

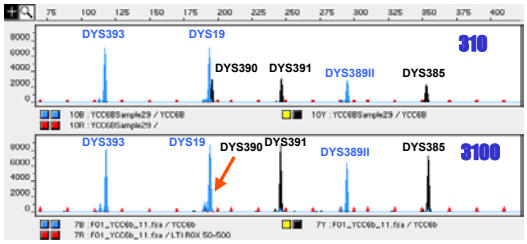
- Syringe – leaks cause capillary to not fill properly
- Capillary storage & wash – **it dries, it dies!**
- Pump block – cleaning helps insure good fill
- Change the running buffer regularly

YOU MUST BE CLEAN AROUND A CE!

Spectral Calibration

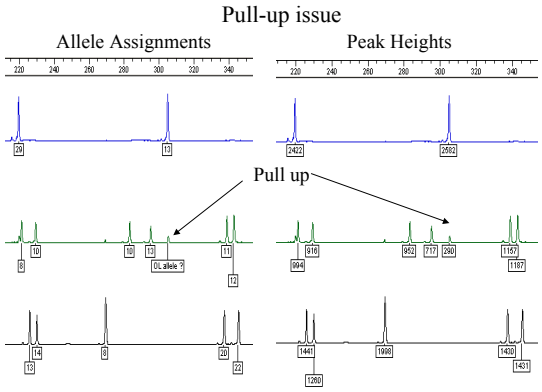
- Performed:
  - New dye set on the instrument
  - After Laser or CCD camera has been realigned
  - You begin to see a decrease in the spectral separation (pull-up, pull-down).
- You must have a valid separation matrix on the instrument prior to running samples.

Use of the Correct Matrix is Critical

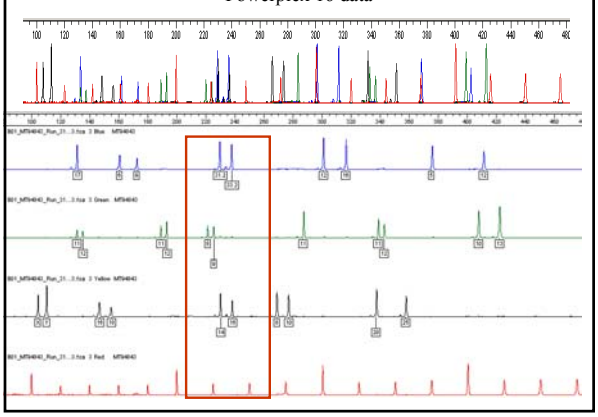


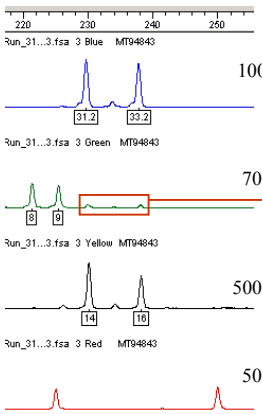
This figure shows the same sample, amplified with the Reliagene Y-Plex™ 6 kit, injected into both the 310 and the 3100. Note that the DYS390 allele, which is labeled with TAMRA as the yellow dye, does not show up in the 3100 result. The matrix used in this case contained NED rather than TAMRA. Thus,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

Pull-up issue



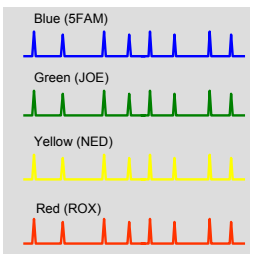
Powerplex 16 data





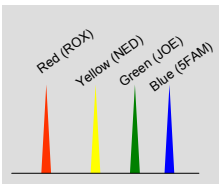
Time for a new matrix

(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

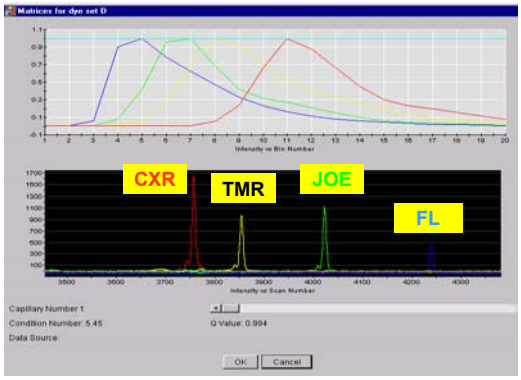
(B) ABI 3100 Matrix (Spectral Calibration) Sample



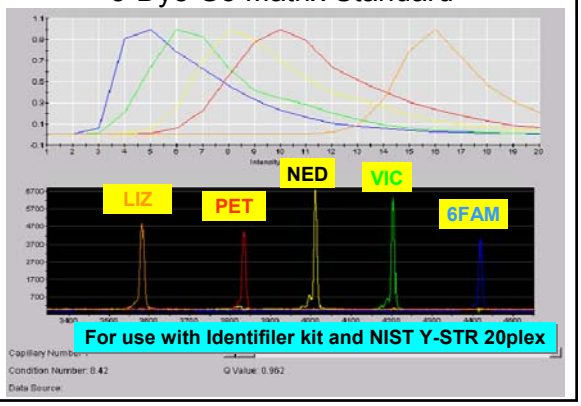
Single sample run containing all dye colors
Only one peak per dye color
Injected into each capillary of the array

A separate spectral calibration file is created for each capillary

Defining the Matrix on the ABI 3100



5-Dye G5 Matrix Standard



Matrices Created on NIST ABI 3100 with Various Dye Combinations

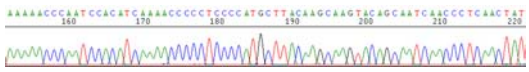
3100 Filters

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

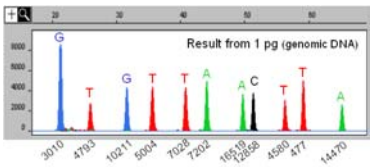
Different ABI 3100 matrix sets used at NIST in order to address a variety of applications and dye combinations.

Other Applications with ABI 3100 and POP6 besides STR Typing

mtDNA Sequencing (HV1)

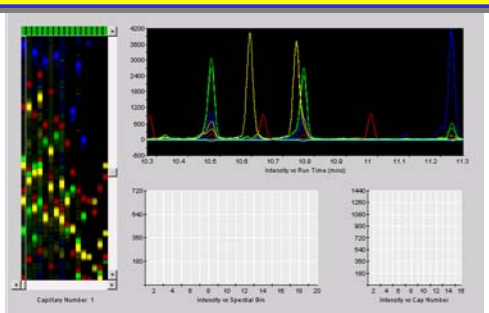


SNaPshot SNP Typing
(Coding Region mtSNP 11plex minisequencing assay)

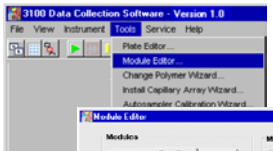


Data from ABI 3100 During the Run

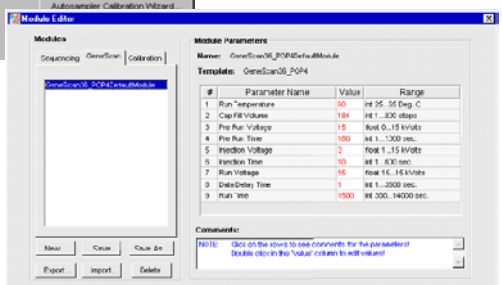
Matrix is applied during the data collection so if there is a problem, the sample must be REINJECTED **after** a new matrix is applied rather than applying a new matrix to any raw data as can be done on the ABI 310…



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

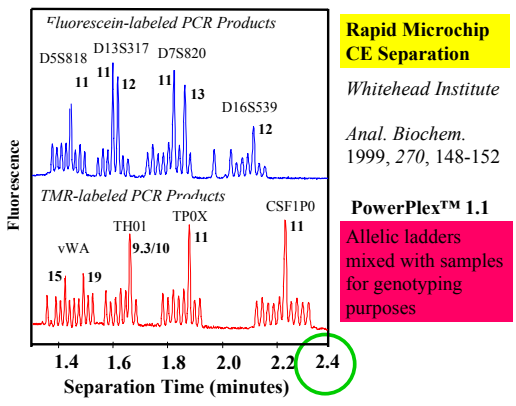
What is under development for STR typing?

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



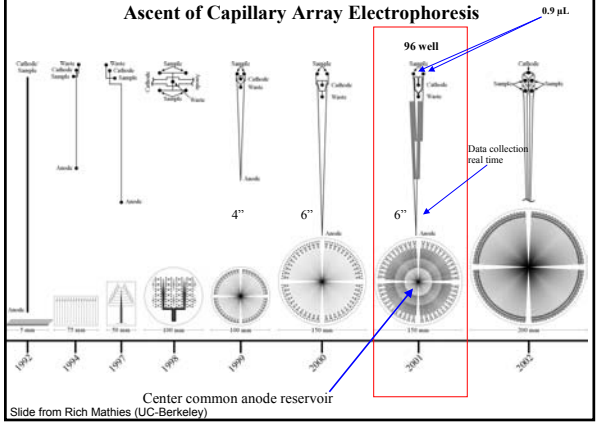
Rapid Microchip CE Separation

Whitehead Institute

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

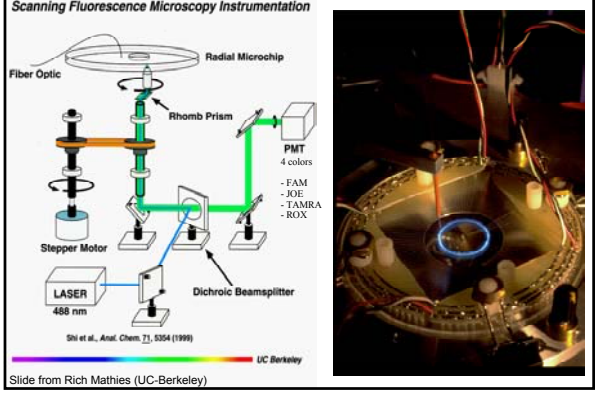
PowerPlex™ 1.1

Allelic ladders mixed with samples for genotyping purposes



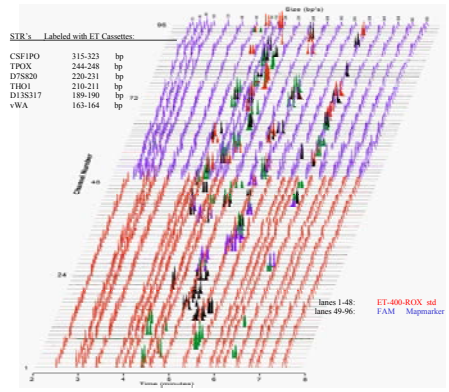
Slide from Rich Mathies (UC-Berkeley)

Berkeley Rotary Confocal Fluorescence Scanner



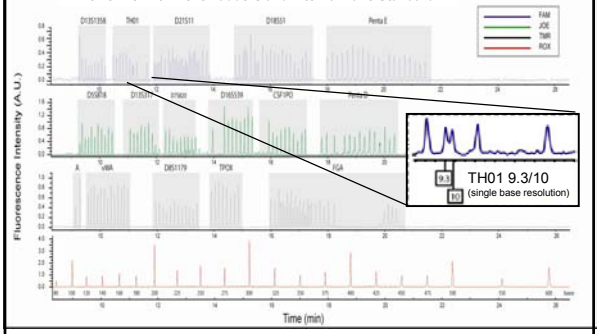
Slide from Rich Mathies (UC-Berkeley)

Typing 96 STR Samples in < 8 Minutes



Slide from Rich Mathies (UC-Berkeley)

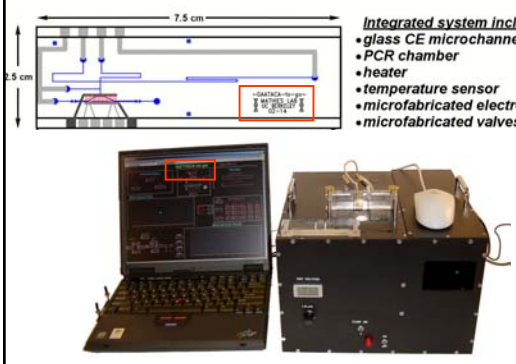
PowerPlex 16 Allelic Ladders and Internal Lane Standard



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

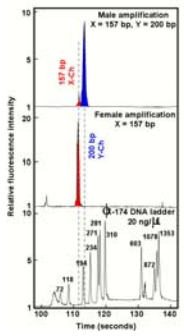
Slide from Rich Mathies (UC-Berkeley)

Portable Genetic Diagnostics Device



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).

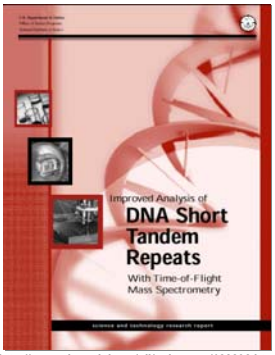
Since January 2003

- 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

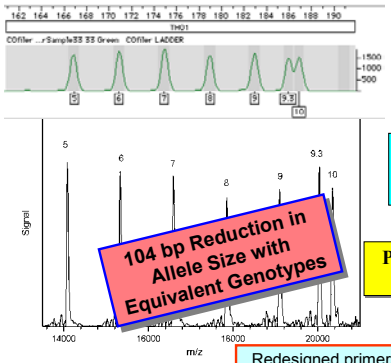


http://www.ojp.usdoj.gov/nij/pubs-sum/188292.htm

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

104 bp Reduction in Allele Size with Equivalent Genotypes

Presented at ISFG 1999 Meeting

Redesigned primers for mass spec work

Timing for Data Collection

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

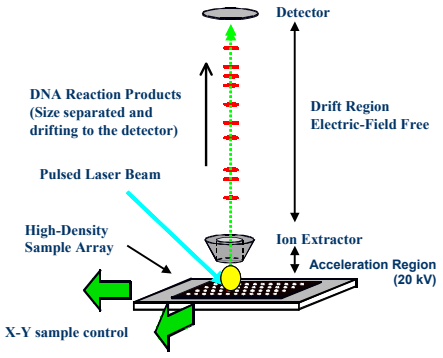
REPEAT process 100+ times

All this occurs in less than 5 seconds per sample

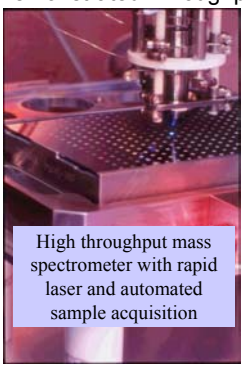
Sum multiple spectra into final sample spectrum

Data processing and genotype determination

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



Demonstrated Throughput at GeneTrace Systems



High throughput mass spectrometer with rapid laser and automated sample acquisition

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

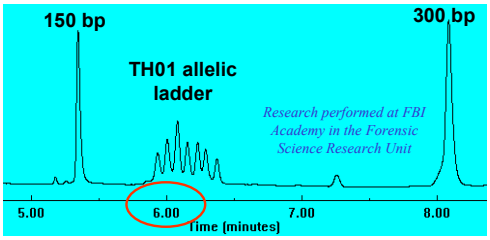
Time required to obtain each genotype…

	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

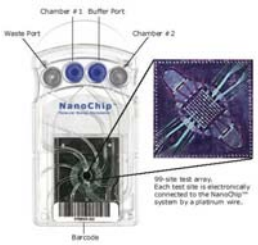
FBI did not start running casework samples using STRs and CE until January 1999

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)