National Institute of Standards and Technology
. . .working with industry to develop and apply technology, measurements and standards

MiniSTR Development
to Aid Testing of Degraded Samples

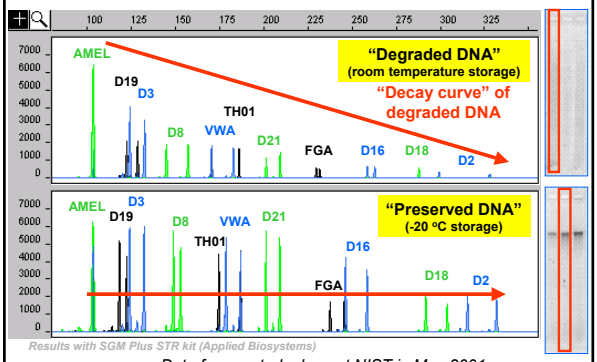
John M. Butler

February 22, 2002
NIJ's 3rd WTC Kinship and DNA Analysis Panel
Albany, New York

john.butler@nist.gov

Comparison of DNA Profiles from Aged Blood Stains

Yield gel results



Data from a study done at NIST in May 2001

Presentation Overview

Development of miniSTRs
- **Validation**
- **Quality Control**
- **Sample Requirements**
- **Potential Pitfalls**
- **Future Plans**

- Mutation Rates
- Statistical Interpretation of Data
- Inconsistencies with Traditional Testing Systems
- Incorporation with Current Systems

Development of miniSTRs

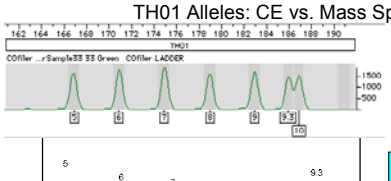
STR Size Reduction
Through Moving Primer Positions Closer to Repeat

Primer positions define PCR product size
Repeat information is independent of amplicon size

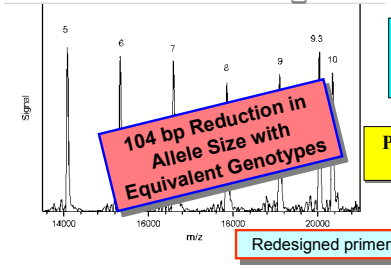
Advantages of Approach:
Size reduction enhances success rate with degraded DNA
Retains same marker information (database compatibility)
Uses highly polymorphic STR loci (high discriminatory power)

Development of miniSTRs: Past Work

TH01 Alleles: CE vs. Mass Spec



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

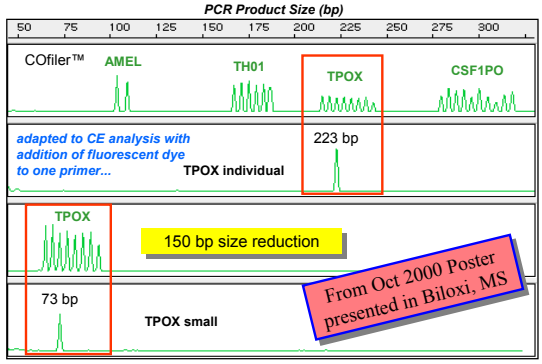


Mass Spec Result
9.3 allele: 203.3 μsec
10 allele: 204.8 μsec

Presented at ISFG 1999 Meeting

Redesigned primers for mass spec work

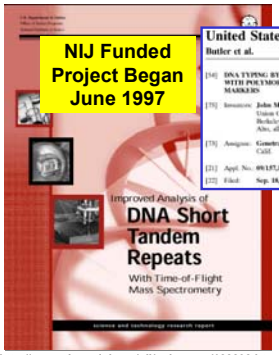
Development of miniSTRs: Past Work



New primer sets are intended to aid with typing degraded DNA samples as well as future microchip CE and mass spectrometry applications…

Development of miniSTRs: Past Work

Publication of miniSTR Primer Sequences



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

Most of the new miniplex primer sequences have already been described in the NIJ report and US Patent 6,090,558 (originally designed for use with STR typing by mass spectrometry)

Selected References on STRs with Degraded DNA

- Whitaker, J.P., et al. (1995) Short tandem repeat typing of bodies from a mass disaster: high success rate and characteristic amplification patterns in highly degraded samples. *BioTechniques* 18: 670-677
- Clayton, T.M., et al. (1995) Further validation of a quadruplex STR DNA typing system: a collaborative effort to identify victims of a mass disaster. *Forensic Sci.Int.* 76: 17-25
- Yoshida, K., et al. (1997) Evaluation of new primers for CSF1PO. *Int.J.Legal Med.* 110: 36-38
- Schmerer, W.M., et al. (1999) Optimized DNA extraction to improve reproducibility of short tandem repeat genotyping with highly degraded DNA as target. *Electrophoresis* 20: 1712-1716
- Wiegand, P. and Kleiber, M. (2001) Less is more--length reduction of STR amplicons using redesigned primers. *Int.J.Legal Med.* 114: 285-287

Smaller PCR product size improves success rates with degraded DNA

Development of miniSTRs

We were in a unique position to do this project

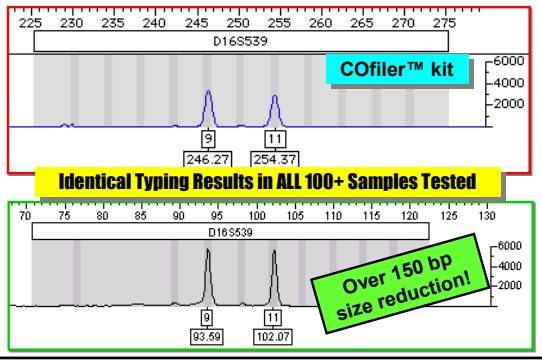
- **NIJ-funding** at NIST and Ohio University
- Previous work with TOF-MS to make STRs smaller
- Knowledge of CODIS STR systems from writing book
- Information on variant and null alleles because of maintaining **STRBase** web site
- Current efforts to rapidly develop multiplex assays
- Improved primer design software and strategies
- Quality control capabilities for PCR primers using TOF-MS and HPLC
- Knowledge of fluorescence dyes and CE
- Genotyper macro writing experience

Development of miniSTR Assays

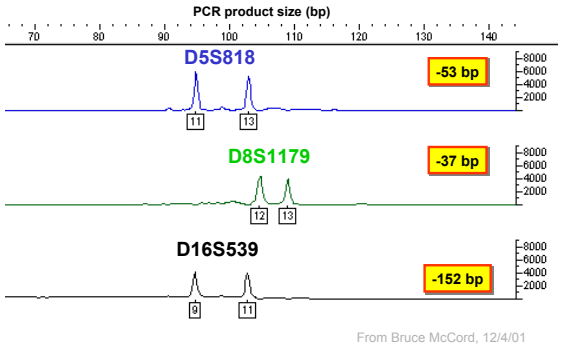
- Project begun in November 2001 at the request of Bob Shaler to aid WTC DNA identifications
- Primers designed to come as close as possible to the repeat region to generate the smallest possible PCR products
- Smaller amplicons offer improved chances of success with degraded DNA samples
- Available as singleplexes or miniplexes (usually one locus per dye color)
- Testing has been performed to demonstrate equivalent genotypes are produced compared with commercial STR kits (*developmental validation*)

Development of miniSTRs: Validation

Demonstrating Equivalent Genotypes with new miniSTRs



Early Miniplex 2 Results



From Bruce McCord, 12/4/01

Development of miniSTRs

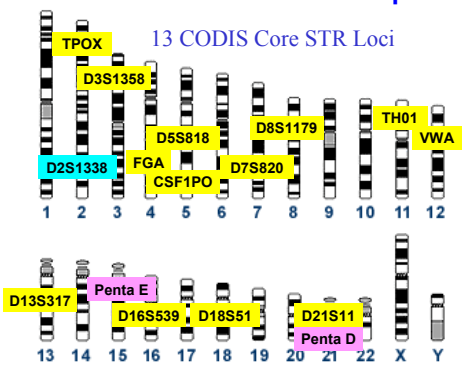
Steps for miniSTR Development

- Design new primers close to repeat
- Purchase and QC primers
- Develop and standardize PCR conditions
- Create allelic ladders
- Develop Genotyper macros
- Test set of samples with new primers versus commercial STR kits for concordance in calls
- Write up protocols

Testing being performed in 2 independent laboratories (collaboration with Bruce McCord, Ohio University)

MiniSTR Markers Developed

13 CODIS Core STR Loci



Development of miniSTRs

Miniplex Primer Sets

Dye combinations were chosen because matrix is commercially available and works well on ABI 310/3100

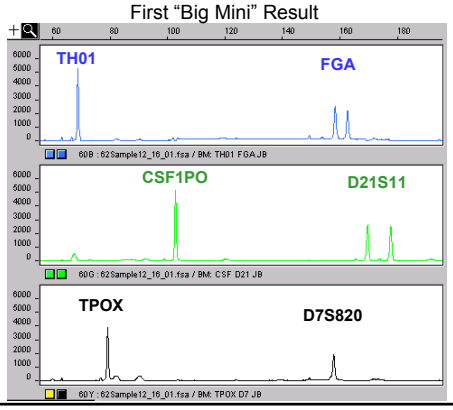
	6FAM	VIC	NED	PET
Miniplex 1	TH01	CSF1PO	TPOX	D3S1358
Miniplex 2	D5S818	D8S1179	D16S539	Penta D
Miniplex 3	FGA	D21S11	D7S820	Penta E
Miniplex 4	VWA	D18S51	D13S317	D2S1338
Miniplex 5	Penta D	Penta E	D2S1338	
"Big Mini"	TH01, FGA	CSF, D21	TPOX, D7	

Only Big Mini supplied to OCME

Testing can be performed in 4-dye or 5-dye combinations using either ROX or LIZ labeled internal size standards

Development of miniSTRs

First "Big Mini" Result



Development of miniSTRs

Primer Sequences for Big Mini STR System

Locus		Big Mini Primer Sequences (5'-3')	Primer Conc.	Distance 3'end from STR repeat
TH01	F	6FAM-CCTGTTCCTCCCTTATTTCCC	1 µM	0
	R	*GTTTCTT*GGGAACACAGACTCCATGGTG	1 µM	1
FGA	F	6FAM-AAATAAAATTAGGCATATTTACAAGC	1 µM	3
	R	GCTGAGTGATTTGTCTGTAATTG	1 µM	23
CSF1PO	F	VIC-ACAGTAACTGCCTTCATAGATAG	0.6 µM	14
	R	GTGTCAGACCCTGTTCTAAGTA	0.6 µM	6
D21S11	F	VIC-ATTCCCCAAGTGAATTGC	1.5 µM	2
	R	GGTAGATAGACTGGATAGATAGACGA	1.5 µM	0
TPOX	F	NED-CTTAGGGAACCCTCACTGAATG	1 µM	-4
	R	*GTTTCTT*GTCCTTGTCAGCGTTTATTTGC	1 µM	5
D7S820	F	NED-GAACACTTGTCATAGTTTAGAACGAAC	1.5 µM	4
	R	*GTTTCTT*TCATTGACAGAATTGCACCA	1.5 µM	65

Development of miniSTRs: Potential Pitfalls

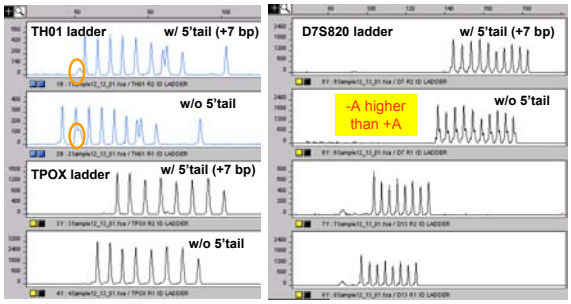
Information on Big Mini STR Loci

GenBank sequence

Locus	Allele Spread (repeat)	Allele Spread (bp)	Start	Stop	Reference Size
TH01	3--14	44	55	98	79 bp
			+7 bp		
FGA	12.2--51.2	152	125	281	159 bp
CSF1PO	6--16	40	89	129	113 bp
D21S11	24--38.2	127	153	209	173 bp
TPOX	5--14	36	65	101	89 bp
			+7 bp		
D7S820	5--15	40	136	176	168 bp
			+7 bp		

From STRBase and *Forensic DNA Typing* Appendix 1

Comparison of Ladders Amplified with and without 5'-tails on reverse primer (tail adds an extra 7 bases)



5'-tail added to reverse primer to promote full adenylation

D7 ladder is improved with 5'-tail; others appear roughly equivalent

Development of miniSTRs: Potential Pitfalls

Reduction in PCR Product Size

Locus	Size Difference (relative to ABI kits)
TH01	-105 bp
FGA	-71 bp
CSF1PO	-191 bp
D21S11	-33 bp
TPOX	-148 bp
D7S820	-117 bp

Not as much size reduction as other STR loci…

Development of miniSTRs

Primer Position Relative to Repeat

Locus		Distance 3'end from Repeat		Comment
CSF1PO	F	**14**		*partial repeat just 5' of repeat*
	R	6		
FGA	F	3		
	R	**23**		*partial repeat just 3' of repeat*
TH01	F	0		
	R	1		
TPOX	F	-4		
	R	5		
VWA	F	0		
	R	0		
D3S1358	F	-1		
	R	-1		
D5S818	F	4	20	**Has been changed due to allele dropout problems**
	R	-5	6	
D7S820	F	4		
	R	**65**		*polyA stretch just 3' of repeat*

Development of miniSTRs

Primer Position Relative to Repeat (cont.)

D8S1179	F	-4	
	R	5	
D13S317	F	**19**	*self-complementary just 5' of repeat*
	R	2	
D16S539	F	0	
	R	16	
D18S51	F	5	
	R	**33**	*partial repeat just 3' of repeat*
D21S11	F	2	
	R	0	
Penta D	F	11	
	R	**19**	polyA stretch just 3' of repeat
Penta E	F	6	
	R	4	
D2S1338	F	3	
	R	3	
D19S433	F	3	
	R	12	

Development of miniSTRs

PCR Product Size Reduction

Locus	Size Difference (bp)	Big Mini
Penta E	-299	
Penta D	-282	
D2S1338	-198	
CSF1PO	-191	*
D16S539	-152	
D18S51	-151	
TPOX	-148	*
D7S820	-117	⊛
TH01	-105	*
D13S317	-105	
FGA	-71	⊛
VWA	-64	
D5S818	-53 +26 = -27	
D8S1179	-37	
D21S11	-33	⊛
D3S1358	-25	
D19S433	0	

Other possible combinations

* *Non-CODIS loci in 16plex kits*

* *Rest of Profiler Plus loci*

Development of miniSTRs: Sample Requirements

PCR Conditions

- 25 uL volume
  - 4.6 uL PCR mix (must add 2 U TaqGold—0.4 uL)
  - 5 uL Big Mini primer mix
  - **up to 15 uL DNA extract**
- PCR mix
  - 1X Gold buffer, 1.5 mM $MgCl_2$, and 200 µM dNTPs (no BSA)
- Thermal Cycling
  - 95 ºC for 10 minutes
  - 28 cycles: 94 ºC for 1 min, 55 ºC for 1 min, 72 ºC for 1 min
  - 60 ºC for 45 minutes
  - 25 ºC forever

More cycles will improve sensitivity…*to be explored*

Development of miniSTRs

Comments on Primer Design

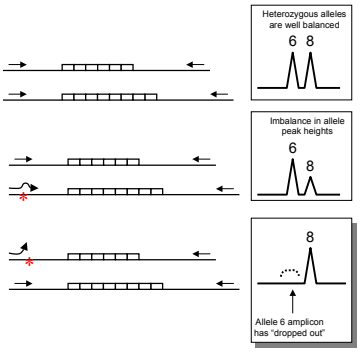
- All known information on flanking sequence variation that has caused null alleles in the past between various kits has been taken into account
- Designed to work as singleplexes or limited miniplexes (to pick up loci that failed when a kit was run on a degraded sample)
- All primer information is made fully available
- Resulted from NIJ-funded work begun in June 1997 with TOF-MS

Development of miniSTRs: Potential Pitfalls

Null alleles

- Allele is present in the DNA sample but fails to be amplified due to a nucleotide change in a primer binding site
- Allele dropout is a problem because a heterozygous sample appears falsely as a homozygote
- Two PCR primer sets can yield different results
- This phenomenon impacts DNA databases but lower stringency matches reduce problem

Development of miniSTRs: Potential Pitfalls



Butler, J.M. (2001) *Forensic DNA Typing*, Figure 6.8

Apparent Null Alleles Observed During Concordance Studies

8/13 CODIS loci affected so far

Locus	Kits Compared	Results	Reference
D13	PP1.1 vs PP16 vs ProPlus	Loss of alleles 9,10, and 11 with PP1.1; fine with PP16 and ProPlus	Promega meeting Oct 2000
D13	PP1.1	Reported 4 bp deletion in 3' flanking region while sequencing a rare allele 7 from 2 Asians	Promega meeting Oct 2000 (P#23)
D16	PP1.1 vs PP16 vs COfiler	Loss of alleles with PP1.1; fine with PP16 and COfiler	Promega meeting Oct 2000
D8	PP16 vs. ProPlus	Loss of allele 16 with ProPlus; fine with PP16	Promega meeting Oct 2000
D8	PP16 vs SGM Plus	Loss of allele 15 with SGM Plus; fine with PP16	Promega meeting Oct 2000
VWA	PP1.1 vs. Profiler	Loss of allele 19 in Profiler; fine with PP1.1	Kline 1998
VWA	PP16 vs ProPlus	Weak amp of allele 19 with ProPlus; fine with PP16	Promega meeting Oct 2000 (P#101)
D5	PP16 vs ProPlus	Loss of alleles 10 and 11 with PP16; fine with ProPlus	ISFG 2001 (P#24)
FGA	PP16 vs ProPlus	Weak amp on allele 21 with ProPlus; fine with PP16	Promega meeting Oct 2000 (P#101)
FGA	SGM vs SGM Plus	Loss of allele 26 with SGM Plus; weak amp of same allele with SGM	Cotton 2000
CSF	PP16 vs COfiler	Weak amp on allele 14 with COfiler; fine with PP16	Promega meeting Oct 2000
CSF	PP16 vs Profiler	Weak amp on allele 8 with PP16; fine with Profiler	Promega meeting Oct 2000
TPOX	PP16 vs Profiler	Weak amp on allele 9 with PP16; fine with Profiler	Promega meeting Oct 2000

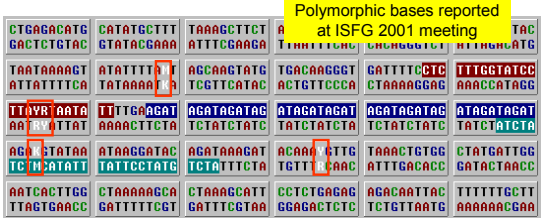
Butler and Vallone, 2001 NIJ Report, Exhibit 109

Development of miniSTRs: Quality Control

First set of D5S818 miniSTR primers

(Miniplex 2)

Polymorphic bases reported at ISFG 2001 meeting



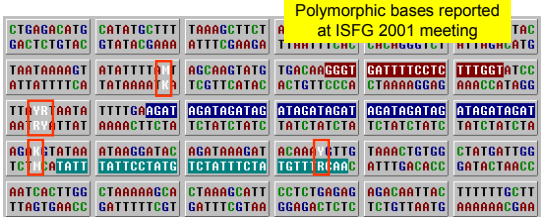
Original miniSTR primers for D5 produced 12 allele dropouts in 109 samples tested

Primers have been moved now to correct this problem

Development of miniSTRs: Quality Control

Newly designed D5S818 miniSTR primers

Polymorphic bases reported at ISFG 2001 meeting



Development of miniSTRs: Quality Control

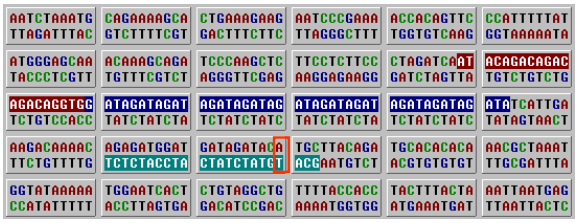
MiniSTR Primer Positions for D8S1179

-37 bp size reduction

Polymorphic nucleotide that causes problems with original ABI kits

Development of miniSTRs: Quality Control

MiniSTR Primer Positions for D16S539



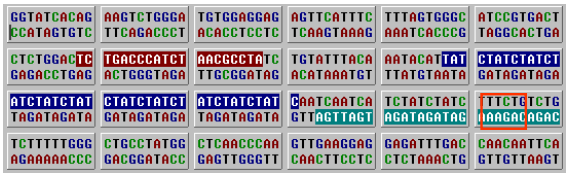
-152 bp

A→T

Comment on PP1.1 null allele site

Development of miniSTRs: Quality Control

MiniSTR Primer Positions for D13S317

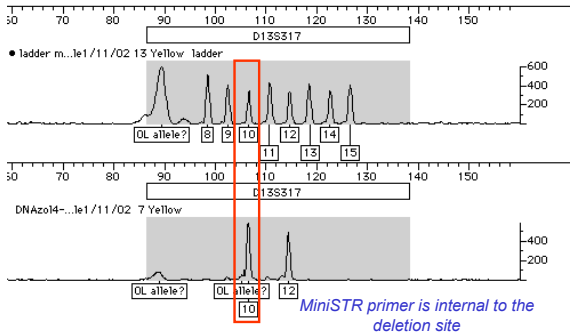


-105 bp

Comment on PP1.1 null allele site

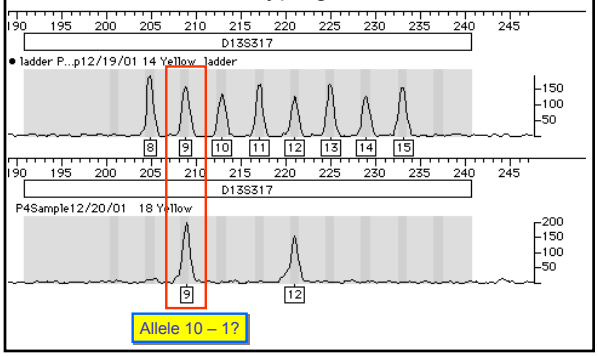
Development of miniSTRs: Potential Pitfalls

Miniplex 4 STR Typing Result at D13S317



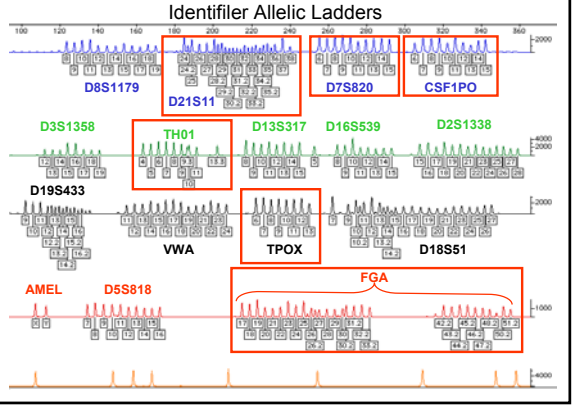
Development of miniSTRs: Potential Pitfalls

Profiler Plus STR Typing Result at D13S317

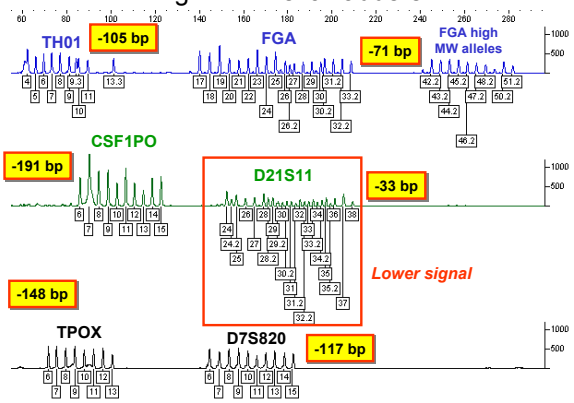


Development of miniSTRs: Sample Requirements

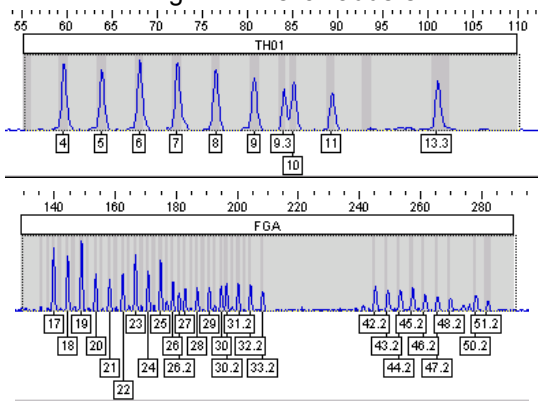
Identifiler Allelic Ladders



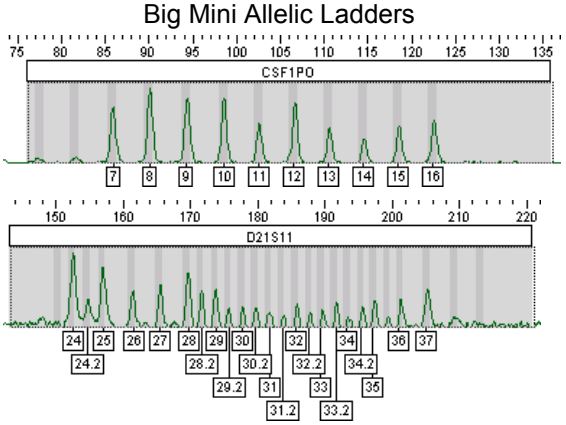
Big Mini Allelic Ladders



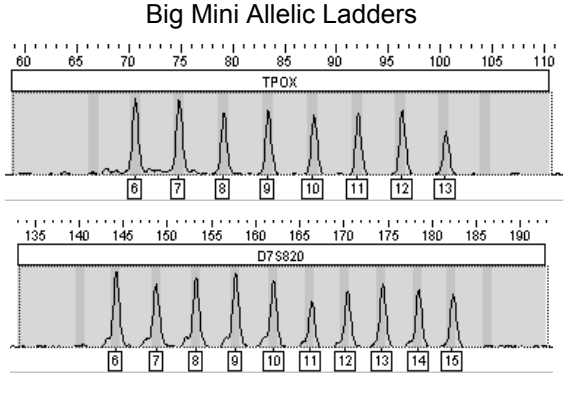
Big Mini Allelic Ladders



Big Mini Allelic Ladders



Big Mini Allelic Ladders



Development of miniSTRs: Validation

Summary of NIST Concordance Studies

16 standard templates (high quality DNA)
compared to Profiler, SGM Plus, and PowerPlex 16 results

16 CEPH family samples (high quality DNA)
compared to Profiler Plus and COfiler results

92 aged blood stains (degraded DNA; samples stored at room temp on untreated paper 14-15 years)
compared to PowerPlex 16 results

No signficant heterozygote peak imbalance

Development of miniSTRs: Validation

TH01 Genotyper Concordance Data on 16 Samples

Sample Info	Big Mini TH01		PP16 TH01		Profiler TH01		SGM Plus TH01	
216	9.3	9.3	9.3	9.3	9.3	9.3	9.3	9.3
219	6	7	6	7	6	7	6	7
220	6	7	6	7	6	7	6	7
223	7	7			7	7	7	7
265	8	8	8	8	8	8	8	8
266	7	7	7	7	7	7	7	7
267	7	9.3			7	9.3	7	9.3
272	7	9	7	9	7	9	7	9
273	7	10	7	10	7	10	7	10
275	7	8	7	8	7	8	7	8
277	6	6	6	6	6	6	6	6
278	7	9	7	9	7	9	7	9
DD	6	7	6	7	6	7	6	7
JB	6	6	6	6	6	6	6	6
DJ	9	9.3	9	9.3	9	9.3	9	9.3
349	7	8	7	8	7	8	7	8

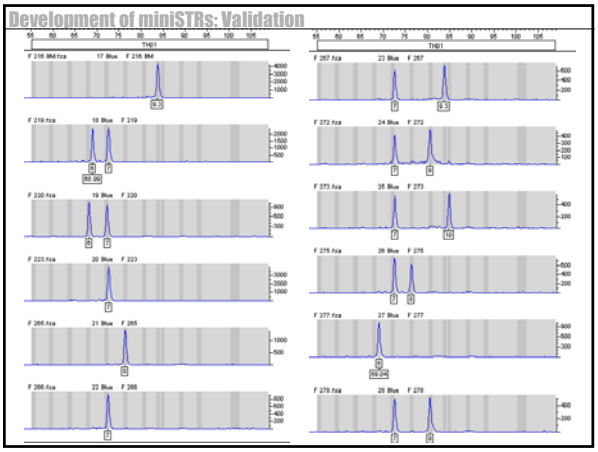
all sample genotypes are completely concordant
(samples 223 and 267 were not tested with PowerPlex 16)

Development of miniSTRs: Validation

FGA Concordance Data

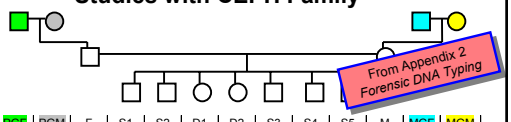
Big Mini FGA		PP16 FGA		Profiler FGA		SGM Plus FGA	
22.2	24	22.2	24	22.2	24	22.2	24
24	27	24	27	24	27	24	27
18	24	18	24	18	24	18	24
22	22			22	22	22	22
24	25	24	25	24	25	24	25
21	25	21	25	21	25	21	25
20	23			20	23	20	23
21	23	21	23	21	23	21	23
20	22.2	20	22.2	20	22.2	20	22.2
22	22	22	22	22	22	22	22
20	23	20	23	20	23	20	23
18.2	23	18.2	23	18.2	23	18.2	23
19	23	19	23	19	23	19	23
21	22	21	22	21	22	21	22
21	22	21	22	21	22	21	22
19	20	19	20	19	20	19	20

Development of miniSTRs: Validation



Development of miniSTRs: Validation

Concordance and Family Inheritance Studies with CEPH Family

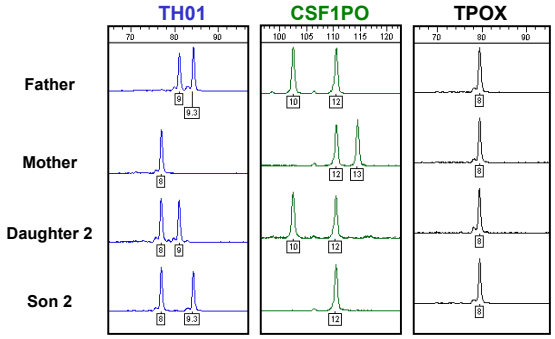


Marker	PGF	PGM	F	S1	S2	D1	D2	S3	S4	S5	M	MGF	MGM
CSF1PO	11,12	10,10	10,12	12,13	12,12	10,12	10,12	12,13	12,12	12,12	12,13	12,13	10,13
FGA	20,22	20,21	20,20	20,21	20,24	20,24	20,24	20,24	20,24	20,21	21,24	21,24	21,22
TH01	9.3,9.3	7,9	9,9.3	8,9	8,9.3	8,9	8,9	8,9	8,9	8,9	8,8	8,8	7,8
TPOX	8,8	8,8	8,8	8,8	8,8	8,8	8,8	8,8	8,8	8,8	8,8	8,8	8,9
VWA	16,16	17,19	16,17	16,17	16,17	17,17	16,16	16,17	17,17	16,17	16,17	16,16	16,17
D3S1358	14,15	17,18	14,18	15,18	16,18	14,15	14,15	15,18	15,18	15,18	15,16	15,16	15,17
D5S818	10,12	10,12	10,12	11,12	12,13	10,13	12,13	12,13	11,12	12,13	11,13	11,12	9,13
D7S820	13,15	9,11	9,15	9,12	9,9	9,9	9,13	9,12	12,13	12,13	9,12	9,11	9,12
D8S1179	12,13	11,13	13,13	13,13	10,13	10,13	13,13	10,13	13,13	13,13	10,13	10,13	13,13
D13S317	9,13	9,10	9,10	10,12	10,11	9,12	10,11	10,11	10,12	9,11	11,12	11,12	11,12
D16S539	12,13	12,13	13,13	12,13	13,13	12,13	13,13	12,13	13,13	13,13	12,13	9,13	12,12
D18S51	13,13	13,14	13,13	12,13	13,13	13,13	12,13	13,13	13,13	13,13	12,13	13,17	12,12
D21S11	29, 29	28, 29	29, 29	29, 32.2	29, 32.2	29, 32.2	29, 32.2	29, 32.2	29, 32.2	29, 32.2	32.2, 32.2	30, 32.2	28, 32.2
AMEL	X,Y	X,X	X,Y	X,Y	X,Y	X,X	X,X	X,Y	X,Y	X,Y	X,X	X,Y	X,X

Genotype calls for each individual at each of the CODIS 13 STR markers

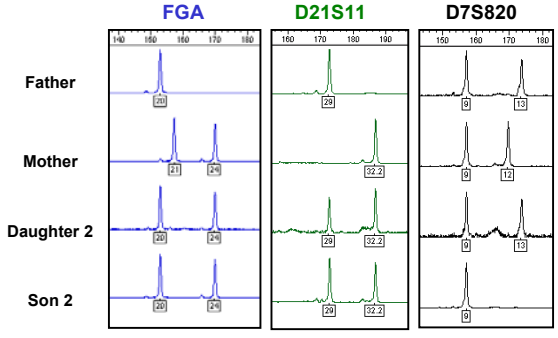
Development of miniSTRs: Validation

CEPH Family miniSTR Results



Development of miniSTRs: Validation

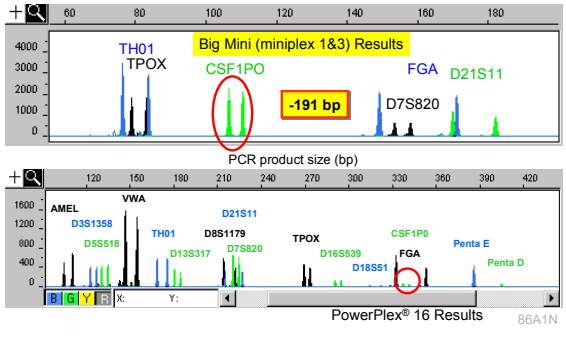
CEPH Family miniSTR Results



Development of miniSTRs: Validation

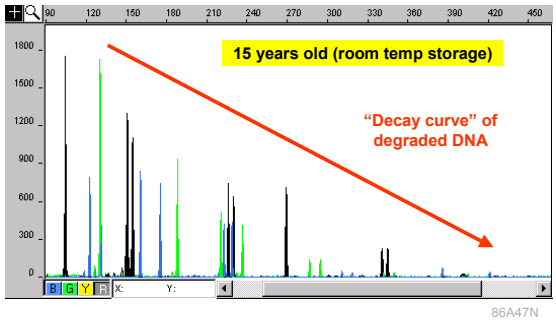
Comparison between miniSTR and Commercial Kit Results

(Aged Blood Stain Known to Contain Degraded DNA)



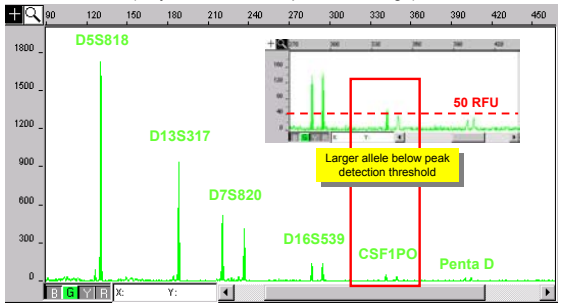
Development of miniSTRs: Validation

PowerPlex® 16 Result on Aged Blood Stain
(15 years at room temperature storage)



Development of miniSTRs: Validation

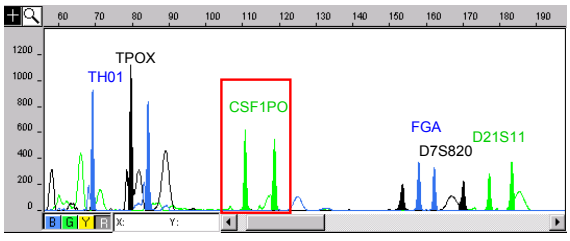
PowerPlex® 16 Result on Aged Blood Stain
(15 years at room temperature storage)



Typed as "12,12"

Development of miniSTRs: Validation

Big Mini Result on Aged Blood Stain
(15 years at room temperature storage)



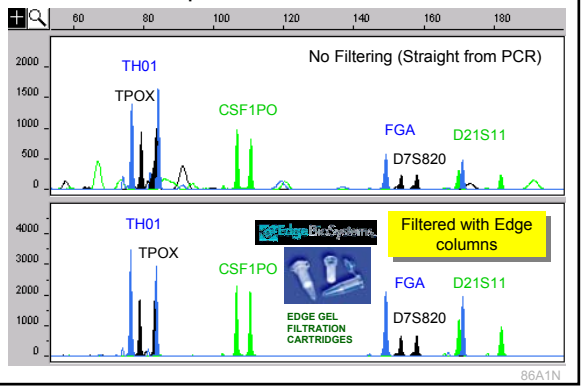
Typed as "12,14" (correct)

Complete concordance where kit gave result

Failures to yield type with kit: D18, Penta E, CSF, Penta D

Development of miniSTRs: Sample Requirements

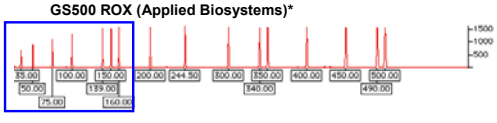
Data Improvement with Filtration



Development of miniSTRs: Sample Requirements

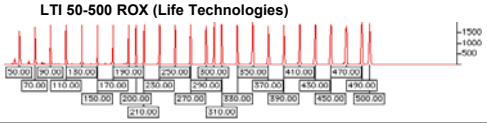
Internal Sizing Standards

GS500 ROX (Applied Biosystems)*



No available size standard is optimized for small STR products if Local Southern sizing methods are used

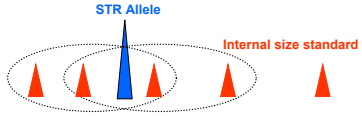
LTI 50-500 ROX (Life Technologies)



Development of miniSTRs: Sample Requirements

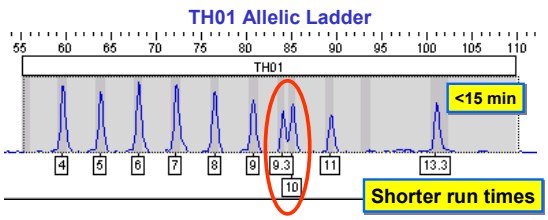
Sizing Algorithm

- Local Southern involves using 2 peak above and 2 peaks below an unknown peak from the internal size standard to make a calculated DNA size
- *Both 35 bp and 50 bp GS500 peaks must be included to type TH01 and TPOX alleles less than 75 bp*
- **Global Southern** can be used



X_1 X_2 $\frac{X_1 + X_2}{2}$ = Size of STR Allele

Additional miniSTR Advantages



Higher resolution available with smaller PCR product size

Smaller STR amplicons will benefit microchip CE separations

Development of miniSTRs: Mutation Rates

Big Mini

Measured Mutation Rates

STR Locus	Maternal Meioses (%)	Paternal Meioses (%)	Null Alleles (%)	Multi-Banded (%)
CSF1PO	**14/47843 (0.03)**	**311/243124 (0.13)**	2/42020 (<0.01)	*None reported*
FGA	**7/8253 (0.01)**	**555/189973 (0.29)**	2/1104 (0.18)	*None reported*
TH01	**5/42100 (0.01)**	**12/74426 (0.02)**	2/7983 (0.03)	0/2646 (<0.040)
TPOX	**2/28766 (0.01)**	**10/45374 (0.02)**	11/43704 (0.03)	13/42020 (0.03)
VWA	20/58839 (0.03)	851/250131 (0.34)	7/42220 (0.02)	1/6581 (0.02)
D3S1358	0/4889 (<0.02)	9/8029(0.11)	*None reported*	*None reported*
D5S818	22/60907 (0.04)	194/130833 (0.15)	3/74922 (<0.01)	*None reported*
D7S820	**14/50827 (0.03)**	**193/131880 (0.15)**	1/42020 (<0.01)	1/406 (0.25)
D8S1179	5/6672 (0.07)	29/10952 (0.26)	*None reported*	*None reported*
D13S317	33/59500 (0.06)	106/69598 (0.15)	52/62344 (0.08)	*None reported*
D16S539	12/42648 (0.03)	40/48760 (0.08)	3/52959 (<0.01)	0/1165 (<0.09)
D18S51	8/8827 (0.09)	29/9567 (0.30)	*None reported*	*None reported*
D21S11	**12/6754 (0.18)**	**17/6980 (0.24)**	1/203 (0.49)	*None reported*

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

*Data used with permission from American Association of Blood Banks (AABB) 1999 Annual Report.

Issues

- **Dye artifacts**
  - ABI has offered to share information on their primer purification procedures *(Feb 12 meeting with John Butler)*
  - Temporary fix with post-PCR purification
- **Allelic ladders**
  - Mecki Prinz was approached by Rhoda Roby (ABI) at AAFS meeting and offered ABI allelic ladder materials
- **Availability in commercial kit form**
  - Promega and Applied Biosystems are both aware of this work
- **STR patents?**
  - No problem while this project is still research…

Development of miniSTRs: Validation

NYC OCME Big Mini Validation Plan

(from Mecki Prinz, Feb 8, 2002)

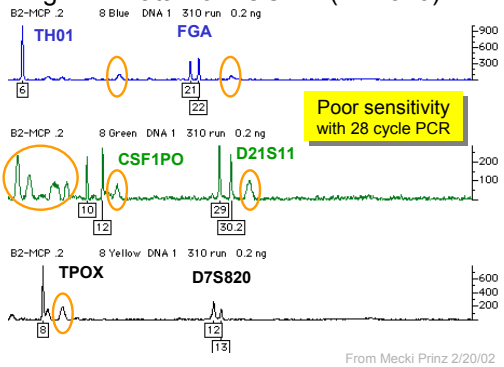
- Test Amplification
  - 1 ng of 6 known samples
  - 377 vs. 310 data

D21S11 weak allelic ladder
Dye artifact peaks removed with Microcon 100

- Sensitivity Titration
  - 5 ng, 2 ng, 1 ng, 0.5 ng, 0.2 ng, 0.1 ng, 0.05 ng in duplicate
- Degraded DNA
  - 24 DNA IQ tissue samples selected from AA587; low yields with Profiler Plus and COfiler
- Concordance Study
  - 25 samples exchange between Albany SP and OCME
- Sizing Precision and Stutter Rates
  - gathered from previous experiments

Development of miniSTRs: Validation and Potential Pitfalls

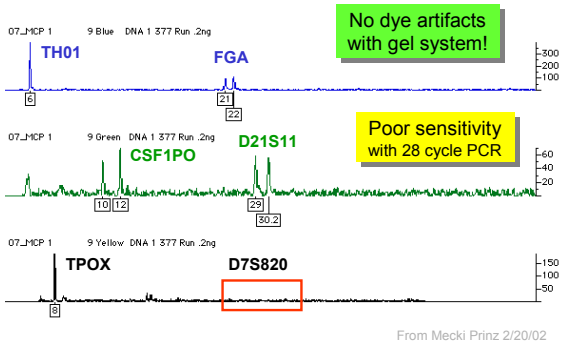
Big Mini Data from OCME (ABI 310)



From Mecki Prinz 2/20/02

Development of miniSTRs: Validation and Potential Pitfalls

Big Mini Data from OCME (ABI 377)



From Mecki Prinz 2/20/02

Development of miniSTRs: Future Plans

Future Plans with miniSTRs

- NYC OCME -- testing Big Mini pilot materials (primers, ladders, macros, protocols)
- AFDIL -- collaboration to investigate miniplex sensitivity and concordance with field samples
- Ohio University -- work on new primers and miniplex sets and better understanding of degradation effects
- NIST – solve dye blob problem and develop new optimized markers ("Autoplex")
- *Publish developmental validation of miniSTRs including all primer sequences*

Paper in preparation on Big Mini development and testing

Future Plans: Improving Stats, etc.

Plans for Improved STR Markers – the "Autoplex" (going beyond the CODIS 13)

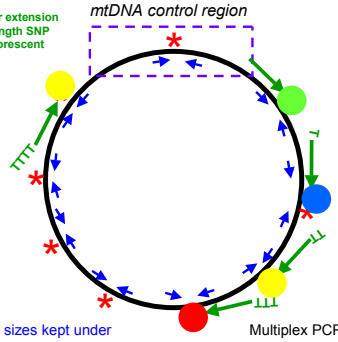
- New markers with smaller allele ranges, low stutter, and better characteristics for small PCR products (will make use of Human Genome Project information)
- Additional STRs to aid in large mass disasters to provide higher discrimination power than is possible with 13 CODIS loci
- Coverage of all chromosomes (22 autosomes + X/Y)
- Dual development of primer sets to enable null allele detection
  - large megaplex system for population data collection
  - miniplex systems to aid casework situations

How many more would be markers would be useful?

Other Potential Assays

Coding Region mtSNP 10plex Assay

Multiplex primer extension with different length SNP primers and fluorescent ddNTPs

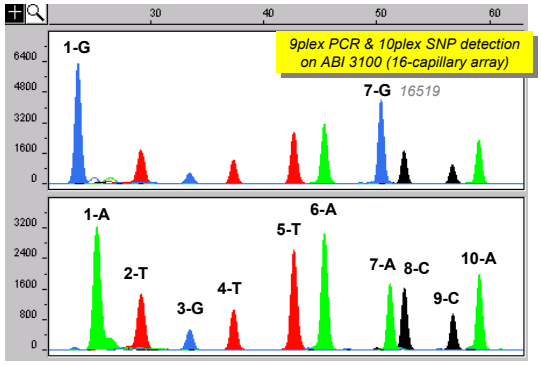


PCR product sizes kept under 150 bp to enable success with degraded DNA samples

Multiplex PCR used to co-amplify all regions of interest at once

Other Potential Assays

Coding Region mtSNP 10plex Assay



STRBase

Short Tandem Repeat DNA Internet Database

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

General Information	Forensic Interest Data	Supplemental Info
•Intro to STRs (downloadable PowerPoint)	•FBI CODIS Core Loci	•Reference List 1479
•STR Fact Sheets	•DAB Standards	•Technology Review
•Sequence Information	•NIST SRM 2391	•Addresses for Scientists
•Multiplex STR Kits	•Published PCR Primers	•Links to Other Web Sites
•Variant Allele Reports	•Y-Chromosome STRs	
	•Population Data	
	•Validation Studies	

Information on miniSTRs will be posted in the future