National Institute of Standards and Technology

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Some Interesting Point Mutations and Deletions Found Through STR Allele Sequencing

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Disclaimer

- This project was supported by NIJ **Grant Number 1999-IJ-R-A094 and 2003-IJ-R-029**, which is an interagency agreement between NIJ and the NIST Office of Law Enforcement Standards.
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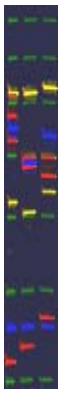
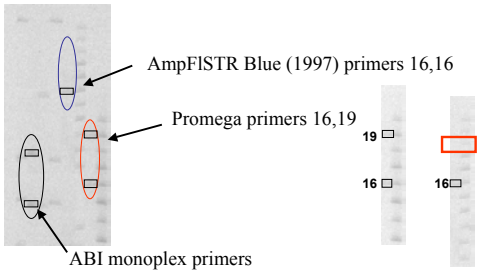


Outline for Presentation

- Explanation of Null and Variant Alleles
- Variant Allele Cataloging and Characterization on STRBase
- STR Allele Sequencing Approach
- Examples

vWA Allele Dropout Observed

What started our interest in sequencing variant alleles



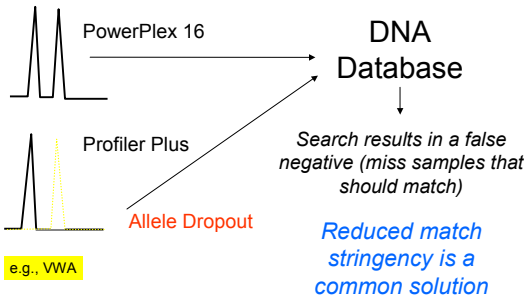
Kline, M.C., Jenkins, B. & Rodgers, S. (1998) Non-amplification of a vWA allele. *J Forensic Sci.*, 43(1), p250

Null Alleles

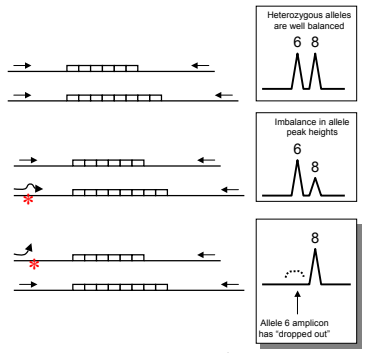
- Allele is present in the DNA sample but fails to be amplified due to a **nucleotide change in a primer binding site**
- Allele dropout is a problem because a heterozygous sample appears falsely as a homozygote
- Two PCR primer sets can yield different results on samples originating from the same source
- This phenomenon impacts DNA databases
- Large concordance studies are typically performed prior to use of new STR kits

For more information, see J.M. Butler (2005) *Forensic DNA Typing, 2nd Edition*, pp. 133-138

Concordance between STR primer sets is important for DNA databases

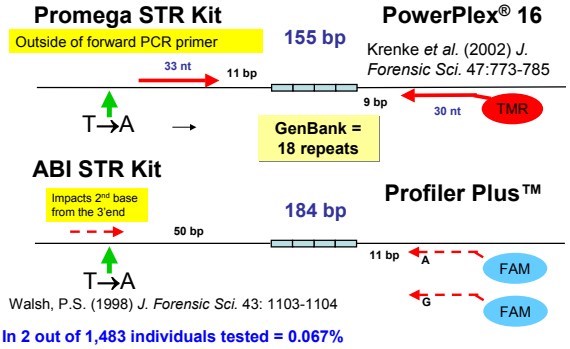


Impact of DNA Sequence Variation in the PCR Primer Binding Site



Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 6.9, ©Elsevier Academic Press

vWA Primer Position Comparisons



Walsh, P.S. (1998) *J. Forensic Sci.* 43: 1103-1104

In 2 out of 1,483 individuals tested = 0.067%

Lazaruk *et al.* (2001) *Forensic Sci Int.* 119:1-10

Apparent Null Alleles Observed During Concordance Studies

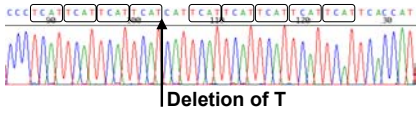
10/13 CODIS loci affected so far

Locus	STR Kits/Assays Compared	Results	Reference
VWA	PP1.1 vs ProPlus	Loss of allele 19 with **ProPlus**; fine with PP1.1	Kline et al. (1998)
D5S818	PP16 vs ProPlus	Loss of alleles 10 and 11 with **PP16**; fine with ProPlus	Alves et al. (2003)
D13S317	Identifiler vs miniplexes	Shift of alleles 10 and 11 due to deletion outside of **miniplex assay**	Butler et al. (2003), Drabek et al. (2004)
D16S539	PP1.1 vs PP16 vs COfiler	Loss of alleles with **PP1.1**; fine with PP16 and COfiler	Nelson et al. (2002)
D8S1179	PP16 vs ProPlus	Loss of alleles 15, 16, 17, and 18 with **ProPlus**; fine with PP16	Budowle et al. (2001)
FGA	PP16 vs ProPlus	Loss of allele 22 with **ProPlus**; fine with PP16	Budowle and Sprecher (2001)
D18S51	SGM vs SGM Plus	Loss of alleles 17, 18, 19, and 20 with **SGM Plus**; fine with SGM	Clayton et al. (2004)
CSF1PO	PP16 vs COfiler	Loss of allele 14 with **COfiler**; fine with PP16	Budowle et al. (2001)
TH01	PP16 vs COfiler	Loss of allele 9 with **COfiler**; fine with PP16	Budowle et al. (2001)
D21S11	PP16 vs ProPlus	Loss of allele 32.2 with **PP16**; fine with ProPlus	Budowle et al. (2001)

From Table 6.2 in J.M. Butler (2005) *Forensic DNA Typing, 2nd Edition*, p. 136

Microvariant "Off-Ladder" Alleles

- Defined as alleles that are not exact multiples of the basic repeat motif or sequence variants of the repeat motif or both
- Alleles with partial repeat units are designated by the number of full repeats and then a decimal point followed by the number of bases in the partial repeat
- Example: **TH01 9.3 allele**: $[TCAT]_4$ -CAT $[TCAT]_5$



Variation in the Flanking Region Can Cause Variant Alleles

D7S820 Example: commonly observed x .3 and x .1 alleles

Likely the result of a variation in the number of T's found in a poly(T) stretch 13 bases downstream of the core GATA repeat.

(Egyed, B. *et al. Forensic Sci. Int.* 2000, 113, 25-27).

http://www.cstl.nist.gov/biotech/strbase/var_d782.htm

Allele Designation	Allele Size	Instrument	Amp Kit*	Contributor	Verification/Conformation Method(s)	Notes	Frequency
12.1 [3]	281.65	ABI 310	PR	Kelly Duffy/R.Rubocki			
12.1 [4]	281.5	ABI 310	PR	Gintautas Svilpa	Observed both from suspect and crime scene stain		1
12.1 [5]	283.85	ABI 377	PS	Catherine Allor	Reamplified and Reanalyzed	Paternity samples only	1 in 11100
12.3	285.43	ABI 377	CO	Kelly Solis, Texas DPS	Re-extraction	Convicted offender	1 in 68000
13.1	286.8	ABI 310	PR, MP	Margaret Kline	Reamplified with two kits.		1 in 600 samples
13.1 [2]	287.58	ABI 377	CO, PS	Nicole Swinton	Re-extracted and Reamplified		

Allele	Frequency at the time of reporting
12.3	frequency 1 in 68000
13.1	frequency 1 in 600

Variation in the Flanking Region Can Cause Variant Alleles

D7S820 Example: commonly observed x .3 and x .1 alleles

GATAGAACACTTGTCATAGTTTAGAACGAACTAACGATAGATAGATAGATAGATAGATAG

CTATCTTGTGAACAGTATCAAATCTTGCTTGATTGCTATCTATCTATCTATCTATCTATC

13 repeat units = $(GATA)_{13}$

8 T's TTTTTTTT x.3 → 12.3

10 T's TTTTTTTTTT x.1 → 13.1

9T's nominal "on ladder"

ATAGATAGATAGATAGATAGATAGATAGACAGATTGATAGTTTTTTTTTAATCTCACTAAA

TATCTATCTATCTATCTATCTATCTATCTGTCTAACTATCAAAAAAAAATTAGAGTGATTT

Variant Alleles Cataloged in STRBase

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

Off-Ladder Alleles

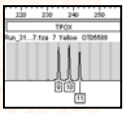
Currently **264** at 13/13 CODIS loci

- CSF1PO (10)
- D2S1338 (3)
- D3S1358 (16)
- D5S818 (5)
- D7S820 (20)
- D8S1179 (4)
- D13S317 (8)
- D16S539 (10)
- D18S51 (28)
- D19S433 (8)
- D21S11 (21)
- FESFPS (1)
- FGA (62)
- HUMTH01 (4)
- Penta D (8)
- Penta E (6)
- TPOX (7)
- VWA (5)

Tri-Allelic Patterns

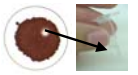
Currently **59** at 13/13 CODIS loci

- CSF1PO (2)
- D3S1358 (4)
- D5S818 (1)
- D7S820 (3)
- D8S1179 (5)
- D13S317 (3)
- D16S539 (1)
- D18S51 (4)
- D21S11 (4)
- FGA (9)
- HUMTH01 (1)
- TPOX (12)
- VWA (7)



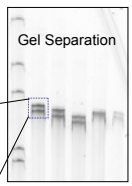
Steps in STR Allele Sequencing

- DNA Extraction
- Amplification with primers external to kit primers
- Gel Cutouts with Heterozygotes
- Re-Amplification
- Amplicon Quantitation
- ExoSAP
- Cycle Sequencing
- Dye Terminator Removal
- F/R Sequence Alignment to Reference Sequence

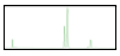


DNA Extraction

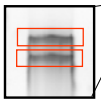
PCR Amplification



Gel Separation



Amplicon Quantitation



Allele Isolation with gel cutouts

Re-Amplification

12 GAAA repeats

DNA sequence analysis

Steps in STR Allele Sequencing

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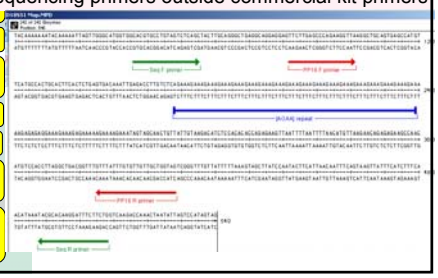
PCR Amplification

Sequencing primers outside commercial kit primers



Steps in STR Allele Sequencing

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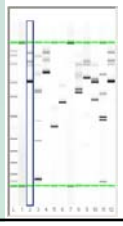
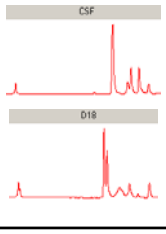
- F/R Sequence Alignment to Reference Sequence

12 samples ~45 minutes

Agilent 2100 Bioanalyzer





CSF1PO
12,14
517 bp

D18S51
15,22
431bp, 455bp

Steps in STR Allele Sequencing

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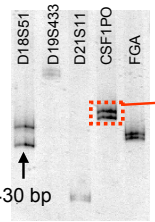
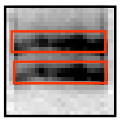
Gel Separation



430 bp

Allele Isolation with gel cutouts

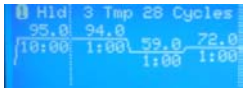


14

12

Re-Amplification

9%T 3%C
gel 32 cm
50 mMTris Formate in the Gel
Sodium Borate Running Buffer



Steps in STR Allele Sequencing

- DNA Extraction
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- Dye Terminator Removal
- F/R Sequence Alignment to Reference Sequence

12 samples ~45 minutes

Agilent 2100 Bioanalyzer

Peak	Mig.Time(secs)	Corr.Area	Size(bp)	Conc.(ng/ul)
1	42.80	97.94	15	4.2
2	93.35	594.47	528	15.0
3	98.40	4.35	645	0.11
4	100.75	139.43	699	3.5
5	102.10	110.07	750	2.8
6	106.05	94.87	946	2.4
7	110.55	70.01	1500	2.1

Steps in STR Allele Sequencing

- DNA Extraction
- Amplification with primers external to kit primers
- Gel Cutouts with Heterozygotes
- Re-Amplification
- Amplicon Quantitation
- ExoSAP
- Cycle Sequencing
- Dye Terminator Removal
- F/R Sequence Alignment to Reference Sequence

ExoSAP Treatment of PCR Products

Removes unconsumed dNTP's and primers

Target 7 ng of the PCR product for sequencing reactions

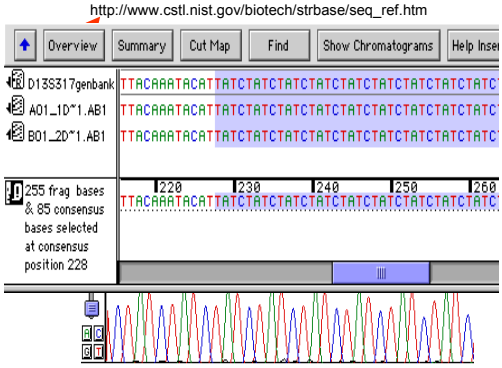
Cycle Sequencing (F primer only)

Cycle Sequencing (R primer only)

DYE Terminator removal

Forward & Reverse Sequence Alignment

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



EXAMPLES

(1) Deletions impacting miniSTRs with D13

(2) D18S51 point mutation causing null alleles in some Middle Eastern individuals

(3) Large D18S51 allele 40

(4) Small D18S51 allele "5.3"

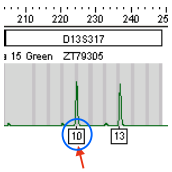
(5) Characterization of DYS635 allele 21.3 variant

Examination of Concordance:

African American sample ZT79305

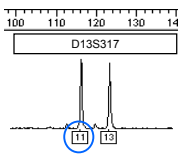
Drabek, J., Chung, D.T., Butler, J.M., McCord, B.R. (2004) Concordance study between miniplex STR assays and a commercial STR typing kit, *J. Forensic Sci.* 49(4): 859-860.

NIST Identifiler data



Really "11-1" allele

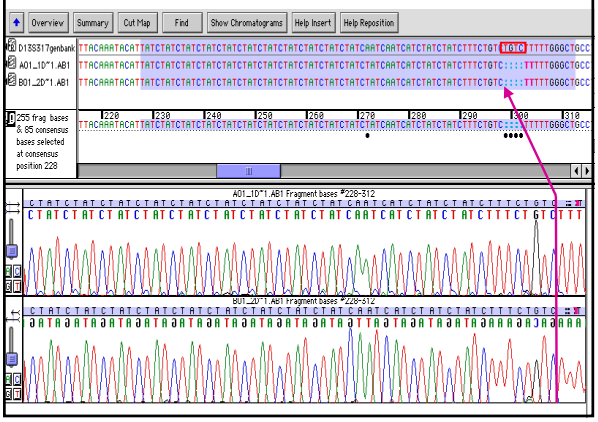
Ohio U miniSTR data



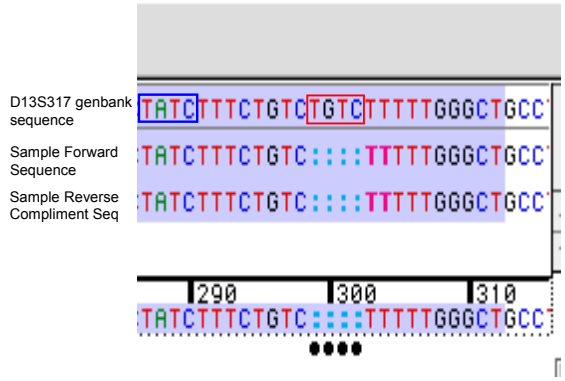
This problem has been seen multiple times by NYC OCME review of WTC BodePlex data

A deletion *outside* the miniSTR primers causes the commercial kit produced allele to appear one repeat smaller…

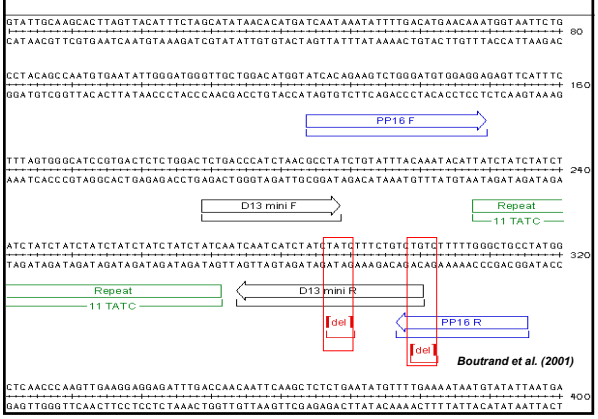
ZT79305: D13S317 TGTC deletion

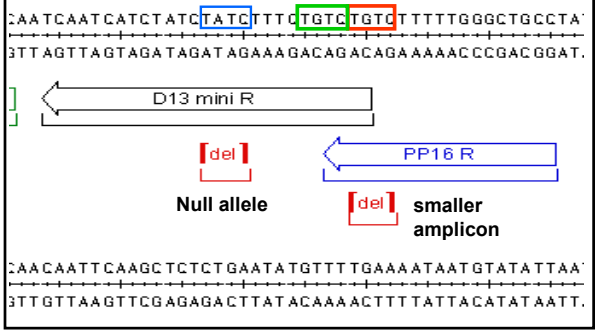


ZT79305: D13S317 TGTC deletion



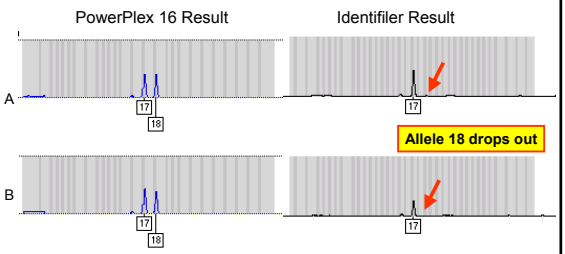
D13S317 Primers and Mutations





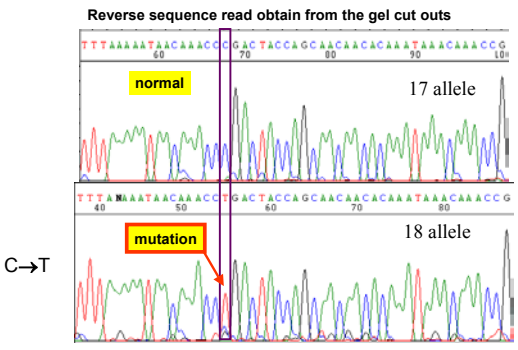
D18S51 Variant Allele

Kuwait Samples



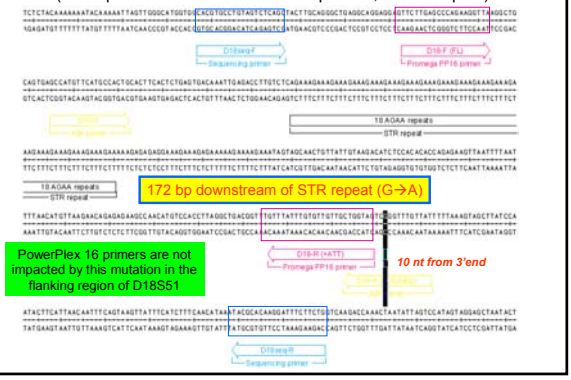
Clayton et al. (2004) **Primer binding site mutations affecting the typing of STR loci contained within the AMPFISTR SGM Plus kit.** *Forensic Sci Int.* 139(2-3): 255-259

Base change ~ 172 bases from D18S51 Repeat results in a Null Allele

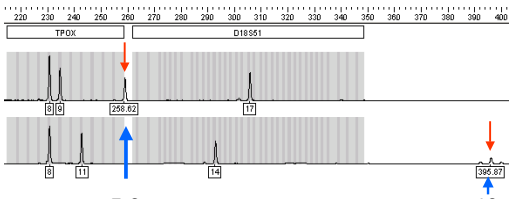


D18S51 Polymorphism

(PP16 primers not affected and ABI primers, allelic dropout)



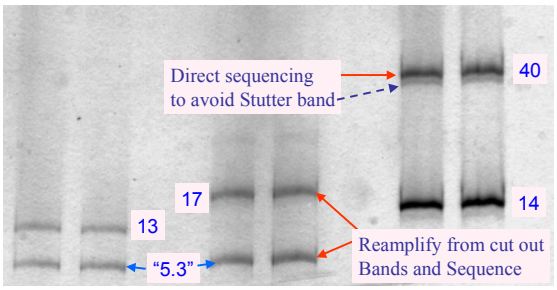
Extreme D18S51 Variants Range 258 – 396 bp



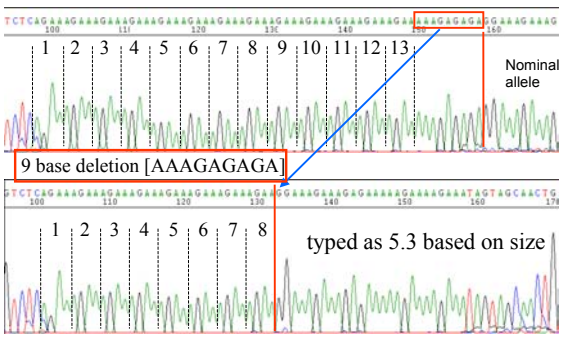
5.3 40

Samples amplified with Identifiler.Nominal range 8 - 28
Small D18 allele just outside of the TPOX allele range.
Large D18 is 46 bp larger than the D18 allele range.

Gel Separation Of D18 Variant Alleles

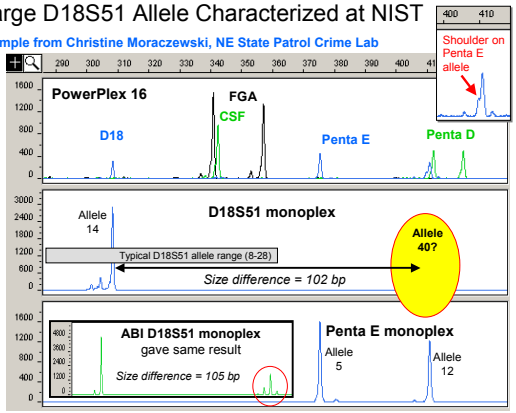


D18S51 sequencing Sample 188



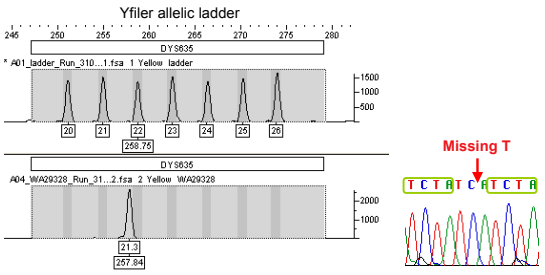
Nominal allele range 8-27 repeats. Sample courtesy DNA Solutions

Large D18S51 Allele Characterized at NIST



DYS635 Variant Allele 21.3

(Y-GATA-C4)

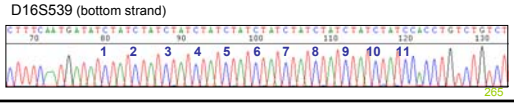


$[TCTA]_4(TGTA)_2[TCTA]_2(TGTA)_2[TCTA]_2(TGTA)_2[TCTA]_5$ TC-A $[TCTA]_2$

Analysis of Common STR Variant Alleles

- We have monoplex primers for all common STR loci and kits
- We have sequencing primers that bind outside of STR kit primer sequence positions to enable view of polymorphic nucleotides that cause primer binding site mutations
- NIJ has funded us to characterize STR variants for the forensic DNA community

D16S539 (bottom strand)



NIST Human Identity Project Team


John Butler
(Project Leader)


Margaret Kline


Jan Redman


Pete Vallone

Former Team Member


Rich Schoske

Former Team Member


Jill Appleby


Mike Coble


Amy Decker

Funding:
Interagency Agreement between National Institute of Justice and NIST Office of Law Enforcement Standards

Sample Suppliers and Collaborators

- **Those who have sent samples:**
- NE State Patrol Crime Lab
- DNA Solutions Inc., OK
- FSS
- Kuwait
- **Mini STR Concordance Collaborator**
  - Bruce McCord, Denise Chung