DNA Mixture Interpretation Principles: Observations from a NIST Scientific Foundation Review
AAFS 2019 Workshop #10 (February 18, 2019; Baltimore, MD)

The Potential of New Technologies

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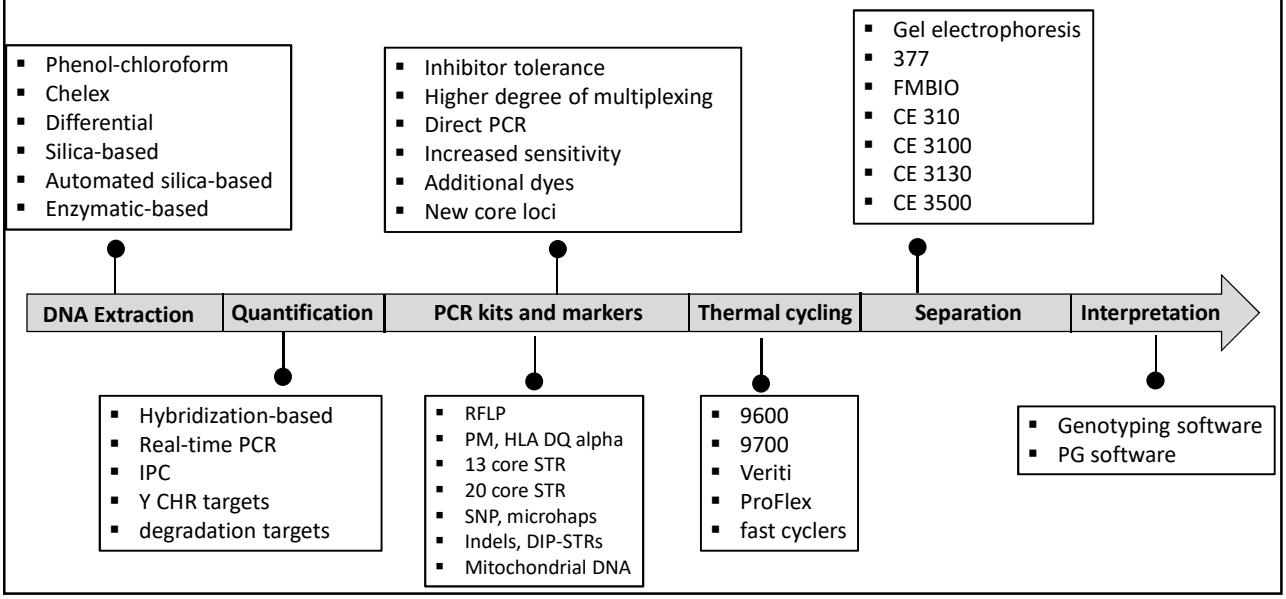
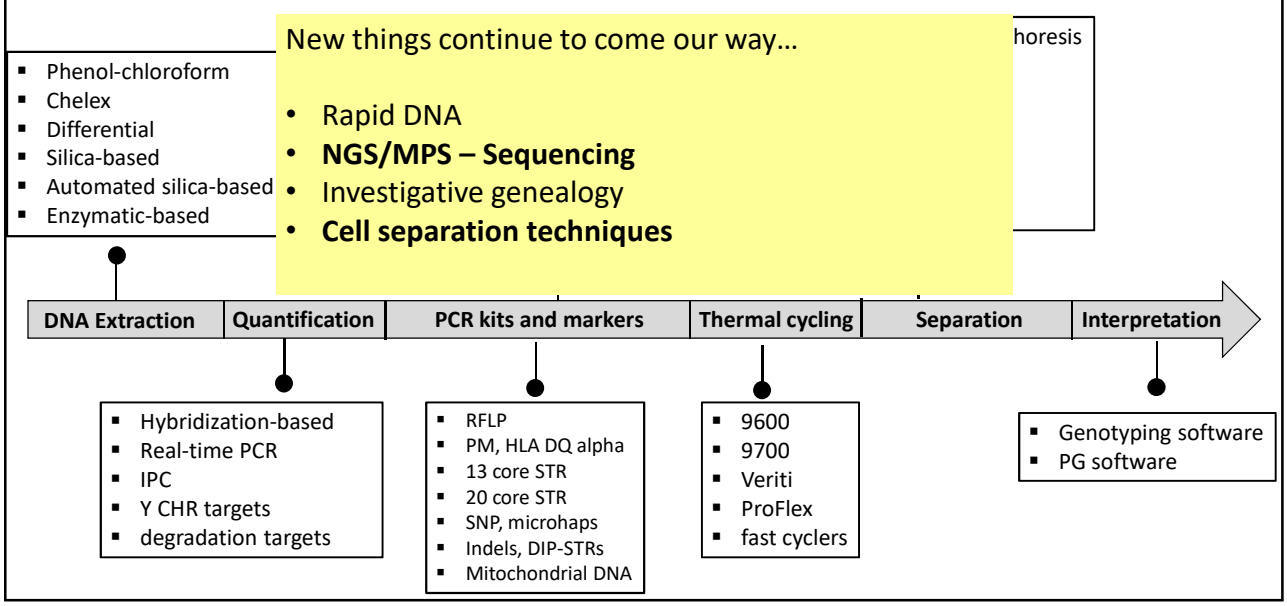
Outline

- Adoption and implementation of new technologies
  - Advances in forensic DNA typing
  - Idealized process
- The mixture problem
  - General illustration
- Sequencing
  - STR and microhaplotype examples
- Cell separation techniques

Advances in Forensic DNA Typing



Advances in Forensic DNA Typing

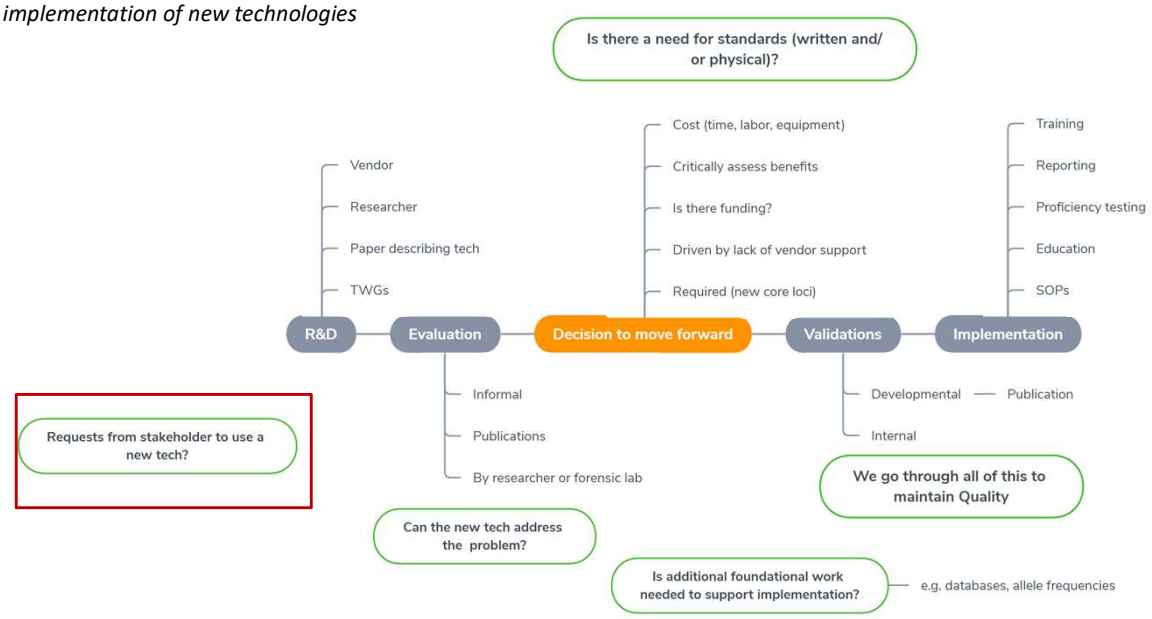


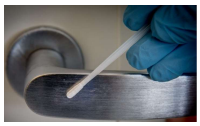
Analysis of a DNA Mixture

- DNA from two or more contributors are deposited
  - DNA may be in the cell or cell free
- Post DNA ***extraction***, the alleles from all contributors are mixed together
  - DNA may be lost/reduced in the purification process
- ***PCR*** amplifies the alleles present post DNA extraction
  - Stochastic effects, degradation, inhibitors
- Currently PCR amplicons are ***separated and detected*** by CE methods
- ***Interpretation*** of the data (community is moving toward probabilistic genotyping)

Challenges as we address more difficult cases: touch DNA, lower template amounts, more contributors…

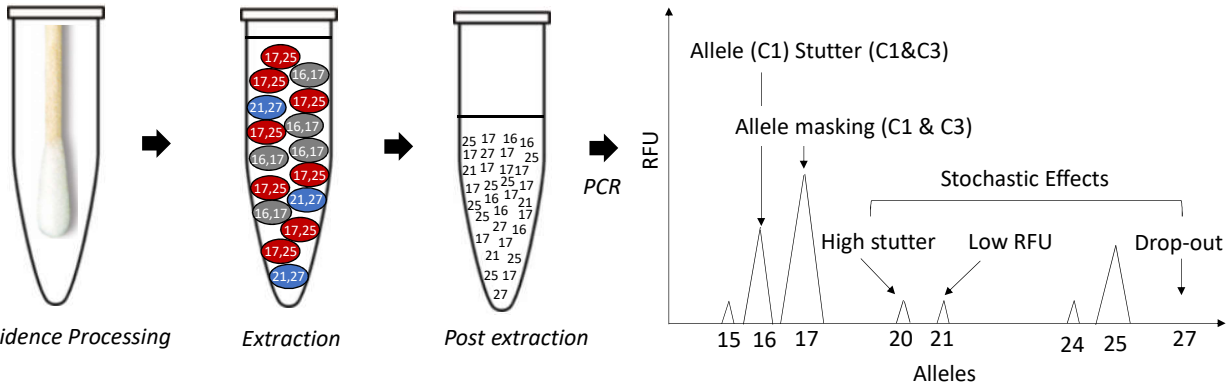
Idealized process for the implementation of new technologies





Collection of evidence

Example
3 person mixture
D2S1338



Contributor 1 with a "16,17" genotype
Contributor 2 with a "21,27" genotype
Contributor 3 with a "17,25" genotype

Sequencing

Next-generation sequencing (NGS)
Massively parallel sequencing (MPS)

Current NGS/MPS platforms and assays allow for the typing of forensically-relevant STR and SNP marker systems

Verogen FGx platform
ForenSeq panel (STRs, SNPs, mito)

Thermo Fisher S5 platform
Precision ID panels (STRs, SNPs, mito)

Promega

PowerSeq™ 46GY System
PowerSeq™ CRM Nested System

Qiagen GeneReader platform
Large SNP panels and mito

Not comprehensive

Benefits of sequencing (general)

- Can provide further resolution of STR alleles
  - Increased polymorphic content
  - Length (CE) -> Sequence (NGS)

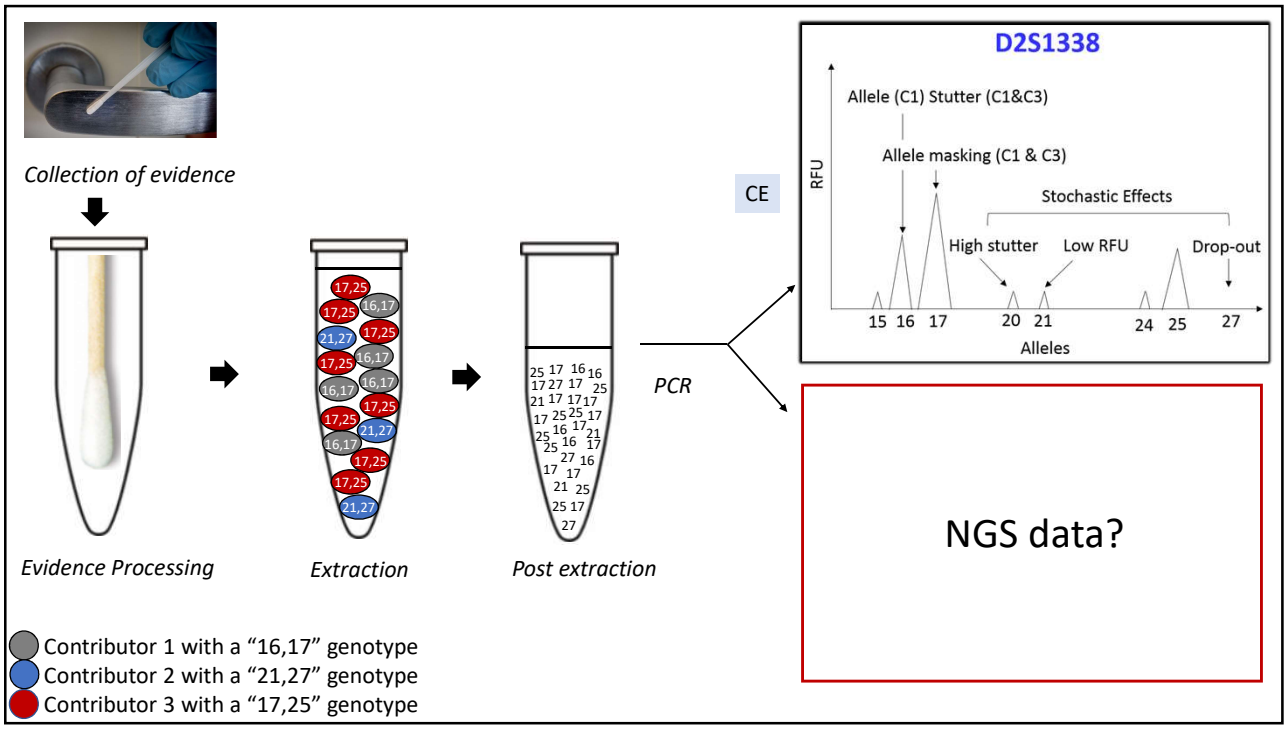
    19 allele -> [GGAA]11 [GGCA]8

More markers
Multiplexing of samples per run
More information per run

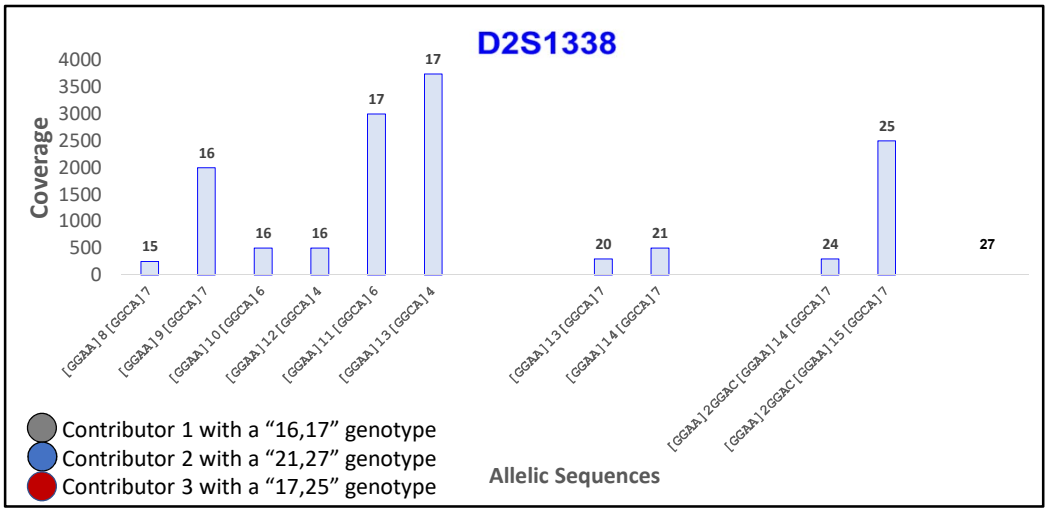
- Technology can be applied to type additional markers systems
  - Additional non-CODIS STR markers
  - Insertion=deletion markers
  - Mitochondrial (control region, whole mitochondrial genome)
  - SNPs
    - Ancestry, Phenotype, ID, **Microhaplotypes**

Sequencing *STRs for Mixtures*

In Comparison to CE methods	Comment
• Additional alleles	• "Unmasking" of alleles identical by length • Improve number of contributor estimates • Sequence-based allele frequencies are applied • Length-based alleles are back compatible to current databases
• Currently using targeted PCR	• Comparable sensitivity to CE • May have an increased input range (> 2 ng) • Subject to stochastic effects
• Stutter products are sequenced	• *Potential* to correlate stutter product(s) to parent allele • Allow for a more accurate modeling of stutter products
• Signal thresholds	• Discern noise (from instrument, PCR, seq, library error) from an allele; determine an AT
• Artifacts	• No dye artifacts; other concerns?
• Shorter PCR amplicons	• Improved performance with degraded samples
• Larger multiplexes	• More loci can be analyzed (autosomal, Y, X, mito)
• Interpretation	• A NGS-based probabilistic genotyping model for STRs?



Looking at the data in 'Sequencing space'



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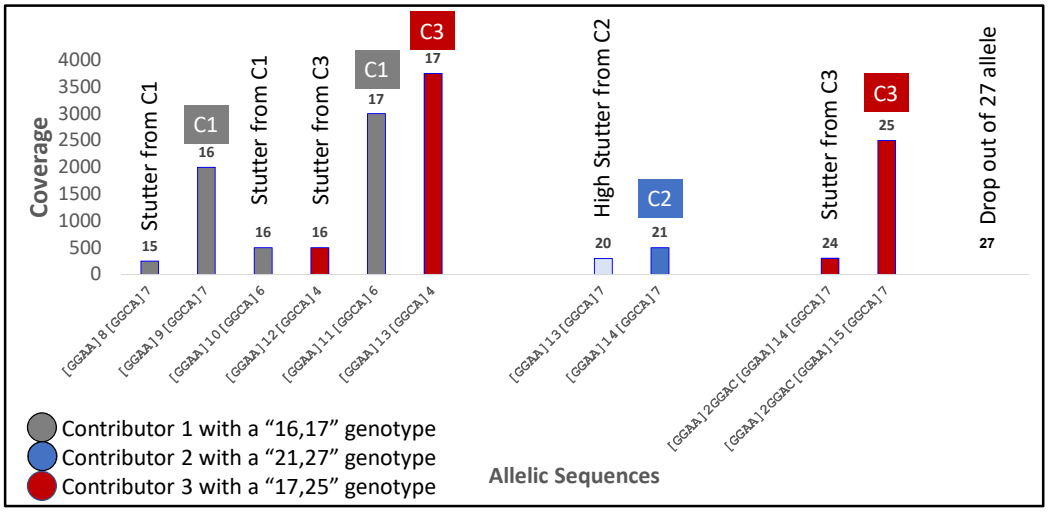
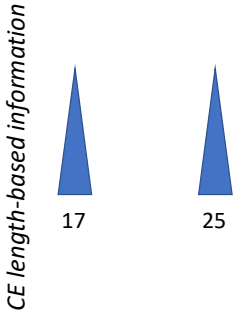


Locus D2S1338 - Length-based allele frequencies



Length	Freq	
15	0.0010	0.1%
16	0.0401	4.0%
17	**0.1419**	**14.2%**
18	0.0705	7.0%
19	0.1486	14.9%
20	0.1327	13.3%
21	0.0666	6.7%
22	0.0752	7.5%
23	0.1182	11.8%
24	0.0970	9.7%
25	**0.0835**	**8.3%**
26	0.0227	2.3%
27	0.0019	0.2%

13 alleles observed

Heterozygous genotype (17, 25)

2pq = 2(0.1419)(0.0835) = 0.0237

…1 in 42

Combined set of allele frequencies taken from Gettings et al. Sequence-based U.S. population data for 27 autosomal STR loci. Forensic Sci Int Genet. 2018 37:106-115

Locus D2S1338 – Sequence-based allele frequencies

Sequence-based information

Heterozygous genotype

17 [GGAA]13 [GGCA]4
25 [GGAA]2 GGAC [GGAA]15 [GGCA]7

2pq = 2(0.0029)(0.0734) = 0.0004

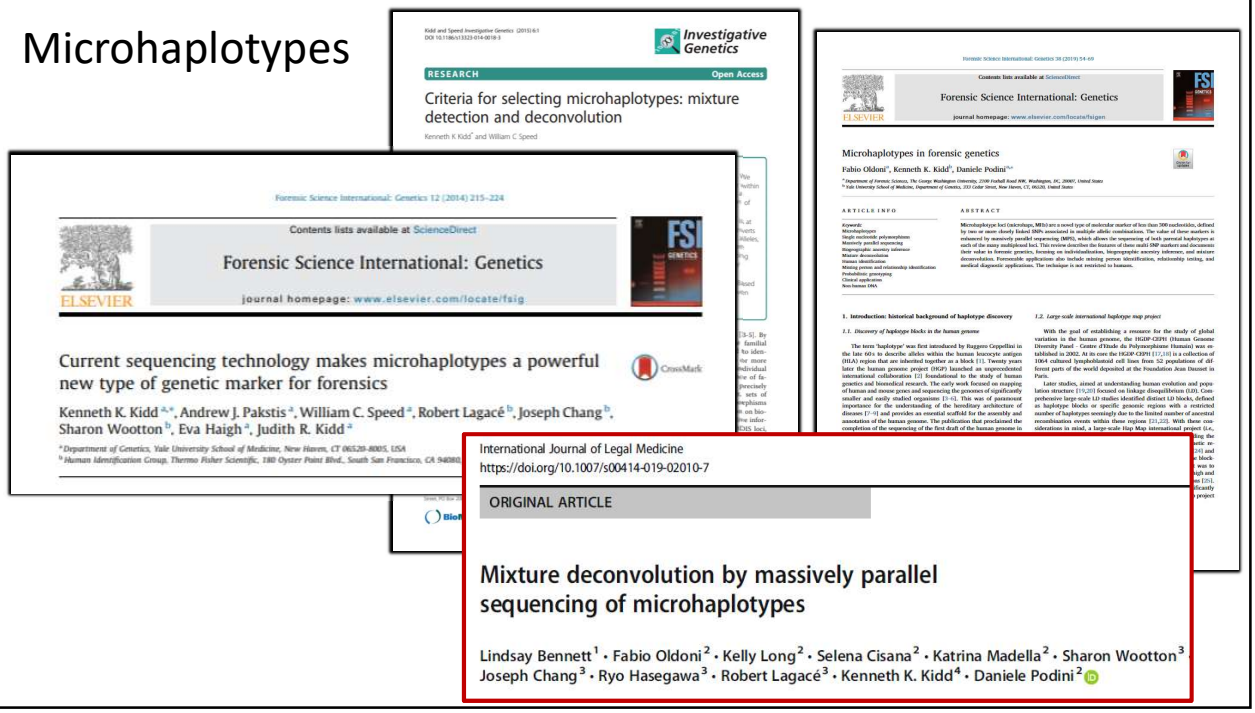
…1 in 2,349

15[GGAA]10 [GGCA]5	0.0010	21[GGAA]14 [GGCA]7	0.0256
16[GGAA]10 [GGCA]6	0.0217	21[GGAA]2 GGAC [GGAA]11 [GGCA]7	0.0232
16[GGAA]12 [GGCA]4	0.0145	21[GGAA]13 [GGCA]8	0.0082
16[GGAA]9 [GGCA]7	0.0019	21[GGAA]2 GGAC [GGAA]12 [GGCA]6	0.0068
16[GGAA]11 [GGCA]5	0.0019	21[GGAA]15 [GGCA]6	0.0014
17[GGAA]11 [GGCA]6	0.1366	21[GGAA]12 [GGCA]9	0.0005
17[GGAA]13 [GGCA]4	**0.0029**	21[GGAA]16 [GGCA]5	0.0005
17[GGAA]10 [GGCA]7	0.0014	21[GGAA]17 [GGCA]4	0.0005
17[GGAA]12 [GGCA]5	0.0010	22[GGAA]2 GGAC [GGAA]12 [GGCA]7	0.0410
18[GGAA]12 [GGCA]6	= 14.2%	22[GGAA]2 GGAC [GGAA]13 [GGCA]6	0.0145
18[GGAA]11 [GGCA]7		22[GGAA]15 [GGCA]7	0.0101
18[GGAA]14 [GGCA]4	0.0024	22[GGAA]14 [GGCA]8	0.0043
18[GGAA]13 [GGCA]5	0.0019	22[GGAA]13 [GGCA]9	0.0039
18[GGAA]8 GAAA [GGAA]2 [GGCA]7	0.0010	22[GGAA]16 [GGCA]6	0.0010
18[GGAA]15 [GGCA]3	0.0005	22[GGAA]2 GGAC [GGAA]14 [GGCA]5	0.0005
19[GGAA]12 [GGCA]7	0.1076	23[GGAA]2 GGAC [GGAA]13 [GGCA]7	0.0960
19[GGAA]13 [GGCA]6	0.0333	23[GGAA]2 GGAC [GGAA]14 [GGCA]6	0.0130
19[GGAA]11 [GGCA]8	0.0024	23[GGAA]16 [GGCA]7	0.0029
19[GGAA]14 [GGCA]5	0.0024	23[GGAA]14 [GGCA]9	0.0024
19[GGAA]2 GGAC [GGAA]10 [GGCA]6	0.0014	23[GGAA]2 GGAC [GGAA]12 [GGCA]8	0.0019
19[GGAA]9 GAAA [GGAA]2 [GGCA]7	0.0005	23[GGAA]15 [GGCA]8	0.0019
19[GGAA]11 GGGA [GGCA]7	0.0005	24[GGAA]2 GGAC [GGAA]14 [GGCA]7	0.0835
19[GGAA]16 [GGCA]3	0.0005	24[GGAA]2 GGAC [GGAA]15 [GGCA]6	0.0106
20[GGAA]13 [GGCA]7	0.0893	24[GGAA]2 GGAC [GGAA]13 [GGCA]8	0.0024
20[GGAA]2 GGAC [GGAA]10 [GGCA]7	0.0121	24[GGAA]15 [GGCA]9	0.0005
20[GGAA]14 [GGCA]6	0.0092	**25[GGAA]2 GGAC [GGAA]15 [GGCA]7**	**0.0734**
20[GGAA]10 GAAA [GGAA]2 [GGCA]7	0.0087	25[GGAA]2 GGAC [GGAA]14 [GGCA]8	0.0072
20[GGAA]12 GGGA [GGCA]7	0.0068	25[GGAA]2 GGAC [GGAA]16 [GGCA]6	0.0029
20[GGAA]12 [GGCA]8	0.0043	26[GGAA]2 GGAC [GGAA]16 [GGCA	= 8.3%
20[GGAA]16 [GGCA]4	0.0010	26[GGAA]2 GGAC [GGAA]15 [GGCA	
20[GGAA]2 GGAC [GGAA]11 [GGCA]6	0.0005	26[GGAA]2 GGAC [GGAA]17 [GGCA]6	0.0014
20[GGAA]2 GGAC [GGAA]9 AGAA [GGCA]7	0.0005	26[GGAA]2 GGAC [GGAA]18 [GGCA]5	0.0005
20[GGAA]15 [GGCA]5	0.0005	27[GGAA]2 GGAC [GGAA]17 [GGCA]7	0.0014
		27[GGAA]2 GGAC [GGAA]16 [GGCA]8	0.0005

Combined set of allele frequencies taken from Gettings et al. Sequence-based U.S. population data for 27 autosomal STR loci. Forensic Sci Int Genet. 2018 37:106-115

67 alleles observed

Microhaplotypes

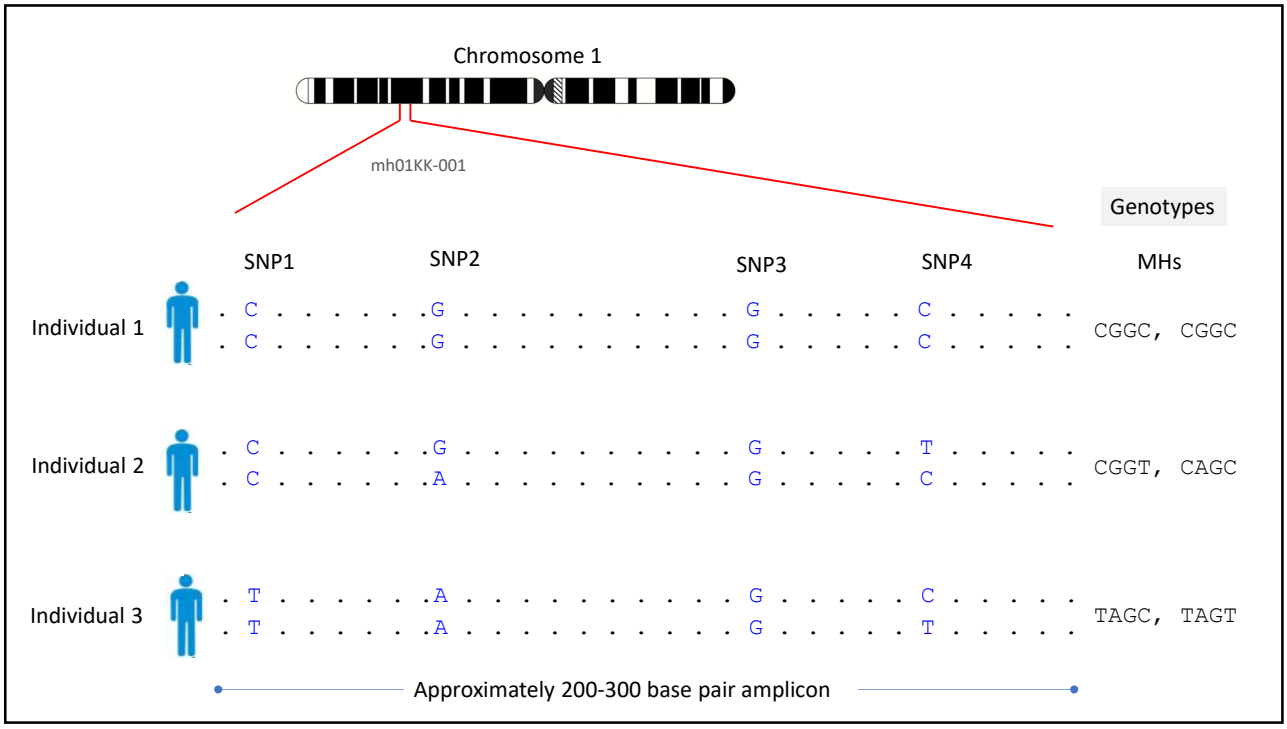


Microhaplotypes

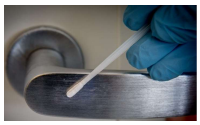
- Novel type of marker of < 300 base pairs
- Defined by two or more closely linked SNPs associated in multiple allelic combinations
  - Similar size amplicons can be used in a multiplex (not length-based alleles)
  - Each allele from a locus will be the same size
- Can also be used for ancestry prediction
- (Typically) fewer alleles than a STR locus

Absence of stutter as the alleles are SNP-defined versus a repeating motif

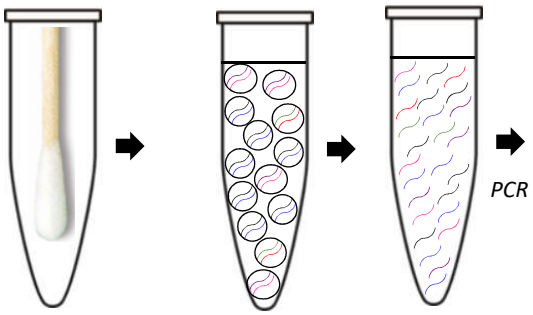
- Still enriched by targeted PCR
- Allele frequencies are published (and still being generated)
- No core law enforcement database
- Will require a framework for interpretation (probabilistic genotyping?)



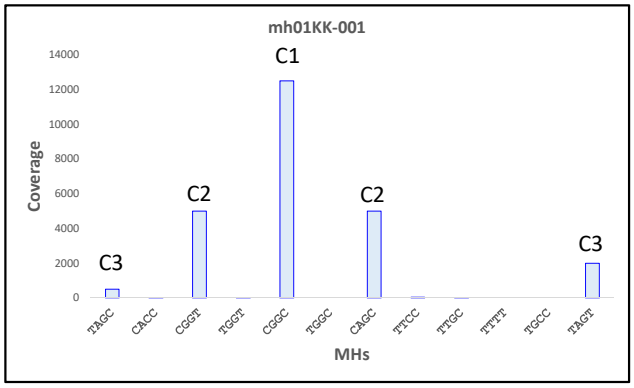
Microhaplotypes



Collection of evidence



Evidence Processing *Extraction* *Post extraction*



Contributor 1 with a "CGGC, CGGC" microhaplotype
Contributor 2 with a "CGGT, CAGC" microhaplotype
Contributor 3 with a "TAGC, TAGT" microhaplotype

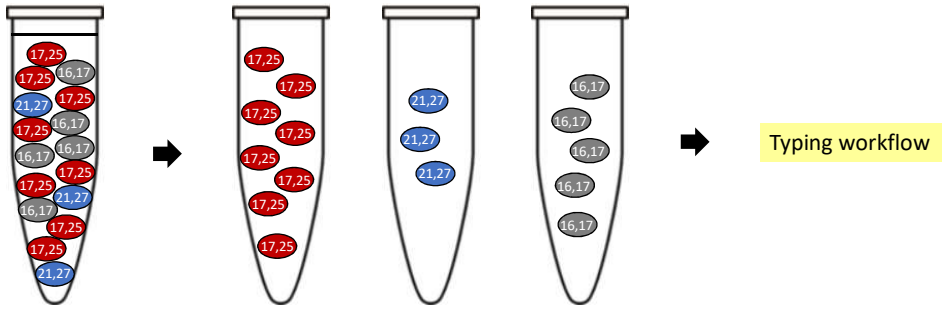
Will still observe some 'noise'
Hb imbalance at lower levels of DNA input (C3)
No stutter artifacts

Sequencing *Microhaplotypes for Mixtures*

In Comparison to CE/STR methods	Comment
~~• Additional~~ Microhaplotype-based alleles	~~• "Unmasking" of alleles identical by length~~ • Improve number of contributor estimates • ***Microhaplotype***-based allele frequencies are applied ~~• Length-based alleles are back compatible to current databases~~
• Currently using targeted PCR	• Comparable sensitivity to CE • May have an increased input range (> 2 ng) • Subject to stochastic effects
~~• Stutter products are sequenced~~ • **No stutter artifacts**	~~• *Potential* to correlate stutter product(s) to parent allele~~ ~~• Allow for a more accurate modeling of stutter products.~~
• Signal thresholds	• Discern noise (from instrument, PCR, seq, library error) from an allele; determine an AT
• Artifacts	• No dye artifacts; other concerns?
• Shorter PCR amplicons (compared to CE)	• Improved performance with degraded samples
• Larger multiplexes	• A need for microhaplotype loci
• Interpretation	• A NGS-based probabilistic genotyping model for MHs?

Physical Separation of Cells

General concept – physical separation/sorting of cells before DNA typing workflow



Physical Separation of Cells – How?!

- Some proposed methods
  - Partition into microreactors
  - Micro-manipulation (needle, laser)
  - Sort based on cell morphology or tagging

Challenges

- Dried cells are more challenging than fresh solutions
- Single cell sampling methods are lower throughput, may require PCR optimization
- Consider the specificity/sensitivity of reagents that bind cells (antibodies)
- Is there DNA in the cell (and what about cell free DNA)?

Short communication Forensic Science International: Genetics 17 (2015) 8–16

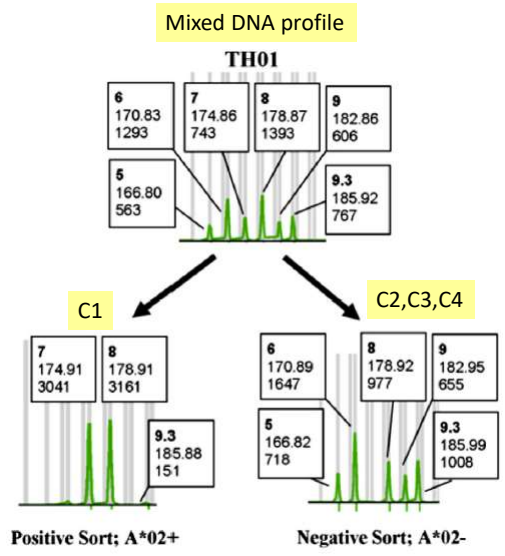
Separation of uncompromised whole blood mixtures for single source STR profiling using fluorescently-labeled human leukocyte antigen (HLA) probes and fluorescence activated cell sorting (FACS)

Lee Dean [a], Ye Jin Kwon [a], M. Katherine Philpott [a], Cristina E. Stanciu [a], Sarah J. Seashols-Williams [a], Tracey Dawson Cruz [a], Jamie Sturgill [b], Christopher J. Ehrhardt [a,*]

[a] Department of Forensic Science, Virginia Commonwealth University, 1015 Floyd Ave, Richmond, VA 23284, USA
[b] School of Nursing, Virginia Commonwealth University, Medical College of Virginia, Richmond, VA 23284, USA

- Four person mixture (C1, C2, C3, C4)
- Binding cells with HLA allele (A*02) antibody
  - C1 is HLA allele (A*02) positive
- FACS separated
- Fractions typed

Contributor genotypes	
C1	7, 8
C2	6, 9
C3	5, 6
C4	8, 9.3



Single source DNA profile recovery from single cells isolated from skin and fabric from touch DNA mixtures in mock physical assaults

Katherine Farash[a], Erin K. Hanson[b], Jack Ballantyne[a,b,c,*]

[a] Graduate Program in Forensic Science, Biochemistry Track, Department of Chemistry, University of Central Florida, PO Box 162366, Orlando, FL 32816-2366, USA
[b] National Center for Forensic Science, PO Box 162367, Orlando, FL 32816-2367, USA
[c] Department of Chemistry, University of Central Florida, PO Box 162366, Orlando, FL 32816-2366, USA

Science & Justice 58 (2018) 191–199

- Avoid 'blind swabbing' that might create mixtures
- A more directed sampling approach ("Smart")

"sensitive, measurable, attainable, relevant, targeted"

- Optimized direct PCR (lysis + PCR)

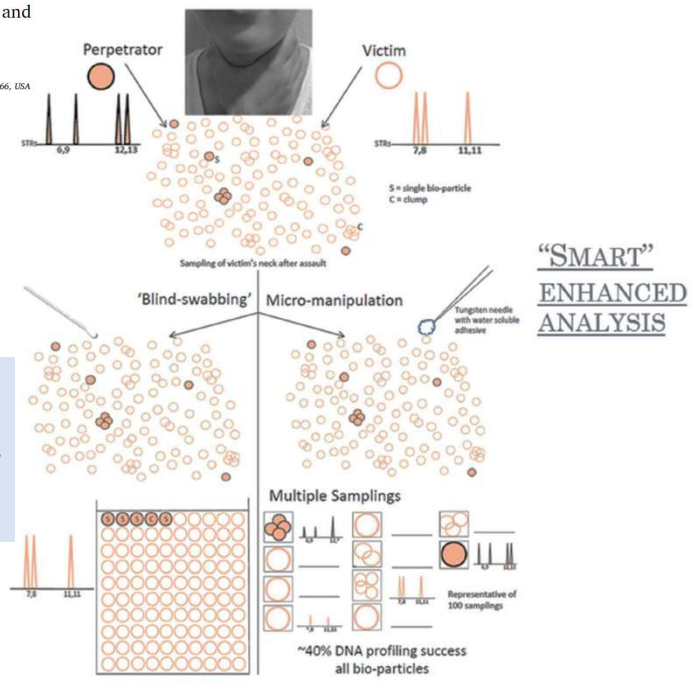
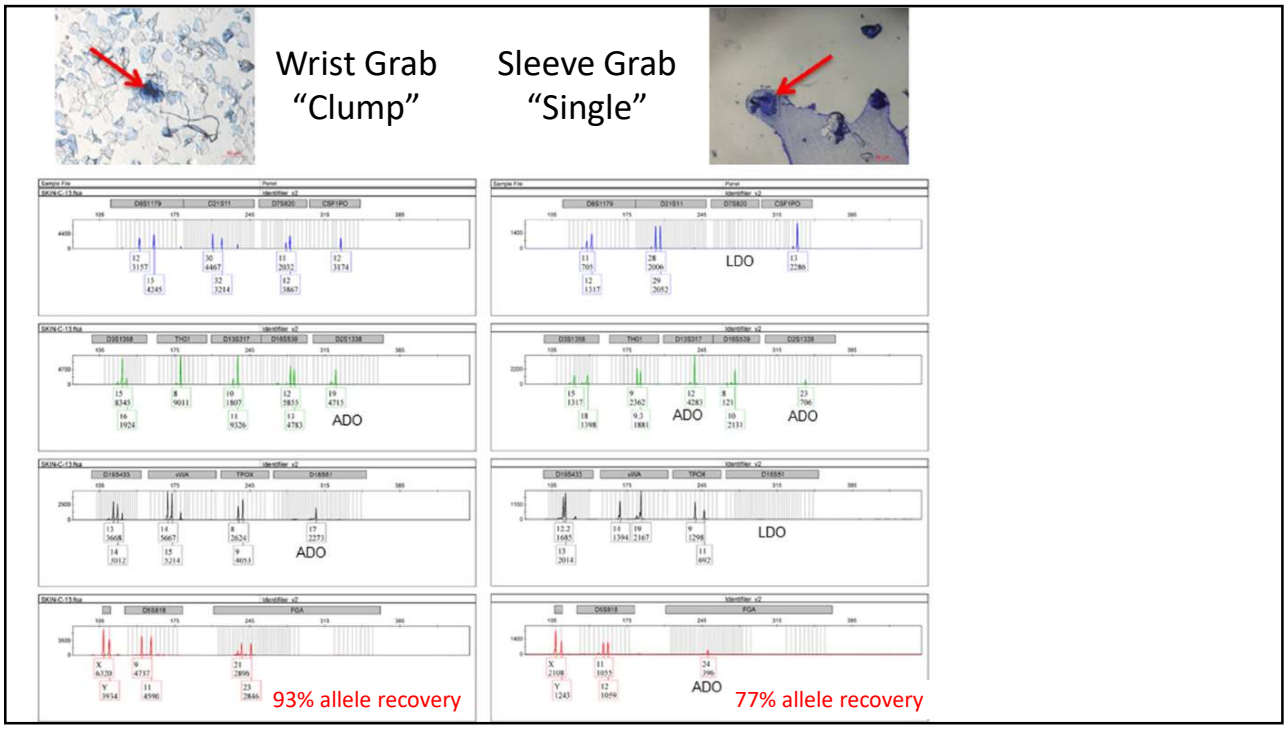
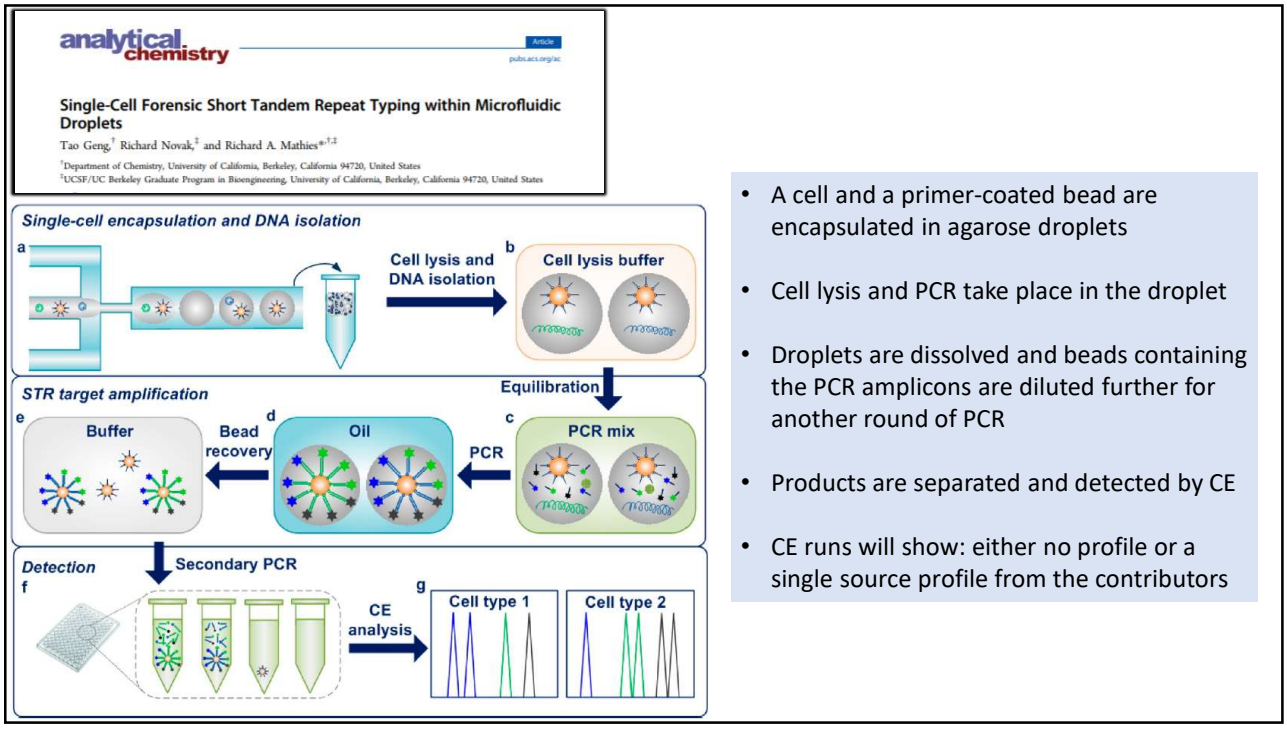
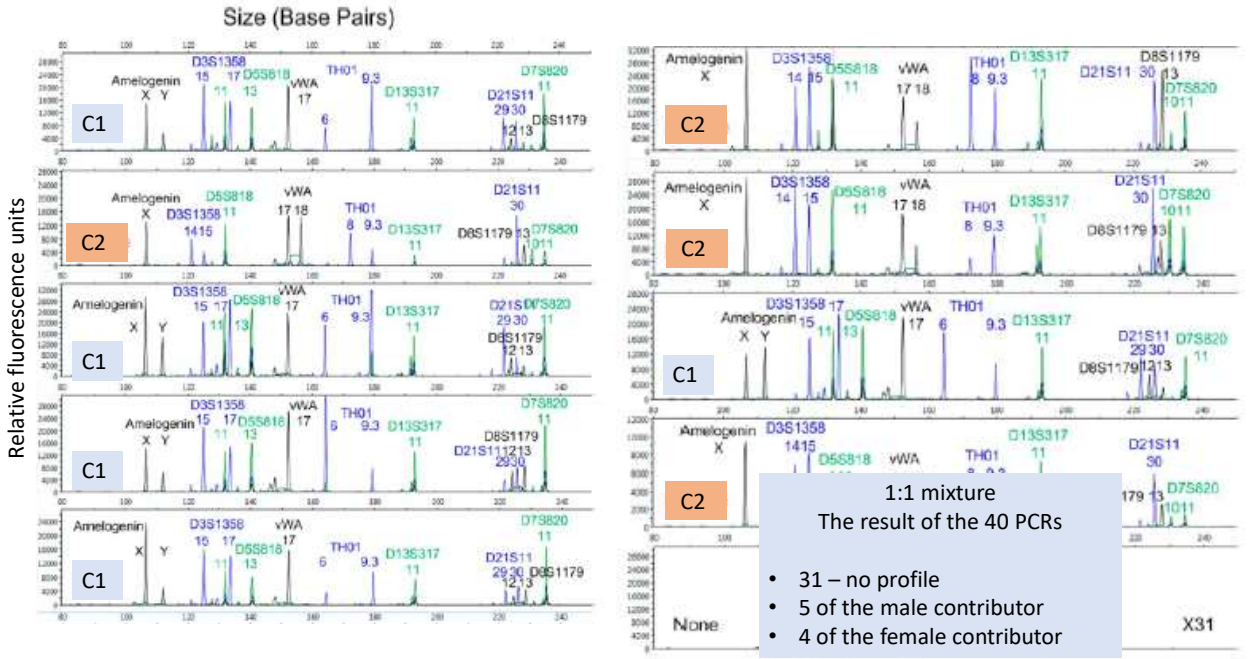


Fig. 1. Schema of standard versus "smart" enhanced analysis of touch DNA. Cells from the victim and perpetrator are indicated as unfilled and filled circles respectively.





- A cell and a primer-coated bead are encapsulated in agarose droplets
- Cell lysis and PCR take place in the droplet
- Droplets are dissolved and beads containing the PCR amplicons are diluted further for another round of PCR
- Products are separated and detected by CE
- CE runs will show: either no profile or a single source profile from the contributors



Thoughts and considerations

- Define and understand the problem
- Understand the potential of the new technology to address the problem
- Consider the cost of implementation as a whole
- There is always something new on the horizon

Thank you for your attention! Questions?

Contact: Peter.Vallone@nist.gov

Thanks to

- **Dr. Sarah Riman (NIST)**
- Members of the NIST mixture review team (John, Hari, Rich, Melissa, and Sheila)
- Funding
  - NIST Special Programs Office: *Forensic DNA*