The Potential of New Technologies

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Leader, Applied Genetics Group, NIST

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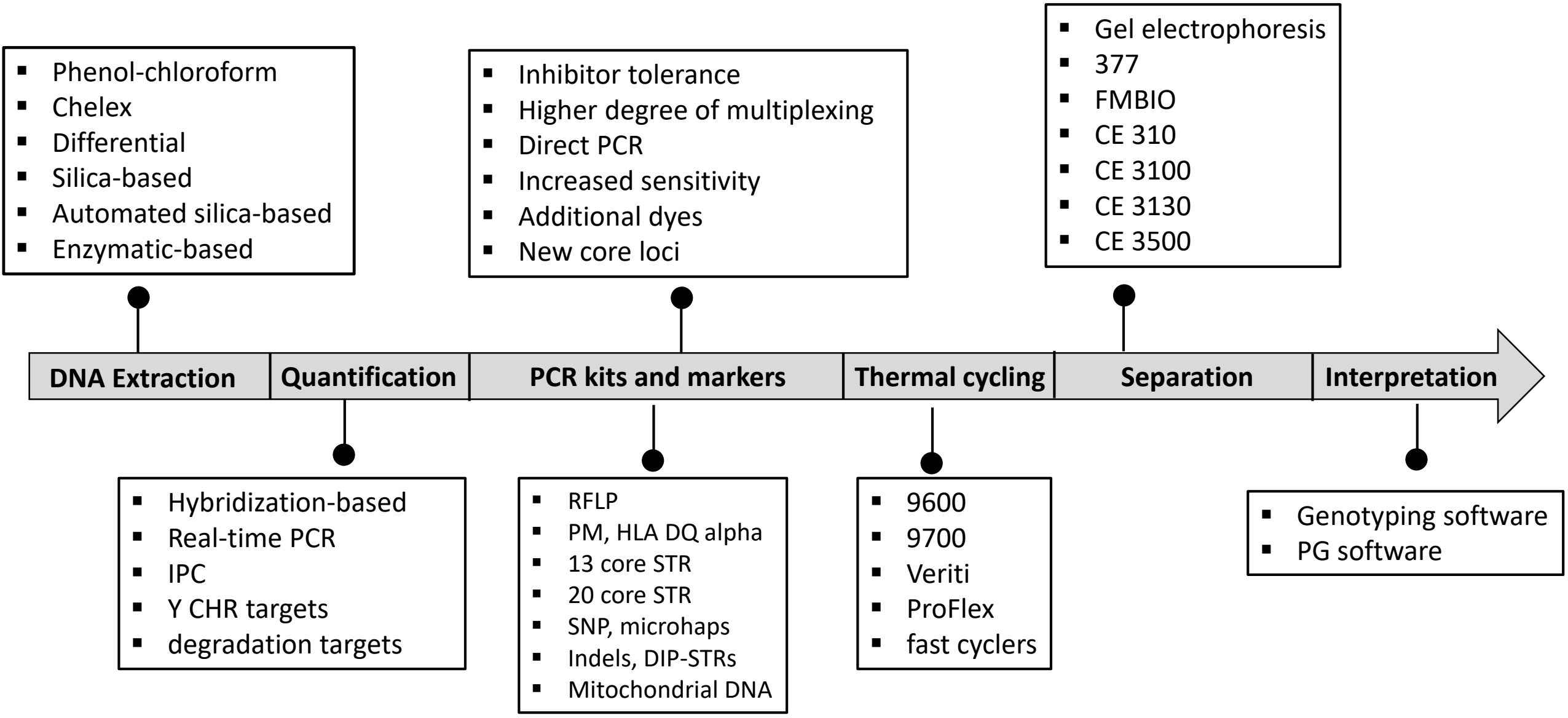
Outline

- 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

New things continue to come our way…

- Rapid DNA
- **NGS/MPS – Sequencing**
- Investigative genealogy
- **Cell separation techniques**

horesis

- Phenol-chloroform
- Chelex
- Differential
- Silica-based
- Automated silica-based
- Enzymatic-based

DNA Extraction | **Quantification** | **PCR kits and markers** | **Thermal cycling** | **Separation** | **Interpretation**

- Hybridization-based
- Real-time PCR
- IPC
- Y CHR targets
- degradation targets

- RFLP
- PM, HLA DQ alpha
- 13 core STR
- 20 core STR
- SNP, microhaps
- Indels, DIP-STRs
- Mitochondrial DNA

- 9600
- 9700
- Veriti
- ProFlex
- fast cyclers

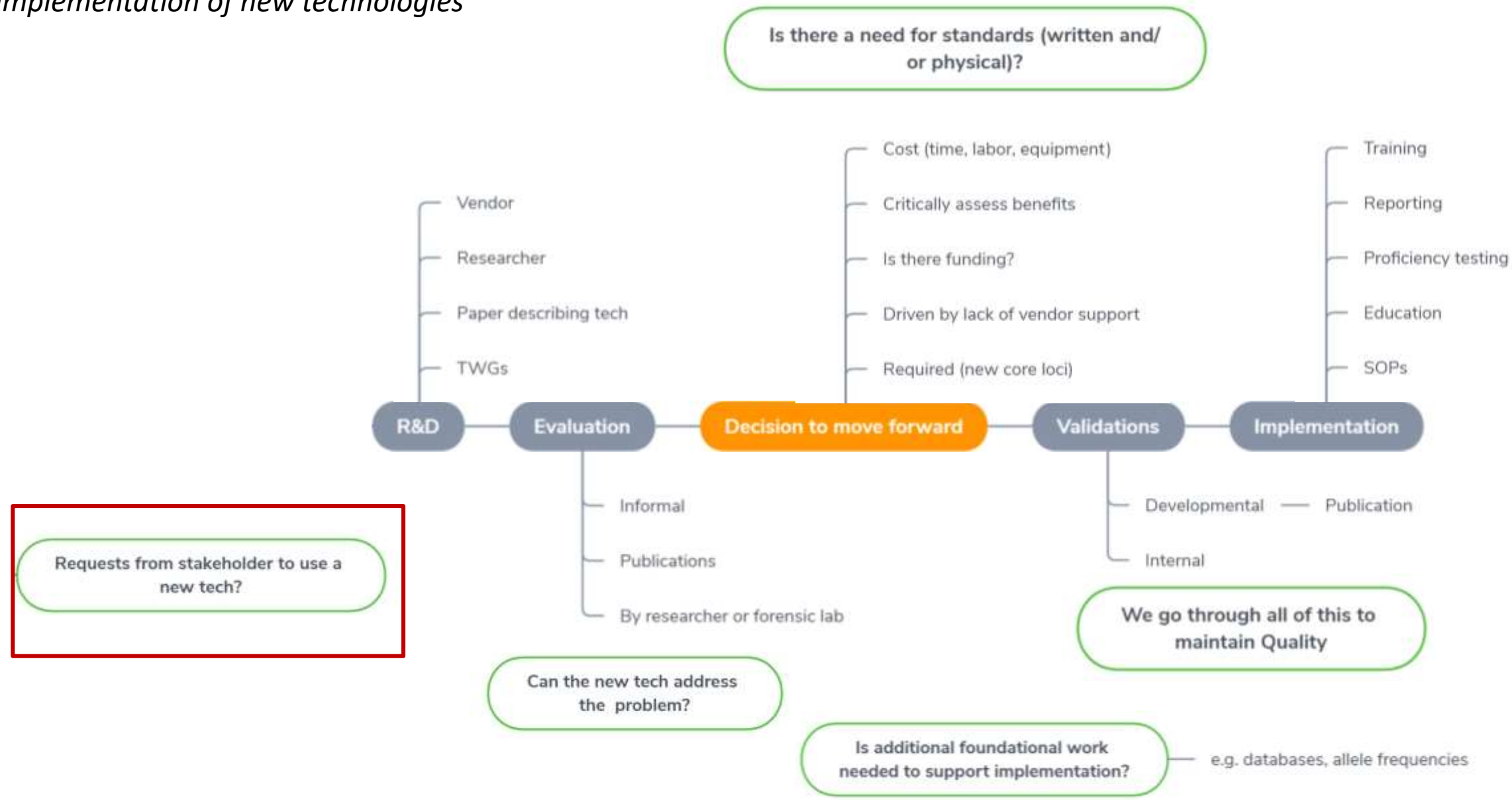
- Genotyping software
- PG software

Analysis of a DNA Mixture

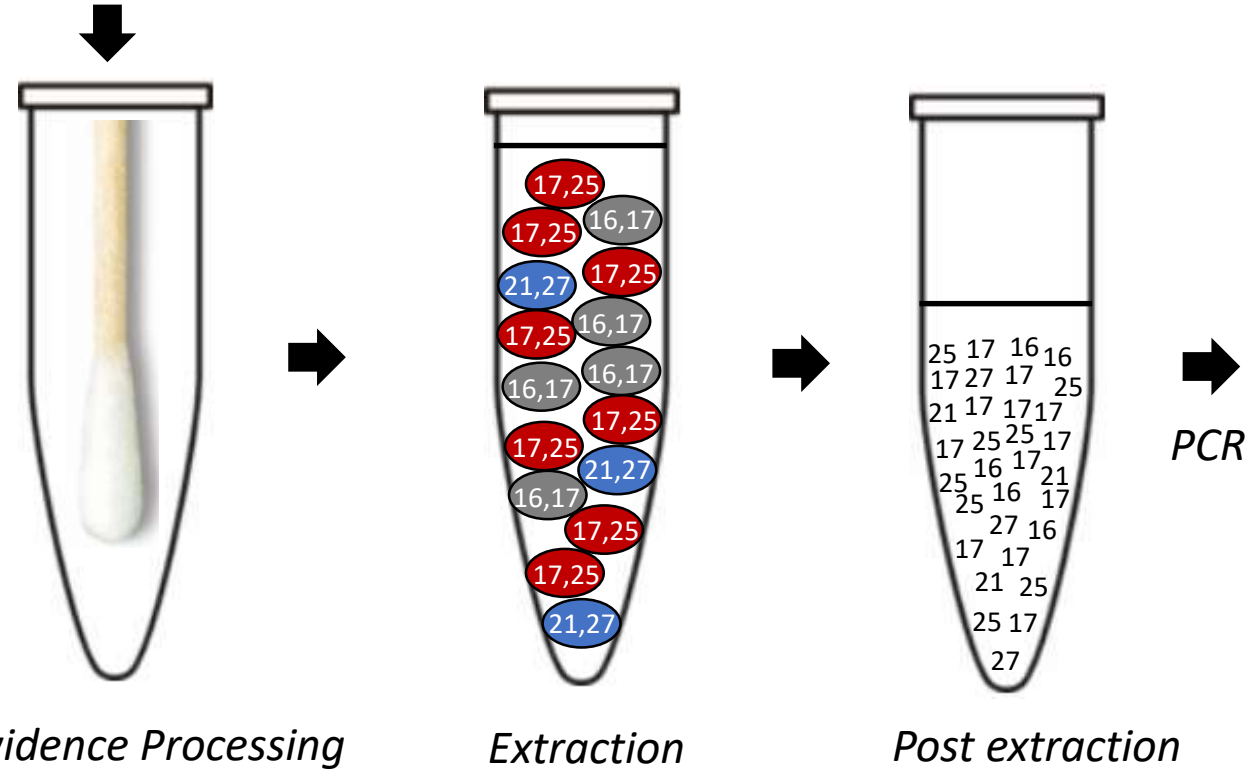
- DNA from two or more contributors are deposited
  - DNA may be in the cell or cell free
- Post DNA ***extraction***, the alleles from all contributors are mixed together
  - DNA may be lost/reduced in the purification process
- ***PCR*** amplifies the alleles present post DNA extraction
  - Stochastic 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

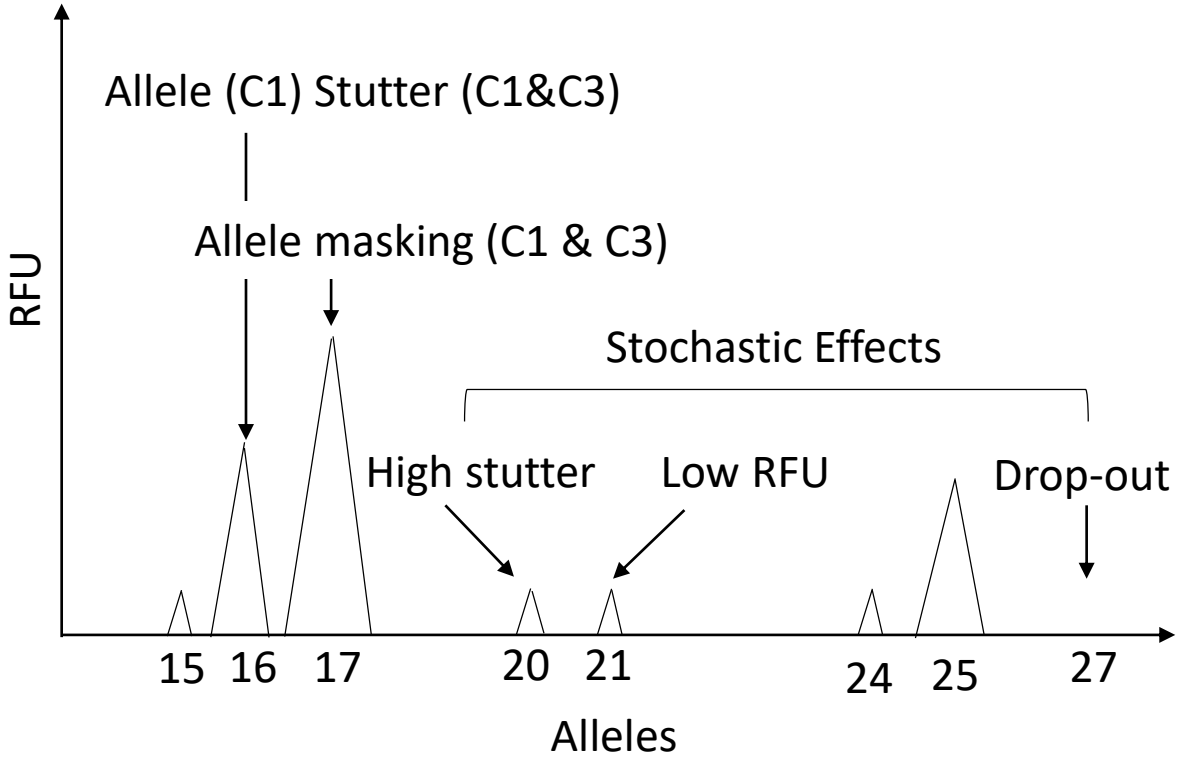


Collection of evidence



Evidence Processing *Extraction* *Post extraction*

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

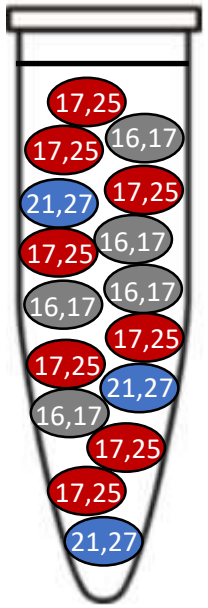
- Technology can be applied to type additional markers systems
  - Additional non-CODIS STR markers
  - Insertion=deletion markers
  - Mitochondrial (control region, whole mitochondrial genome)
  - SNPs
    - Ancestry, Phenotype, ID, **Microhaplotypes**

Sequencing *STRs for Mixtures*

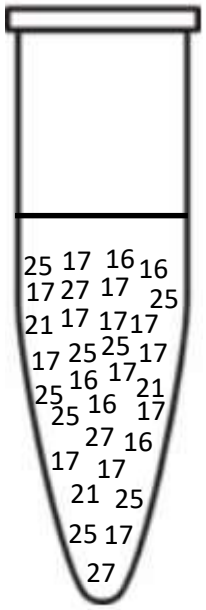
In Comparison to CE methods	Comment
• Additional alleles	• "Unmasking" of alleles identical by length • Improve number of contributor estimates • Sequence-based allele frequencies are applied • Length-based alleles are back compatible to current databases
• Currently using targeted PCR	• Comparable sensitivity 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?

Collection of evidence

Evidence Processing



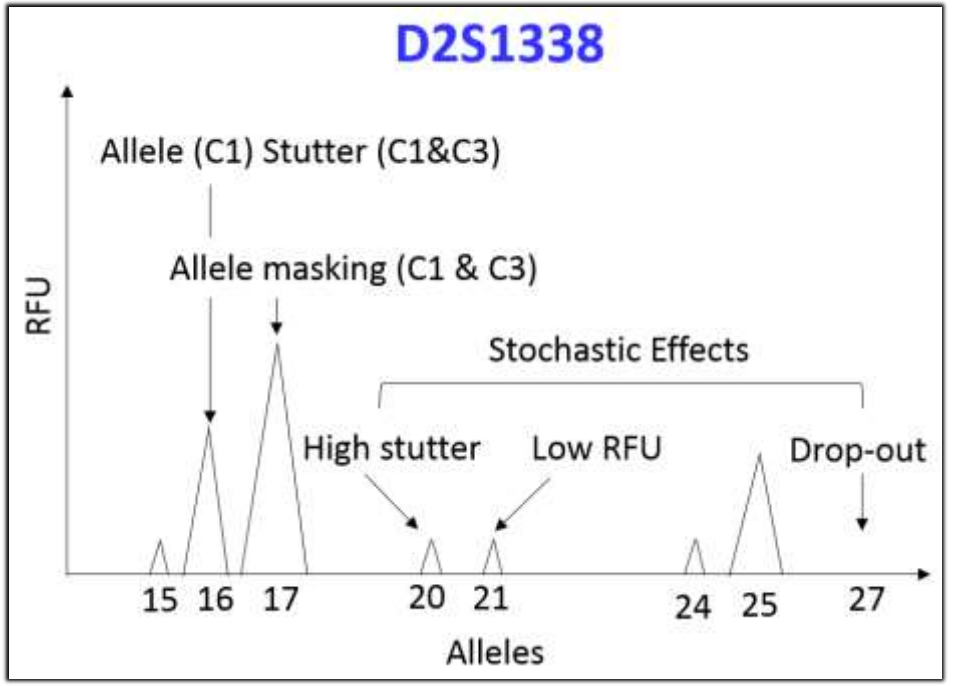
Extraction



Post extraction

PCR

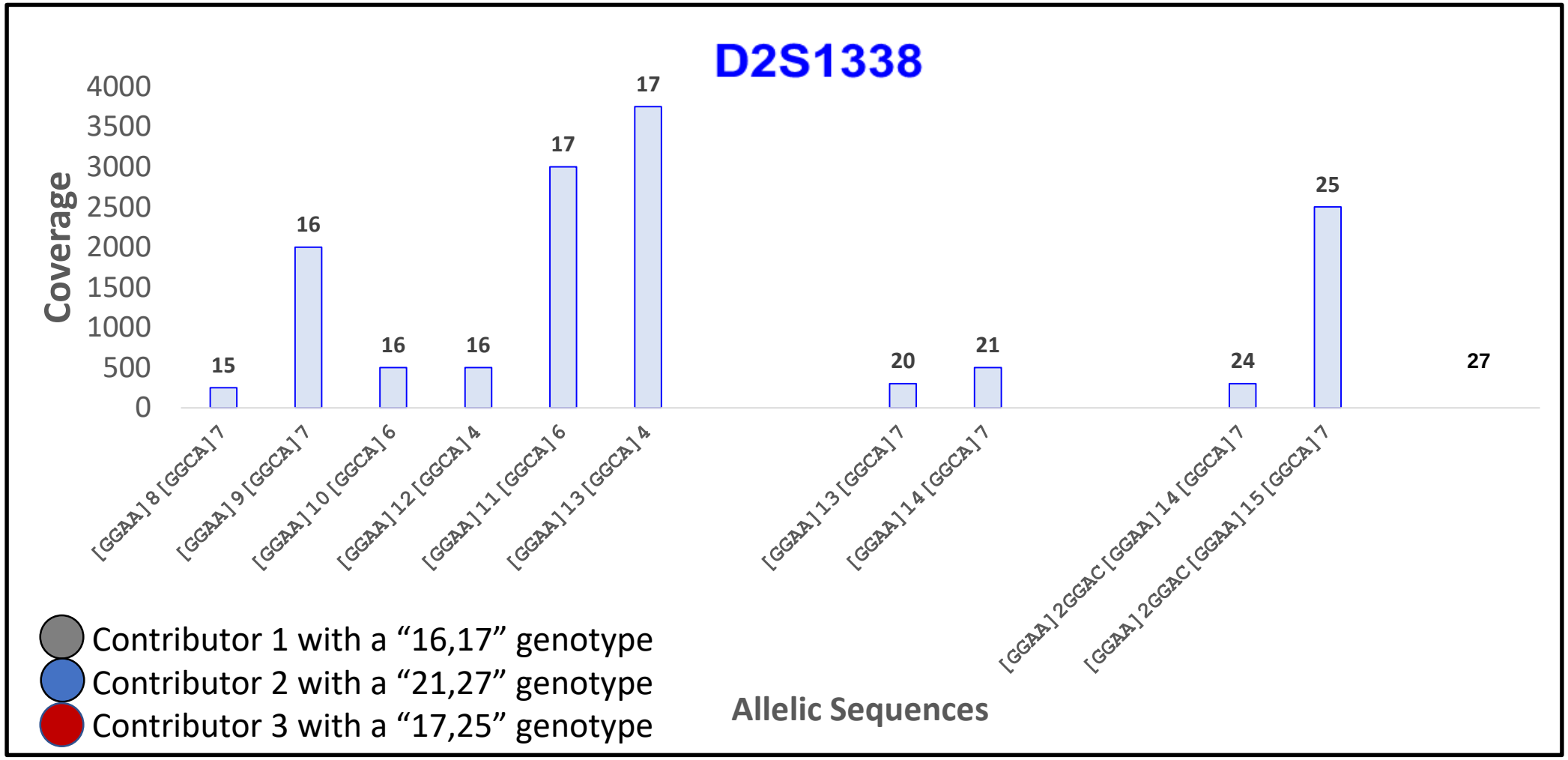
CE



NGS data?

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

Looking at the data in 'Sequencing space'



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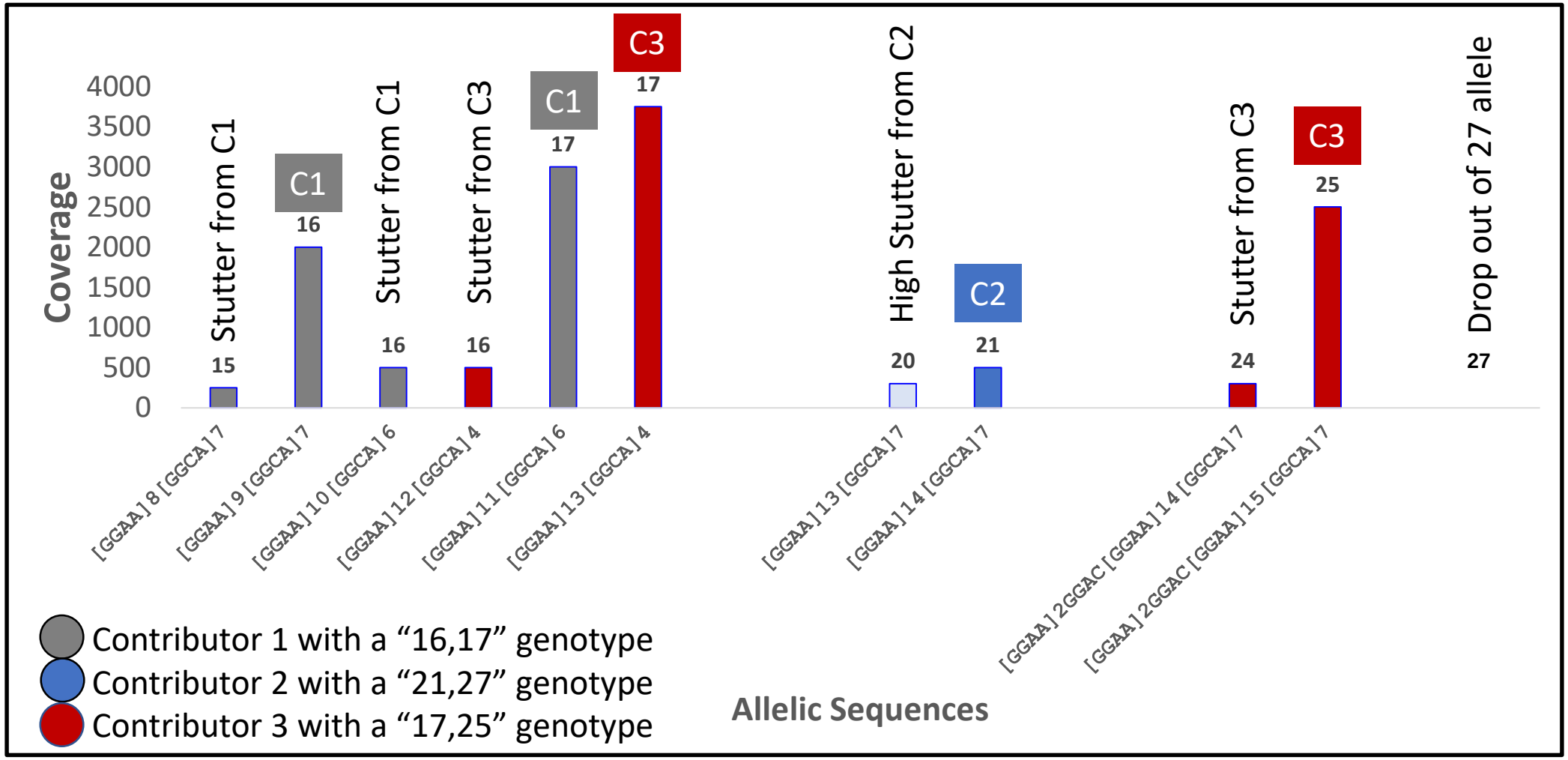
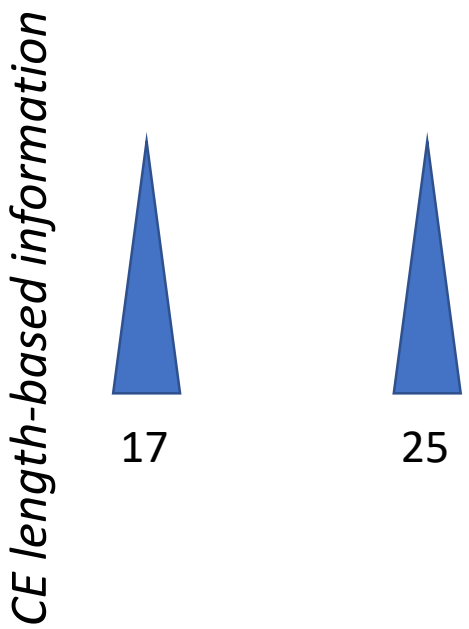


Locus D2S1338 - Length-based allele frequencies



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

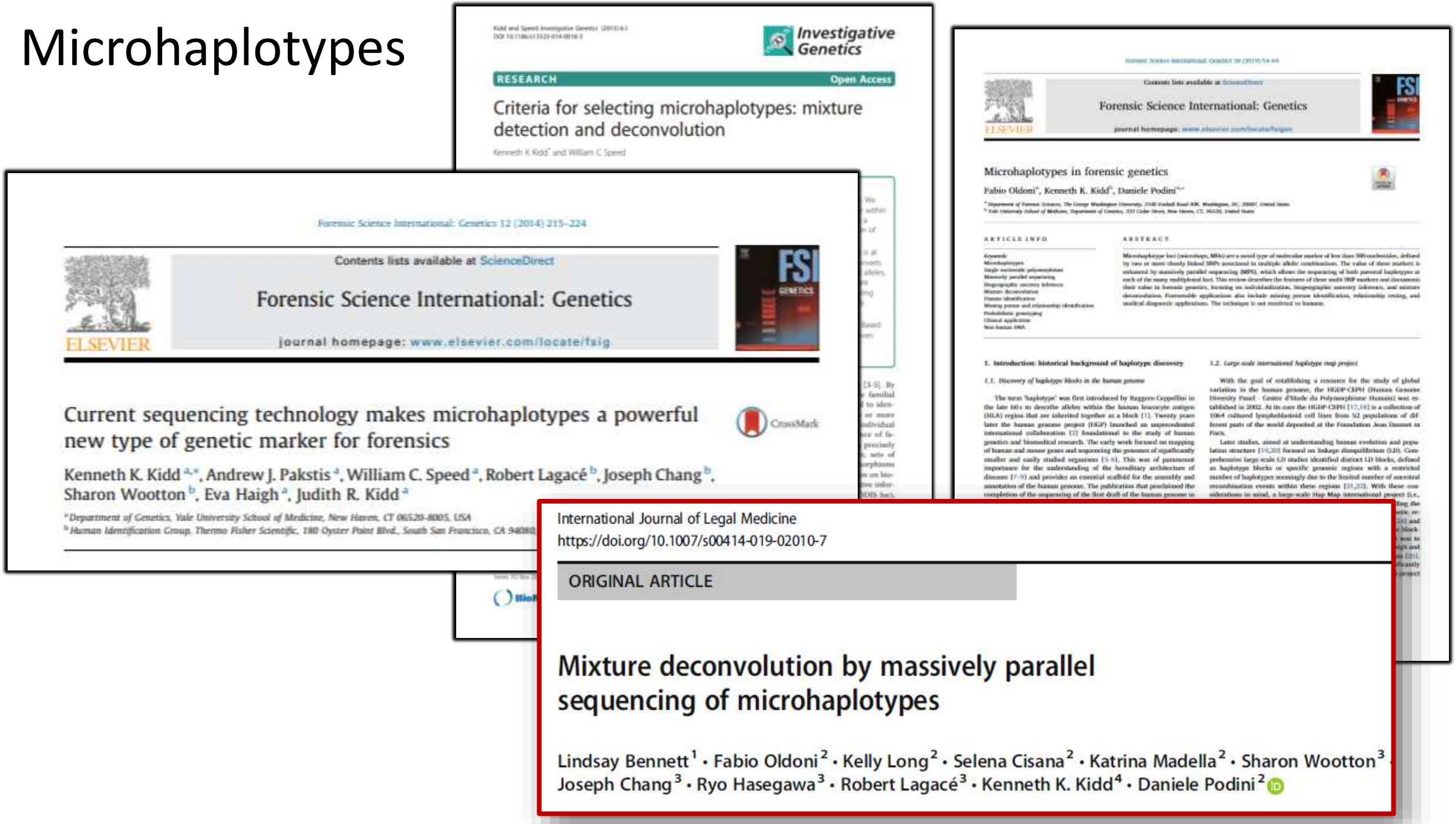
…1 in 2,349

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

67 alleles observed

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

Microhaplotypes



Investigative Genetics

RESEARCH Open Access

Criteria for selecting microhaplotypes: mixture detection and deconvolution

Kenneth K Kidd* and William C Speed

Forensic Science International: Genetics

Microhaplotypes in forensic genetics

Fabio Oldoni, Kenneth K. Kidd, Daniele Podini

Forensic Science International: Genetics 12 (2014) 215–224

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Current sequencing technology makes microhaplotypes a powerful new type of genetic marker for forensics

Kenneth K. Kidd, Andrew J. Pakstis, William C. Speed, Robert Lagacé, Joseph Chang, Sharon Wootton, Eva Haigh, Judith R. Kidd

International Journal of Legal Medicine
https://doi.org/10.1007/s00414-019-02010-7

ORIGINAL ARTICLE

Mixture deconvolution by massively parallel sequencing of microhaplotypes

Lindsay Bennett · Fabio Oldoni · Kelly Long · Selena Cisana · Katrina Madella · Sharon Wootton · Joseph Chang · Ryo Hasegawa · Robert Lagacé · Kenneth K. Kidd · Daniele Podini

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?)

mh01KK-001

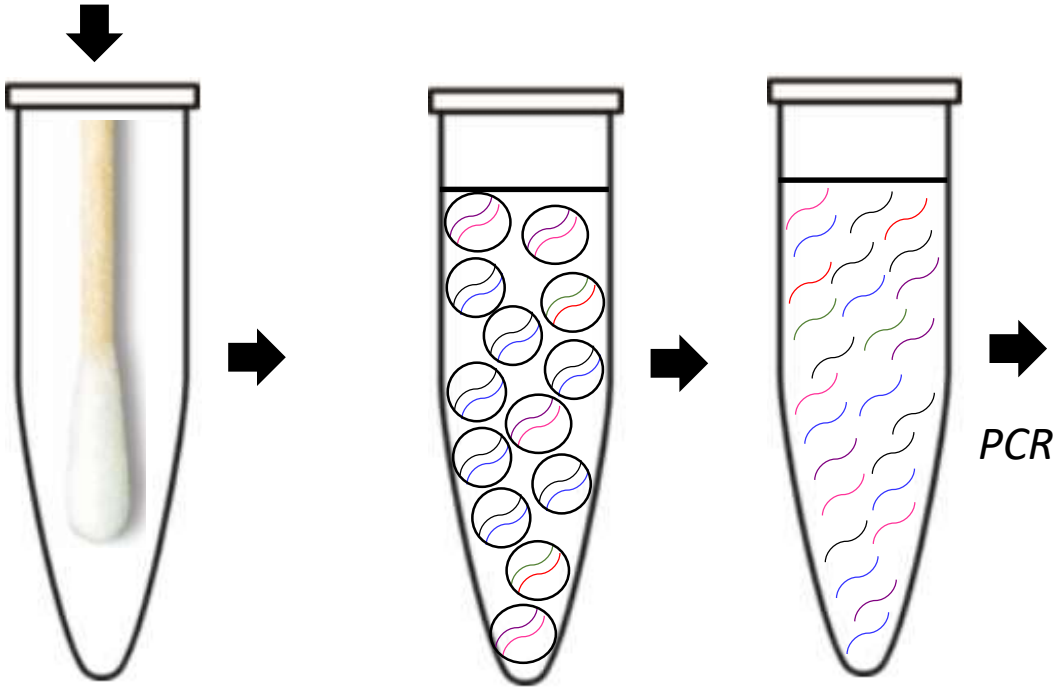
Genotypes

	SNP1	SNP2	SNP3	SNP4	MHs
Individual 1	C	G	G	C	CGGC, CGGC
	C	G	G	C	
Individual 2	C	G	G	T	CGGT, CAGC
	C	A	G	C	
Individual 3	T	A	G	C	TAGC, TAGT
	T	A	G	T	

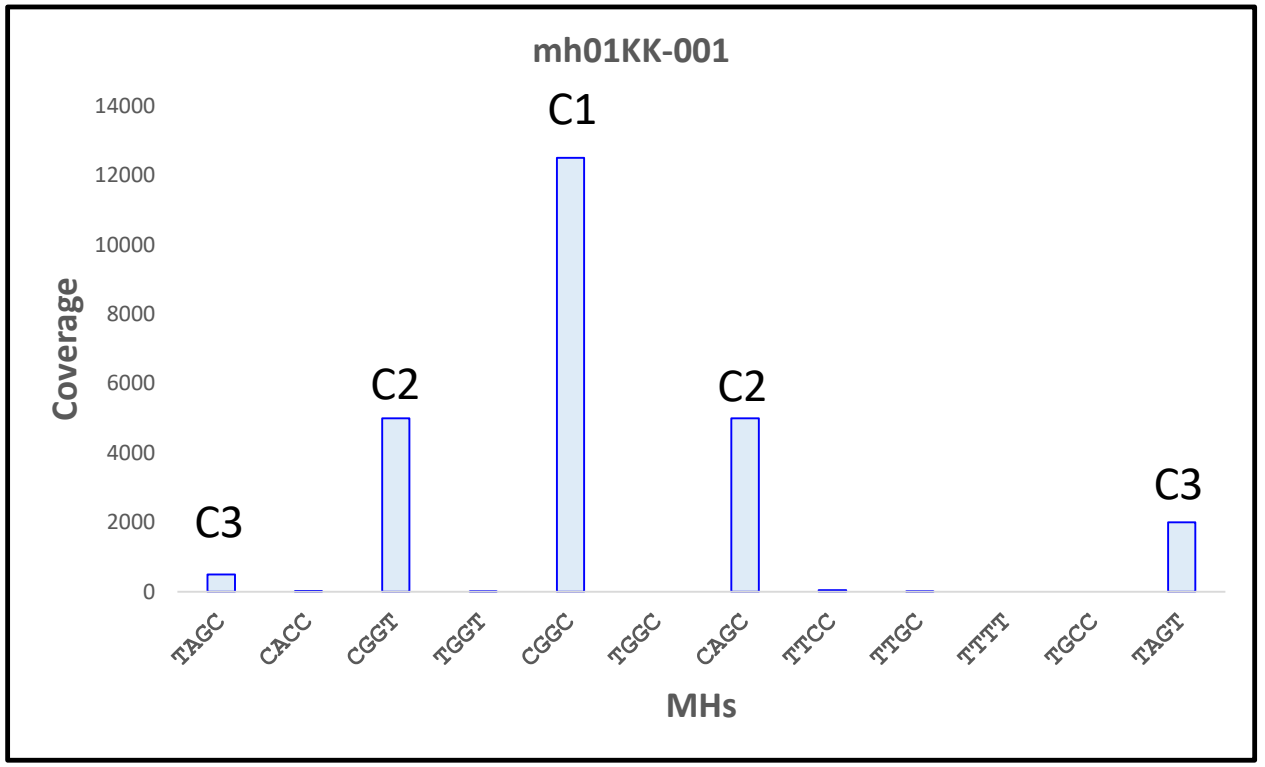
Approximately 200-300 base pair amplicon

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

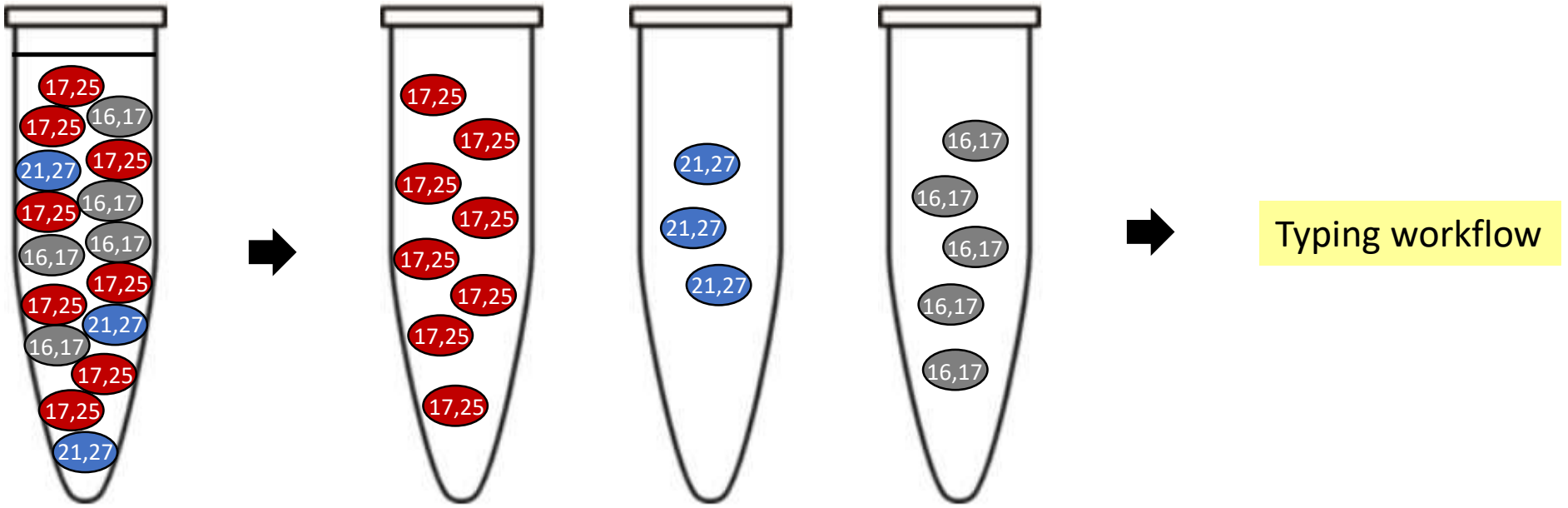
Will still observe some 'noise'
Hb imbalance at lower levels of DNA input (C3)
No stutter artifacts

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

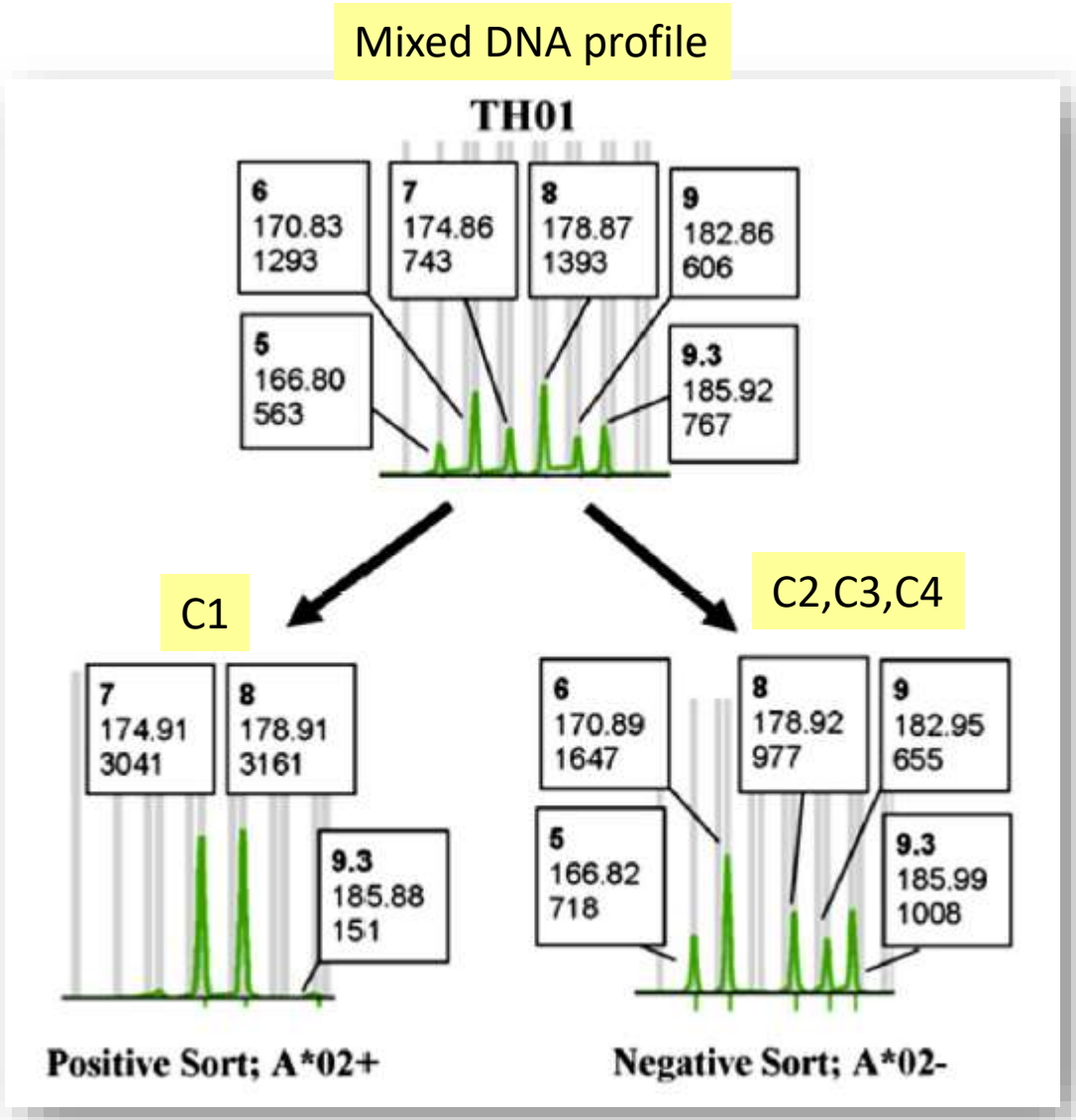
Separation of uncompromised whole blood mixtures for single source STR profiling using fluorescently-labeled human leukocyte antigen (HLA) probes and fluorescence activated cell sorting (FACS)

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)

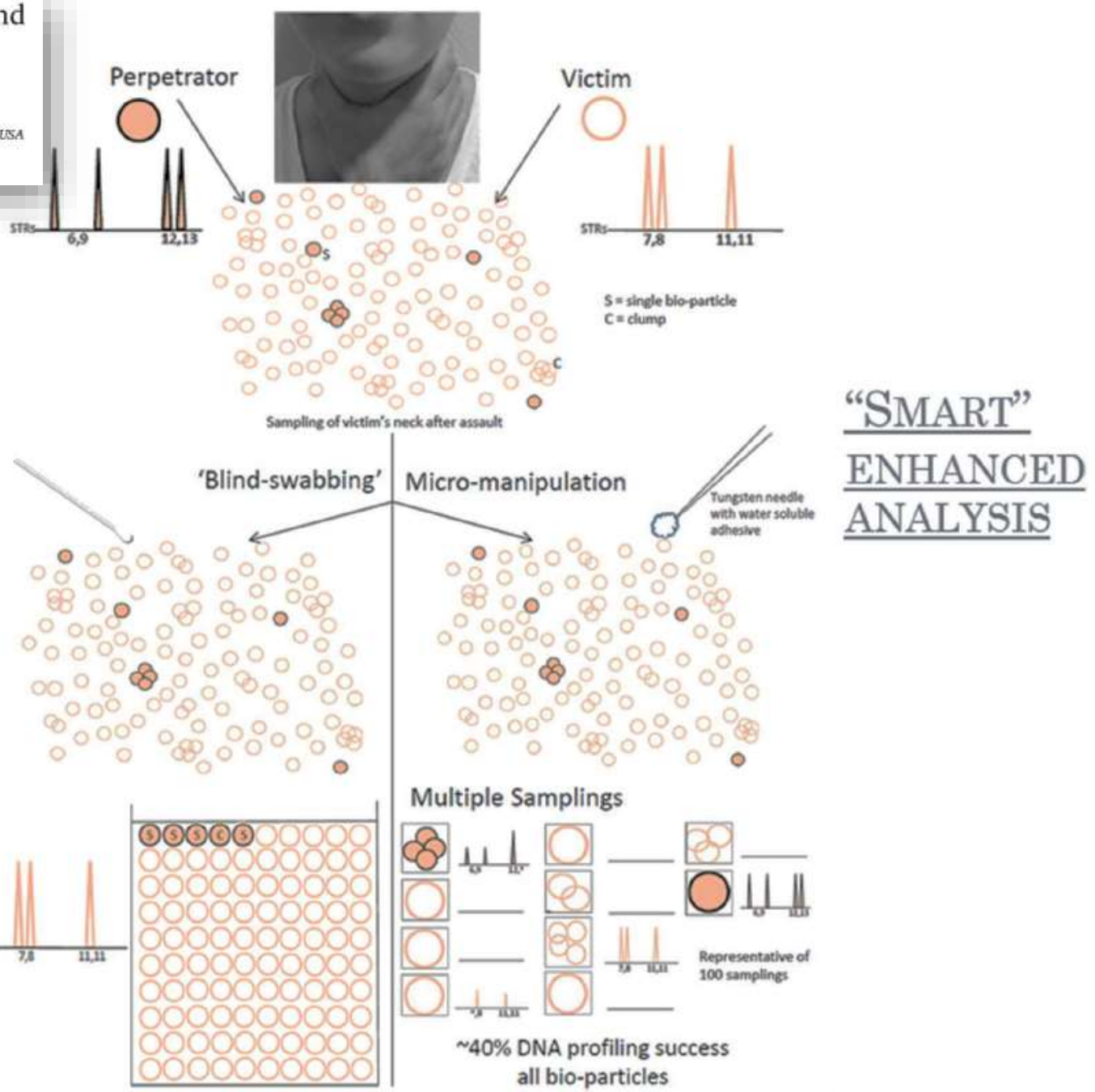
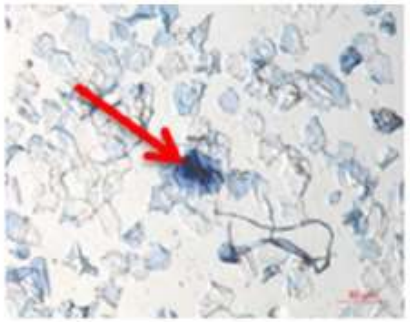
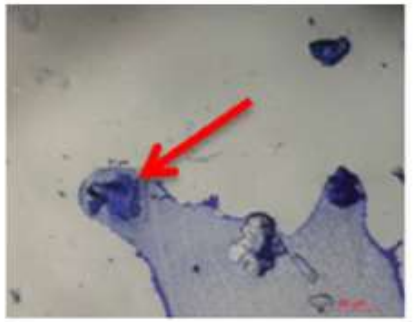


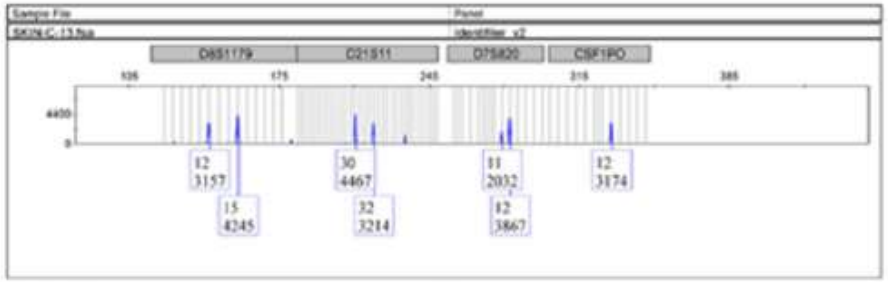
Fig. 1. Schema of standard versus "smart" enhanced analysis of touch DNA. Cells from the victim and perpetrator are indicated as unfilled and filled circles respectively.

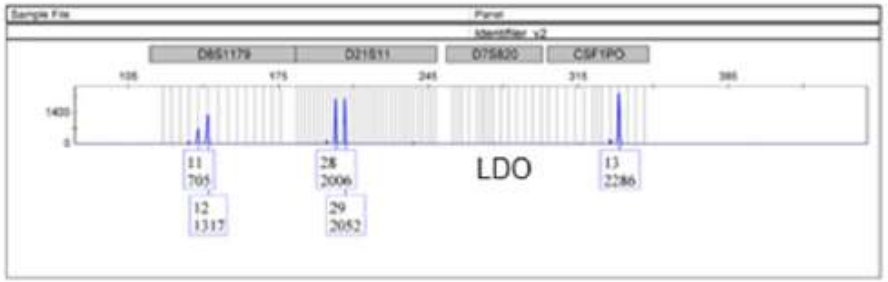


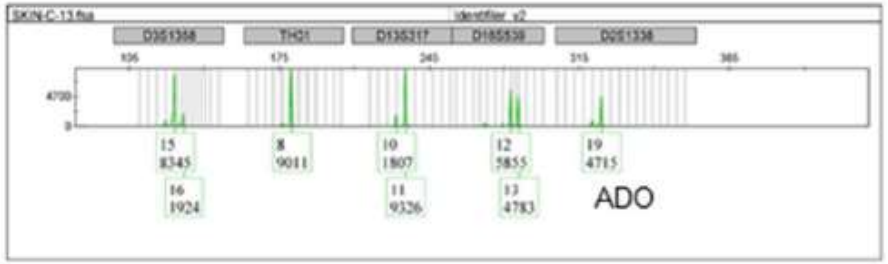
Wrist Grab
"Clump"

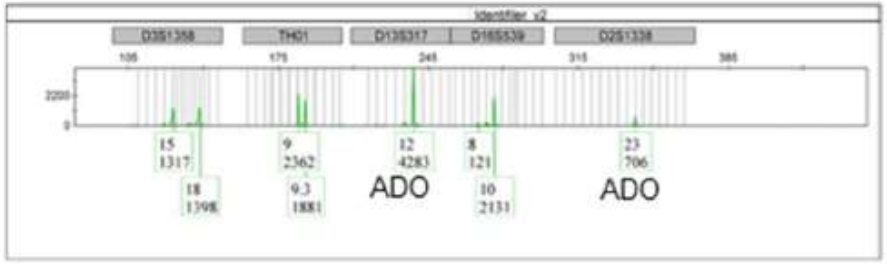
Sleeve Grab
"Single"

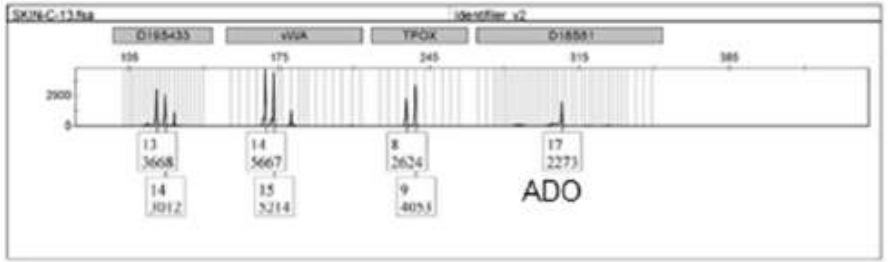


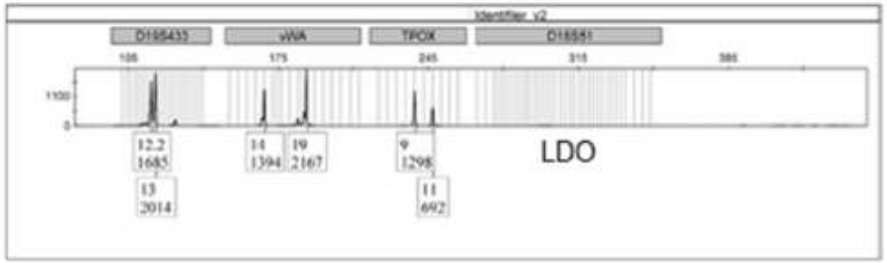


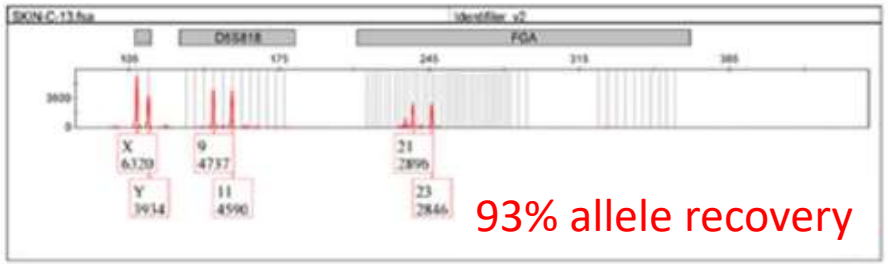




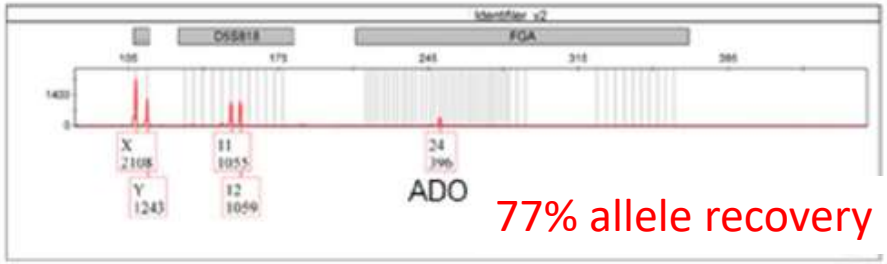








93% allele recovery



77% allele recovery

analytical chemistry
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Single-Cell Forensic Short Tandem Repeat Typing within Microfluidic Droplets

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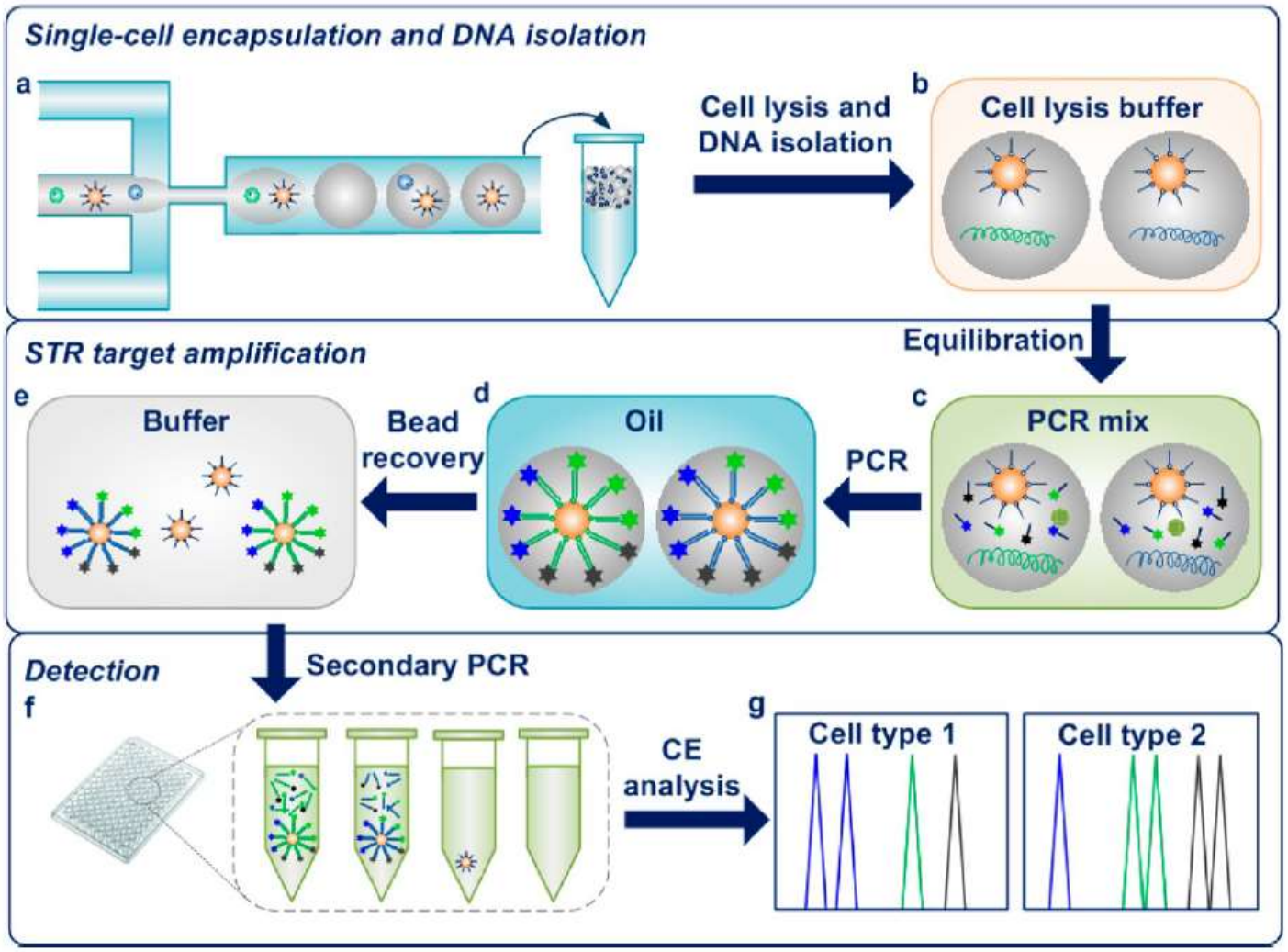
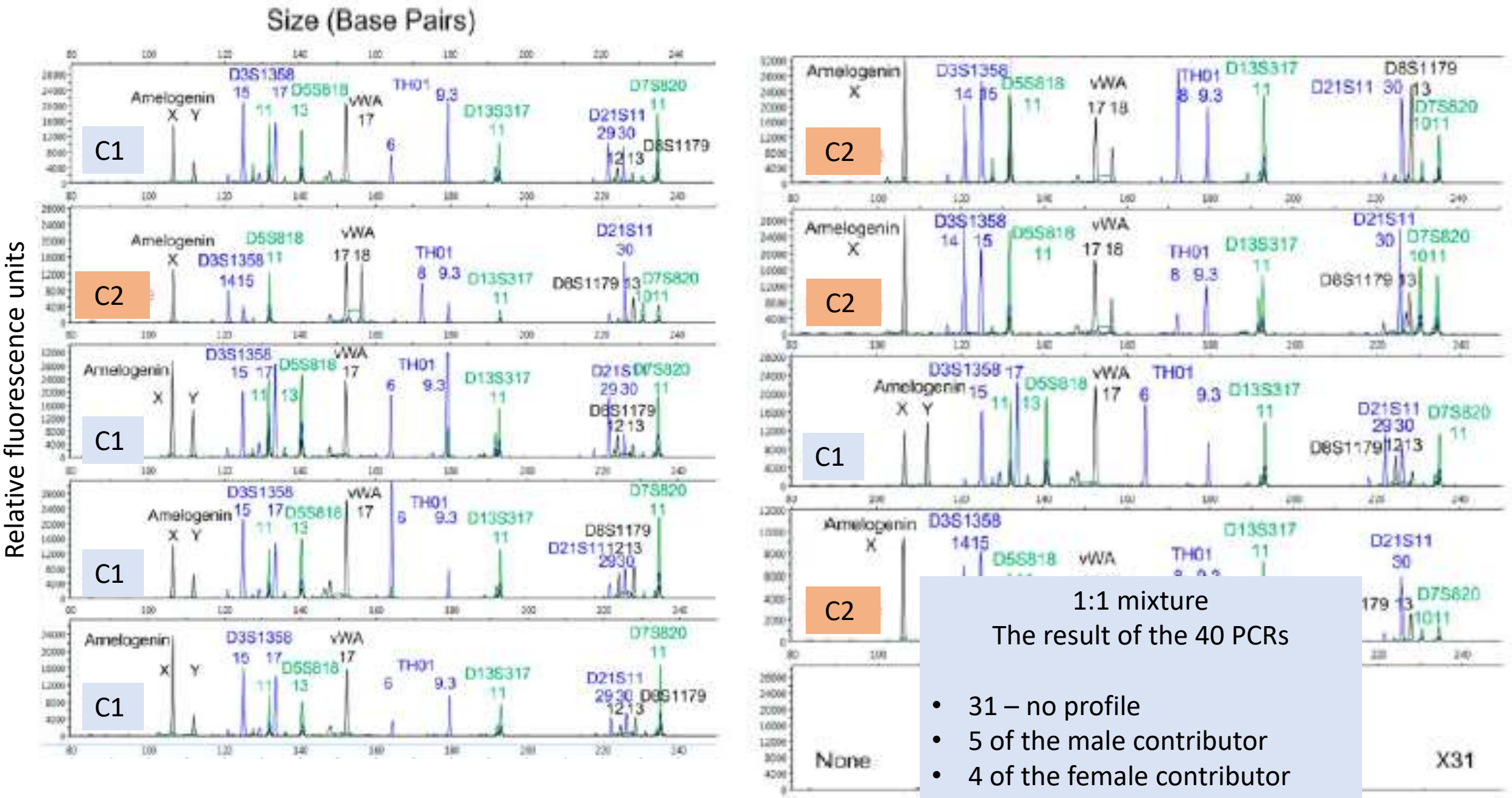


- A cell and a primer-coated bead are encapsulated in agarose droplets
- Cell lysis and PCR take place in the droplet
- Droplets are dissolved and beads containing the PCR amplicons are diluted further for another round of PCR
- Products are separated and detected by CE
- CE runs will show: either no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


Size (Base Pairs)
Relative fluorescence units
C1
C2
C2
C1
C1
C1
C2
C2
C1
None
X31
1:1 mixture
The result of the 40 PCRs
• 31 – no profile
• 5 of the male contributor
• 4 of the female contributor

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

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