Forensic Performance of Short Amplicon Insertion-Deletion (InDel) Markers

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Presentation Outline

1- InDel Polymorphisms: Introduction and Concept.

2- Materials and Methods:

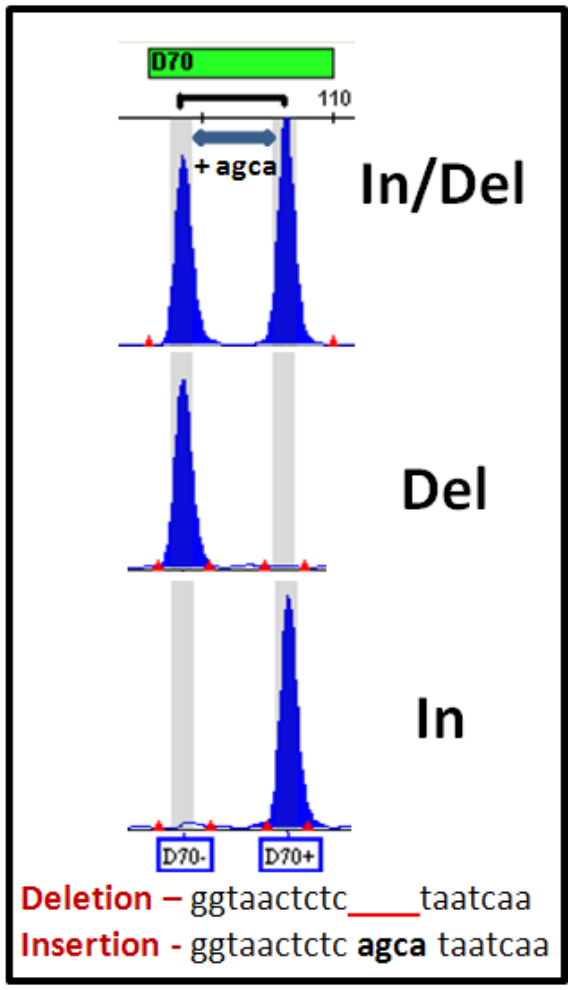
- InDel assays HID-38plex and DIPplex.
- Independence of the markers.

3- Results

- Allele frequency analysis.
- Artificially degraded DNA assay.
- Sequencing of previously unreported variation.

4- Conclusions

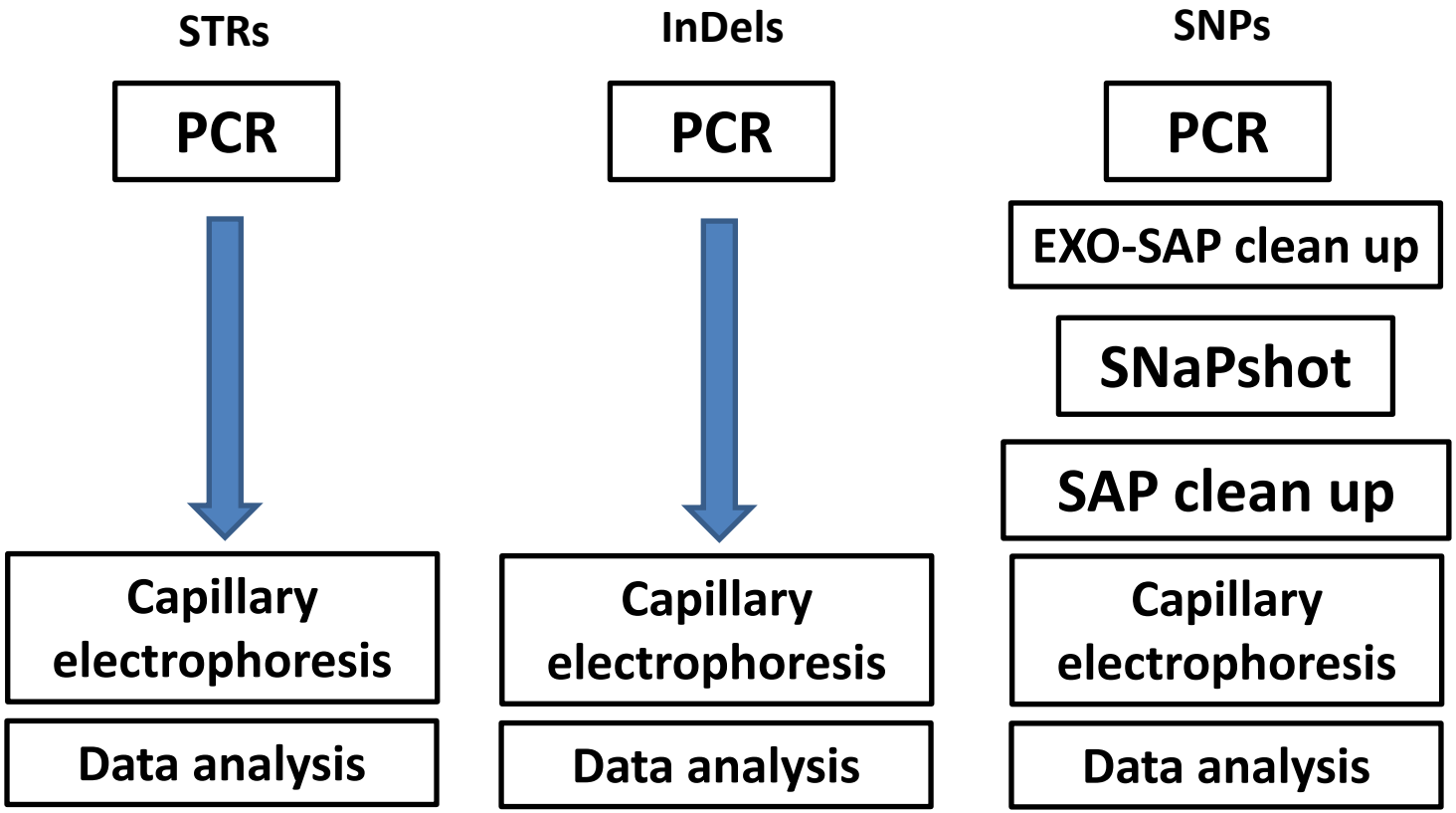
InDel Polymorphisms



- InDels (insertion-deletion) or DIPs (deletion-insertion polymorphisms) are short length polymorphisms, consisting of the presence or absence of a short (typically 1-50 bp) sequence.
- Closely related to SNPs, sharing most of their properties
  - Low mutation rate – ~$2X10^{-8}$
  - Short amplicon PCR – 60 to 160 bp
  - High multiplexing capacity – 30 to 40 markers
- Total number estimated close to 2 million in the human genome.

InDel Polymorphisms

Straight-forward typing methodology



As length polymorphisms, InDels can be typed with a simple direct PCR-to-CE genotyping strategy, using a single multiplexed PCR with dyed-linked primers immediately followed by capillary electrophoresis.

Potential Applications of InDels

Degraded DNA samples
- Short amplicon markers

Missing person cases

Mass fatality cases

Complex pedigree kinship
-High multiplexing capacity
- Low mutation rate

Incest cases

Inmigration cases

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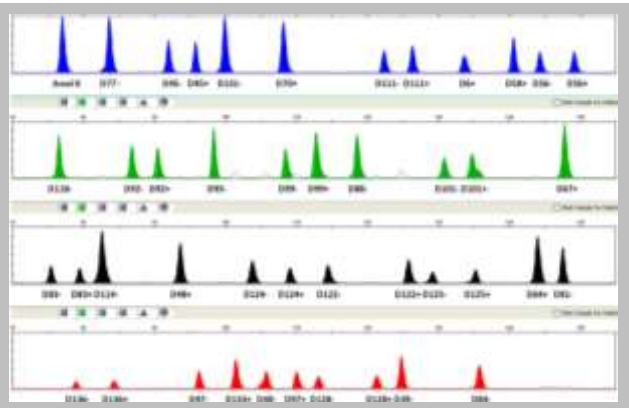
InDel Assays Used in This Study

3130xl data

Qiagen Investigator DIPplex kit

http://www.qiagen.com/products/investigatordipplexkit.aspx

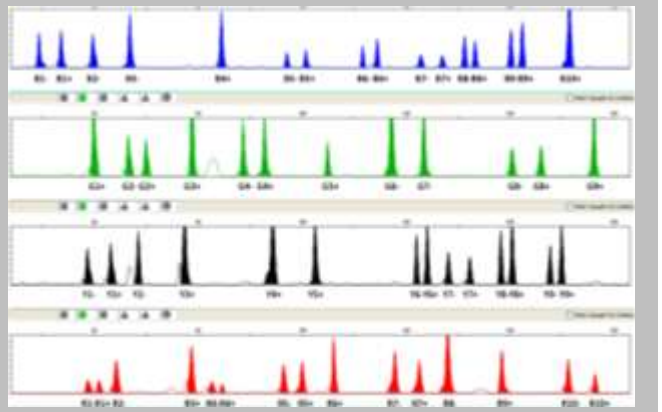
- 31plex PCR
- 30 InDel markers plus amelogenin (on 18 chromosomes)
- Ranging from 75 to 150 bp amplicons



HID-38plex

R. Pereira et al Electrophoresis (2009)

- 38plex PCR
- 38 InDel markers (on 22 chromosomes)
- Ranging from 50 to 155 bp



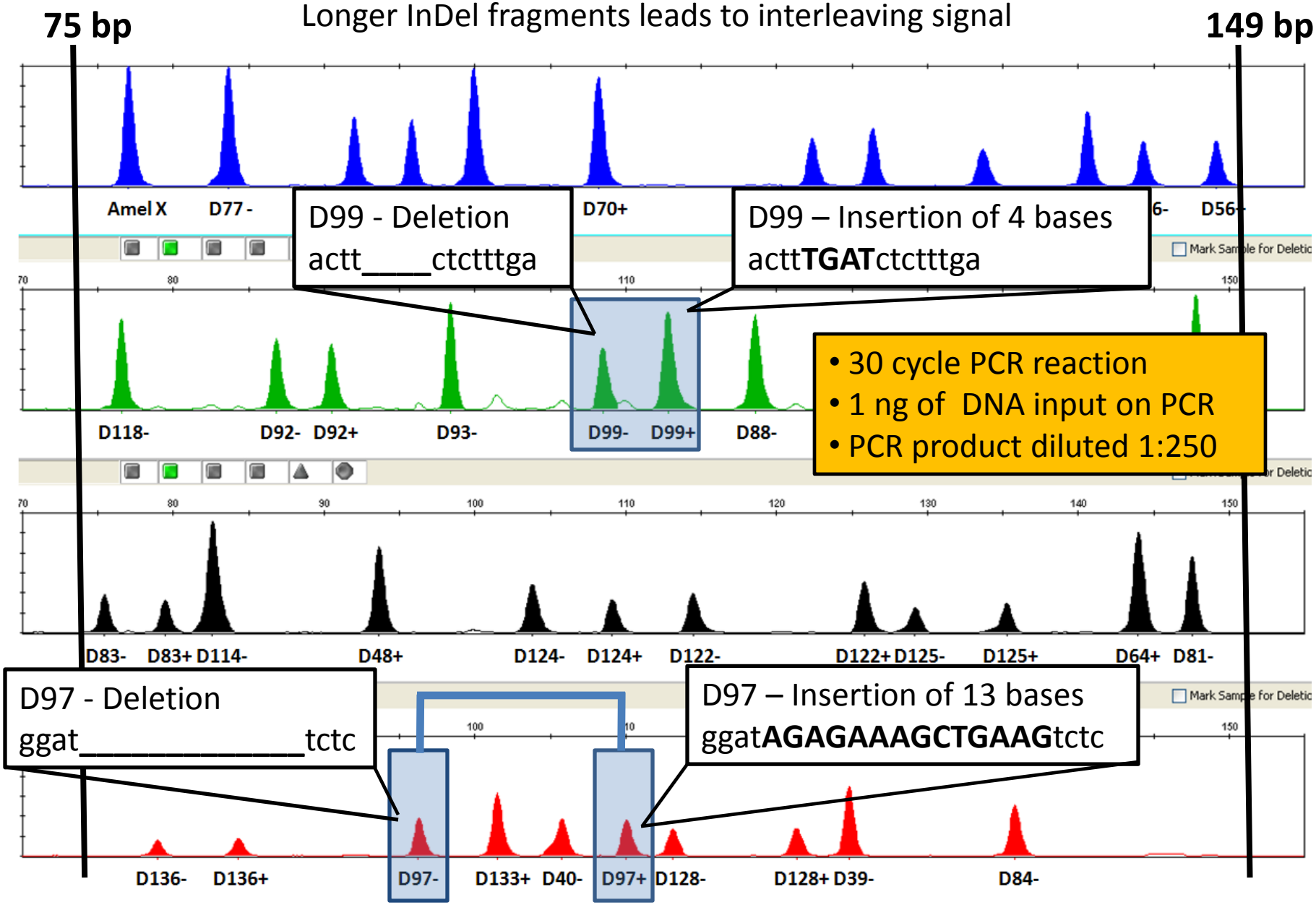
68 InDel markers in total

3000 RFUs

9947a DIPplex Profile

BT matrix standard

Longer InDel fragments leads to interleaving signal



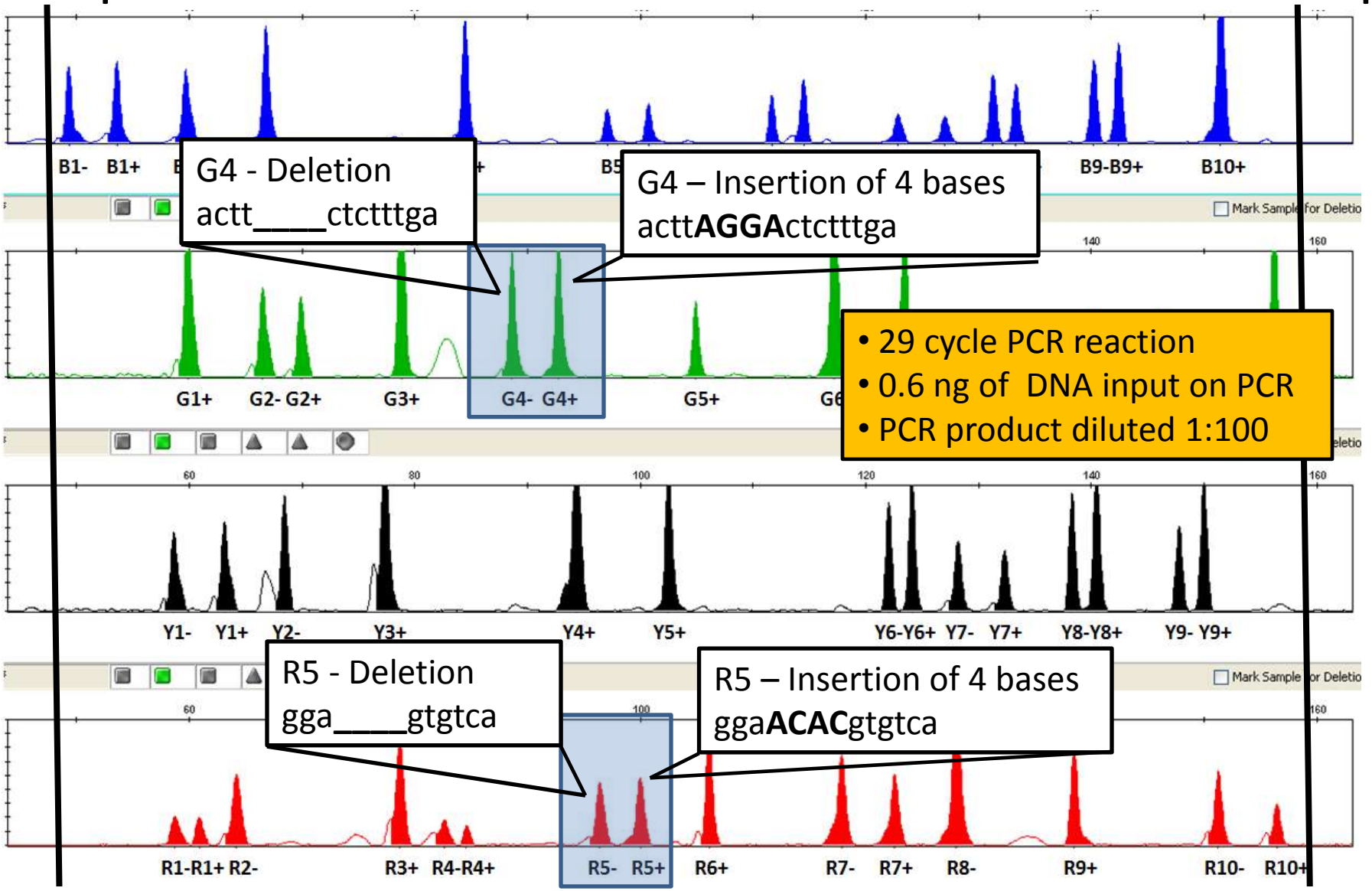
2000 RFUs

G5 matrix standard

9947a HID-38plex profile

Short InDel fragments, No signal interleaving

49 bp **157 bp**



G4 - Deletion
actt____ctctttga

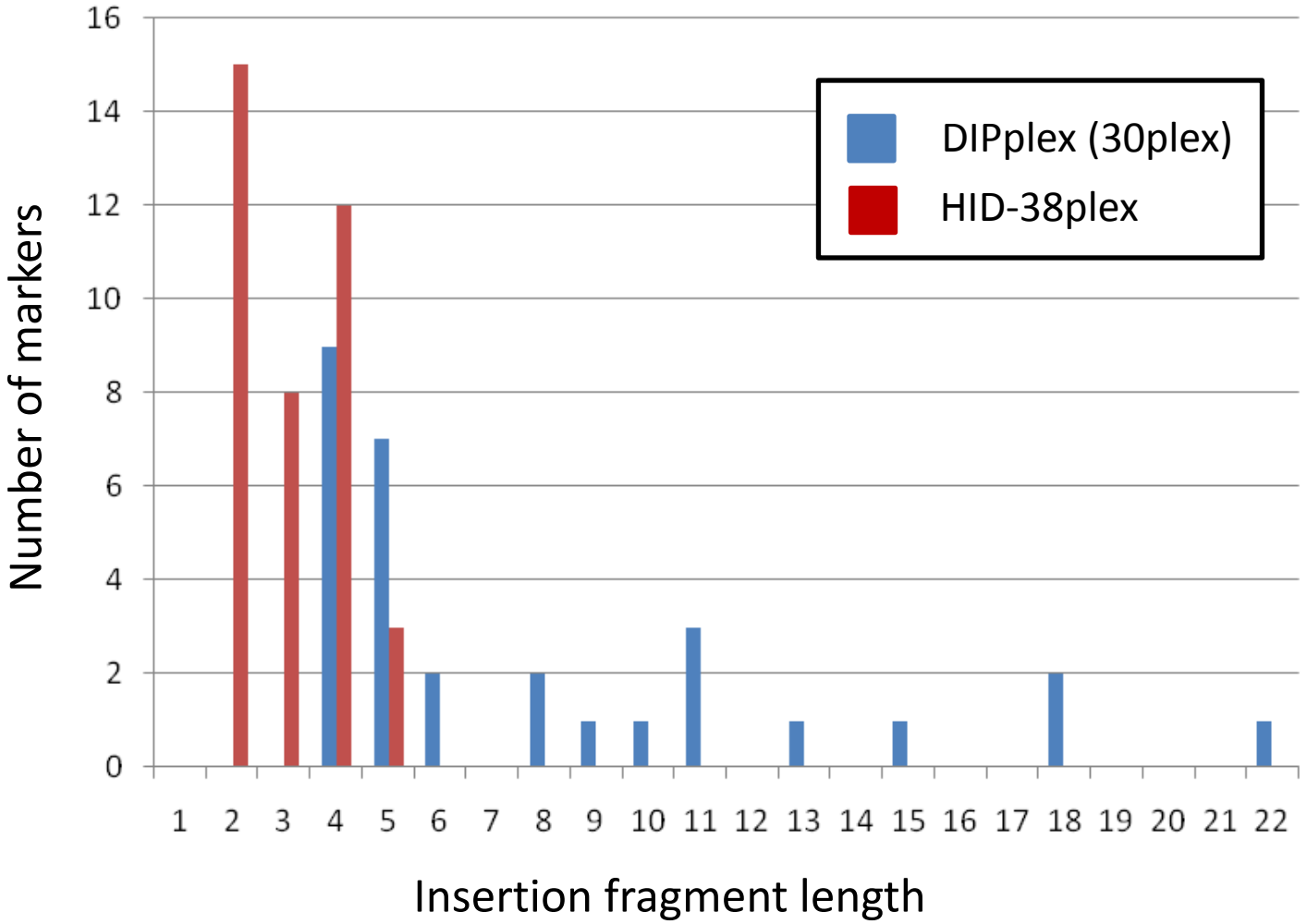
G4 – Insertion of 4 bases
actt**AGGA**ctctttga

- 29 cycle PCR reaction
- 0.6 ng of DNA input on PCR
- PCR product diluted 1:100

R5 - Deletion
gga____gtgtca

R5 – Insertion of 4 bases
gga**ACAC**gtgtca

Allele Spread on both InDel Assays



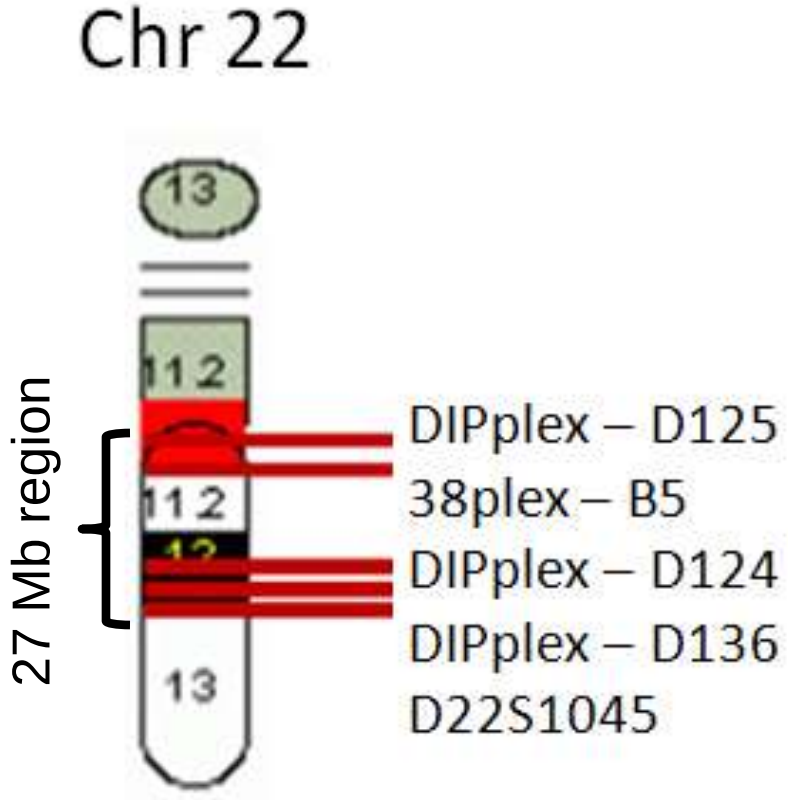
- A shorter Insertion-Deletion fragment length through the assay facilitates the inclusion of a greater number of markers on the same electrophoretic window.
- Moreover, it prevents the interleaving of markers

Genomic Position of the Markers: Linkage Disequilibrium Possibility

- Indels are thought to play a supporting role to current STR assays.

- Due to the high number of markers, some share the same chromosome region.

- Proximity may pose the risk of markers being linked, if so they could not be statistically multiplied together.



Chromosome 22: A fine example

5 markers are located in close positions in a small region:

- 1 HID-38plex InDel.
- 1 forensic STR.
- 3 DIPplex InDels.

Risk of LD should be evaluated.

Genomic Position of the Markers: Linkage Disequilibrium Possibility

Markers separated by less than 10 Mb

6 loci from each InDel assay that are less than 10 Mb from a core STR locus.

DIPplex

CHR	STR	InDel	Physical Distance
5	CSF1PO	RS1305056	6,158,834
6	SE33	RS2307652	8,521,842
8	D8S1179	RS3081400	5,959,018
15	PentaE	RS2307433	7,509,680
22	D22S1045	RS6481	1,747,100
22	D22S1045	RS16363	39,169

HID-38plex

CHR	STR	InDel	Physical Distance
7	D7S820	rs2307978	311,150
11	TH01	rs10688868	1,890,820
12	D12S391	rs1610919	2,352,263
12	vWA	rs1610919	8,838,263
16	D16S539	rs2067208	1,804,212
21	D21S11	rs35605984	4,919,264

When contemplating the possibility of combining the information contained in these InDel markers systems with each other or with core STR loci, we should keep in mind that the proximity between some of these markers could lead to a linkage disequilibrium state.

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Allele Frequency Analysis

We performed population allele frequency analysis with both InDel multiplexes typing the NIST collection of 712 population samples.

Samples from the four representative human groups of the U.S. population have been used. Unrelated individuals of self-declared ancestry.

- **260 African Americans**
- **262 U.S. Caucasians**
- **140 U.S. Hispanics**
- **50 U.S. Asians**

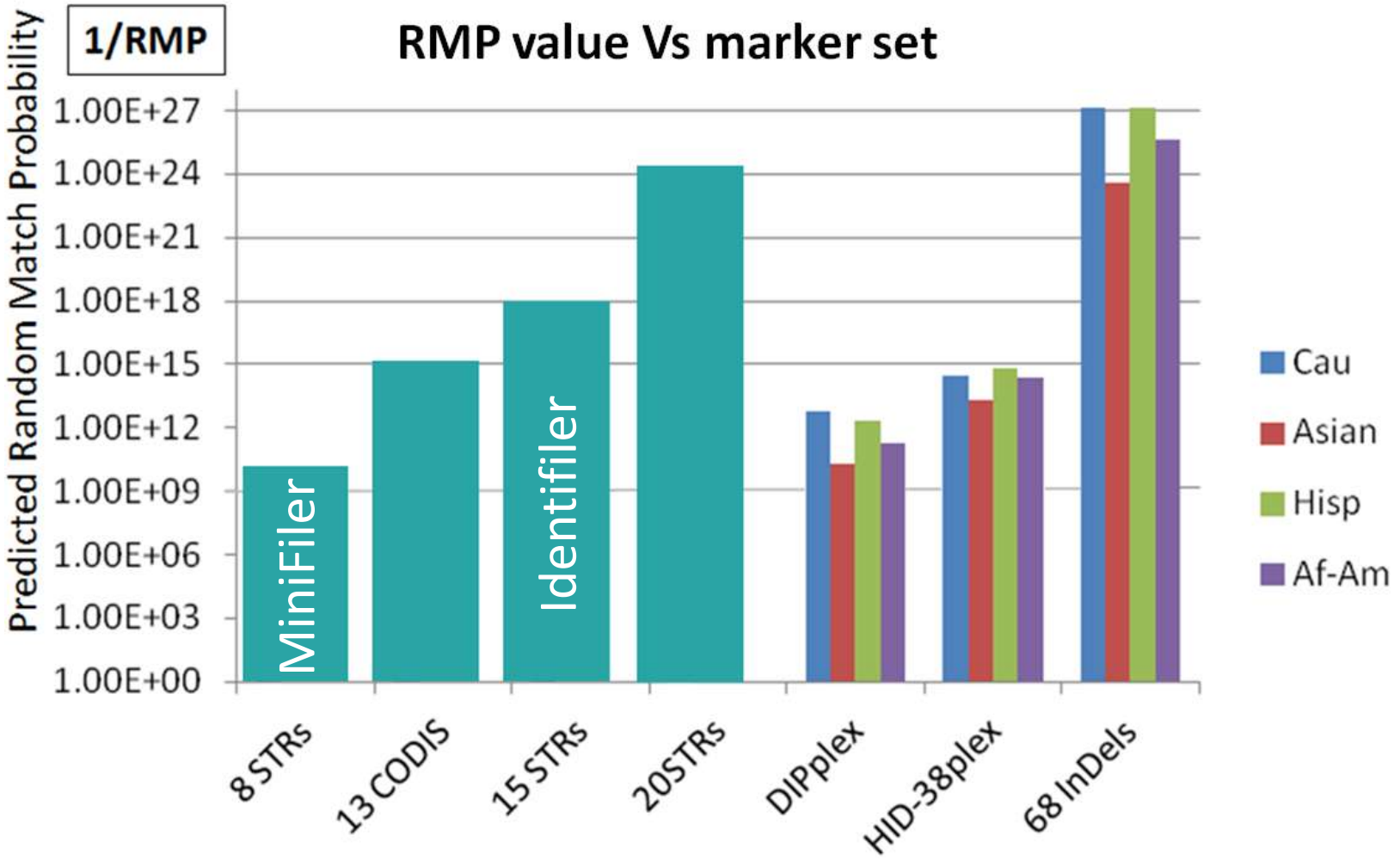
Working under the assumption of full independence of the markers, the following RMP values were calculated.

	U.S. Cauc	U.S. Asian	U.S. Hisp	Af-Am
Mean DIPplex RMP	1.86E-13	4.67E-11	4.88E-13	5.88E-12
Mean HID-38plex RMP	3.67E-15	5.11E-14	1.47E-15	4.74E-15

Allele Frequency Analysis

Although both InDel assays mean RMP value is lower than the 13 CODIS STRs

68 InDel supply discrimination power higher than 20 STRs



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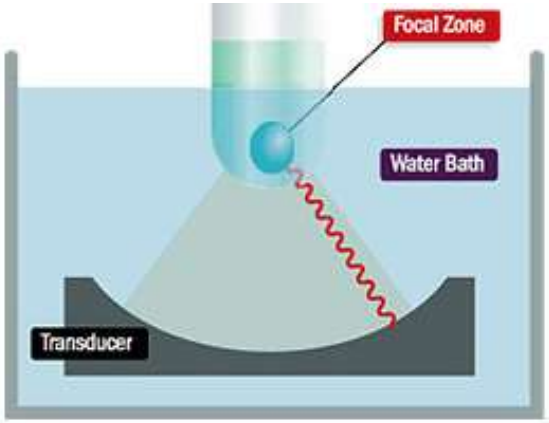
Artificially Degraded DNA Assay

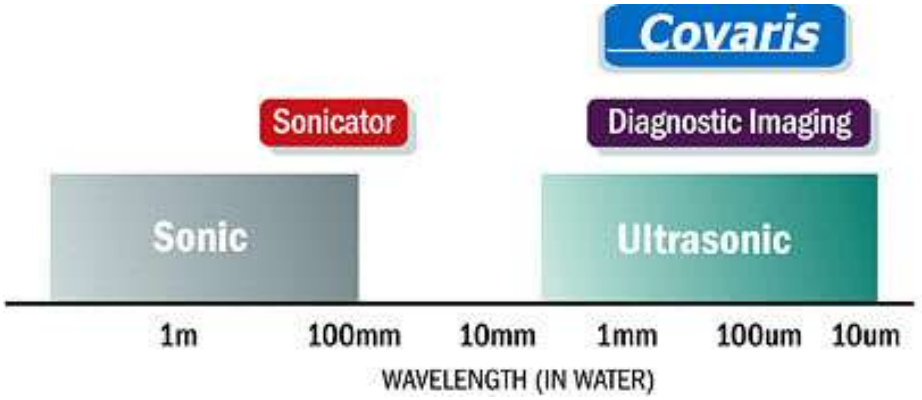
We have conducted several experiments in order to produce mimic DNA degradation samples in a controlled way. Only DNA fragmentation processes were simulated.

Our objective is to compare the short-amplicon InDel typing reactions to establish short-amplicon STRs kit performance.

COVARIS, A focused acoustic DNA shearing technique now employed in Next Generation Sequencing. The method was applied to create appropriately degraded DNA samples in a controlled fashion. For more information see:

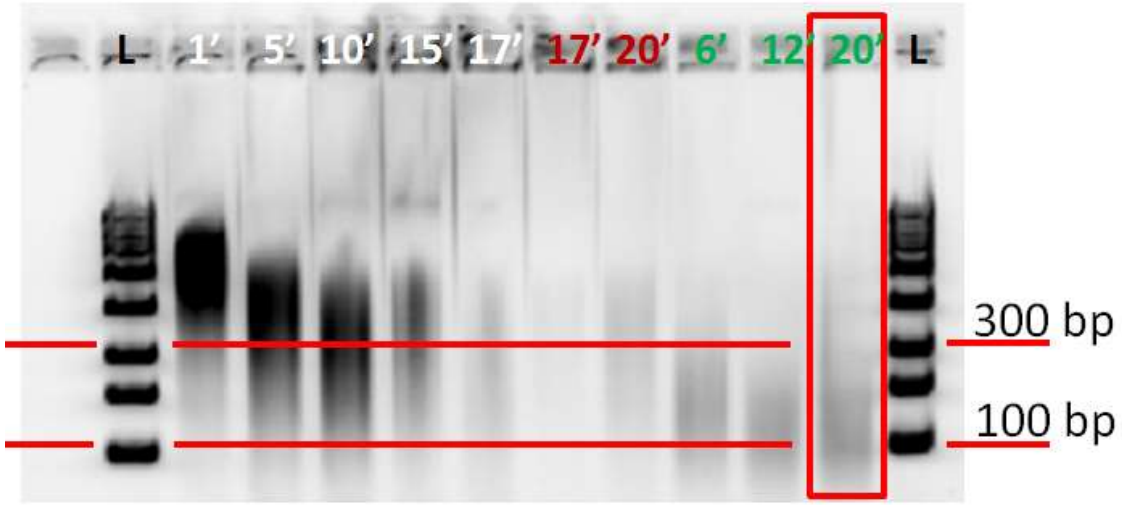
http://www.covarisinc.com/how_it_works.htm





Artificially Degraded DNA Assay

Several protocols have been tried before reaching the desired DNA fragmentation (100-250 bp fragments)



100 cycles per burst / 1mL container

1000 cycles per burst / 1mL container

1000 cycles per burst / 100 µL container

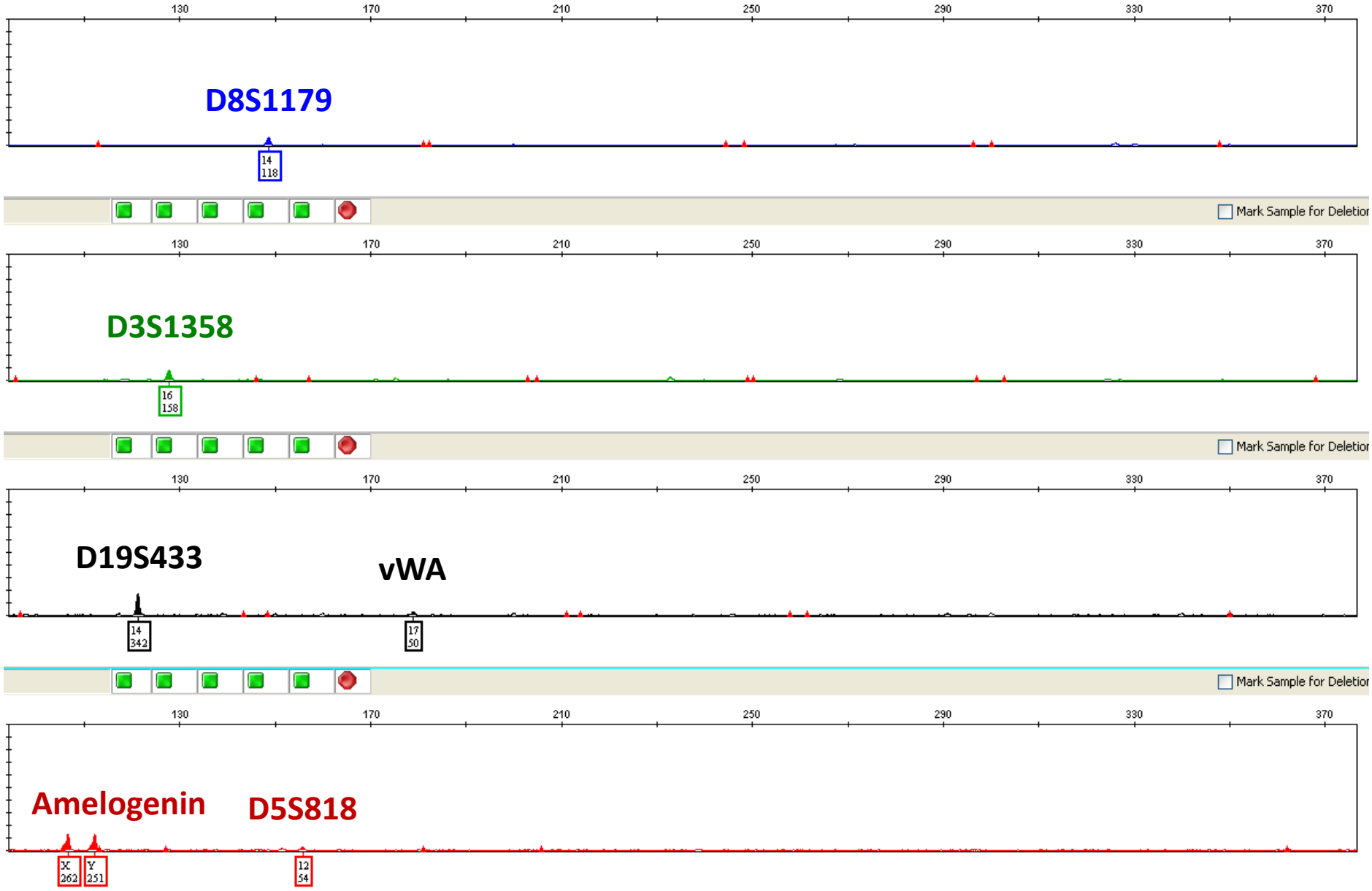
Temperature: 5 ^{0}C
Mode: Frequency sweeping
Duty Cycle: 10 %
Intensity: 10 %
Cycle/Burst: 1000
Time: 20 minutes
DNA: 50 ng
Dilution volume: 100 µL
Tube: glass- 100 µL tube

Only samples corresponding to these conditions (20') were used for the final analysis

All profiles shown scaled to 2000 RFUs

28 PCR cycles

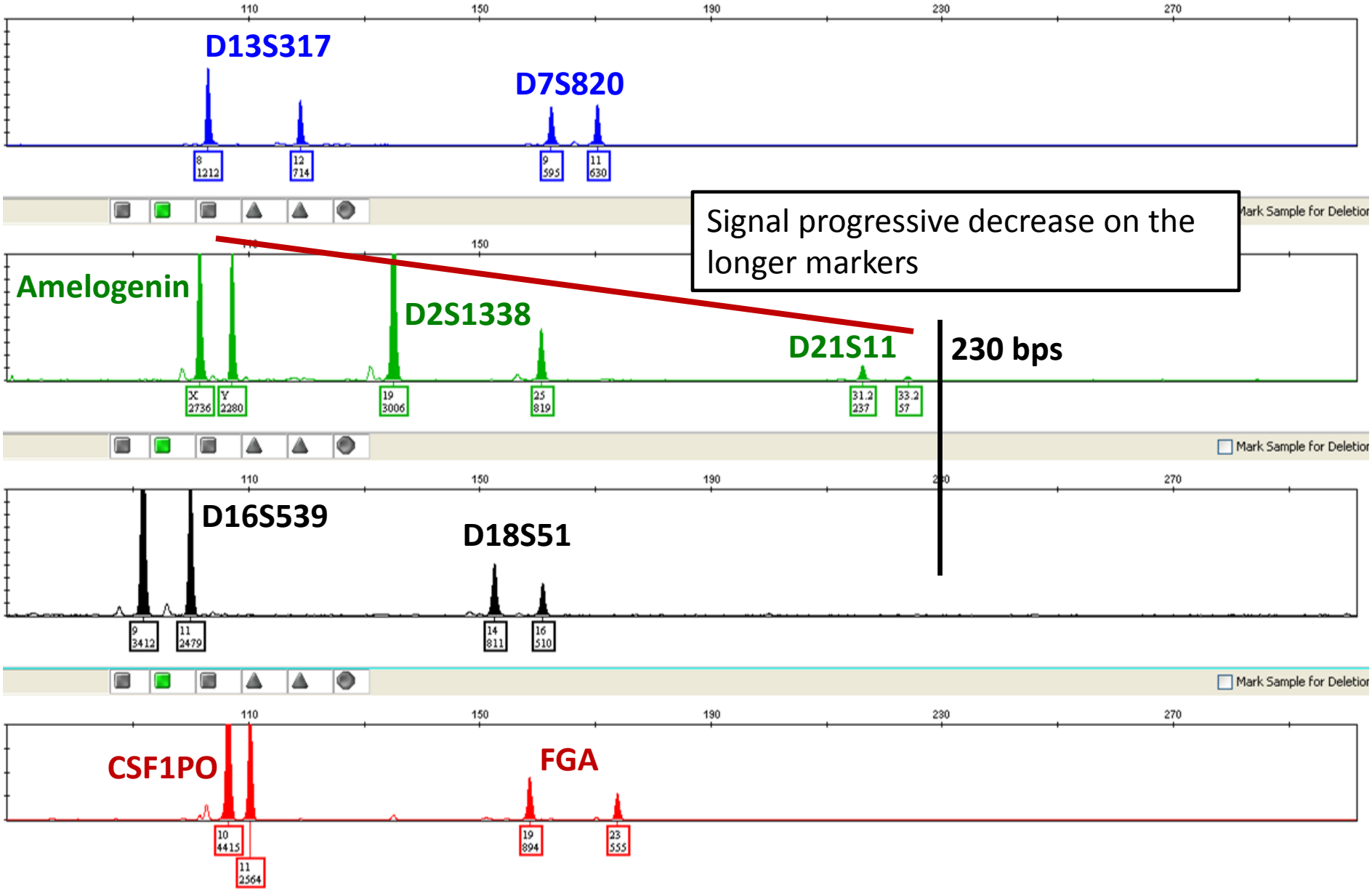
Identifiler – 7 alleles detected



All profiles shown scaled to 2000 RFUs

30 PCR cycles

Minifiler – 16 alleles detected

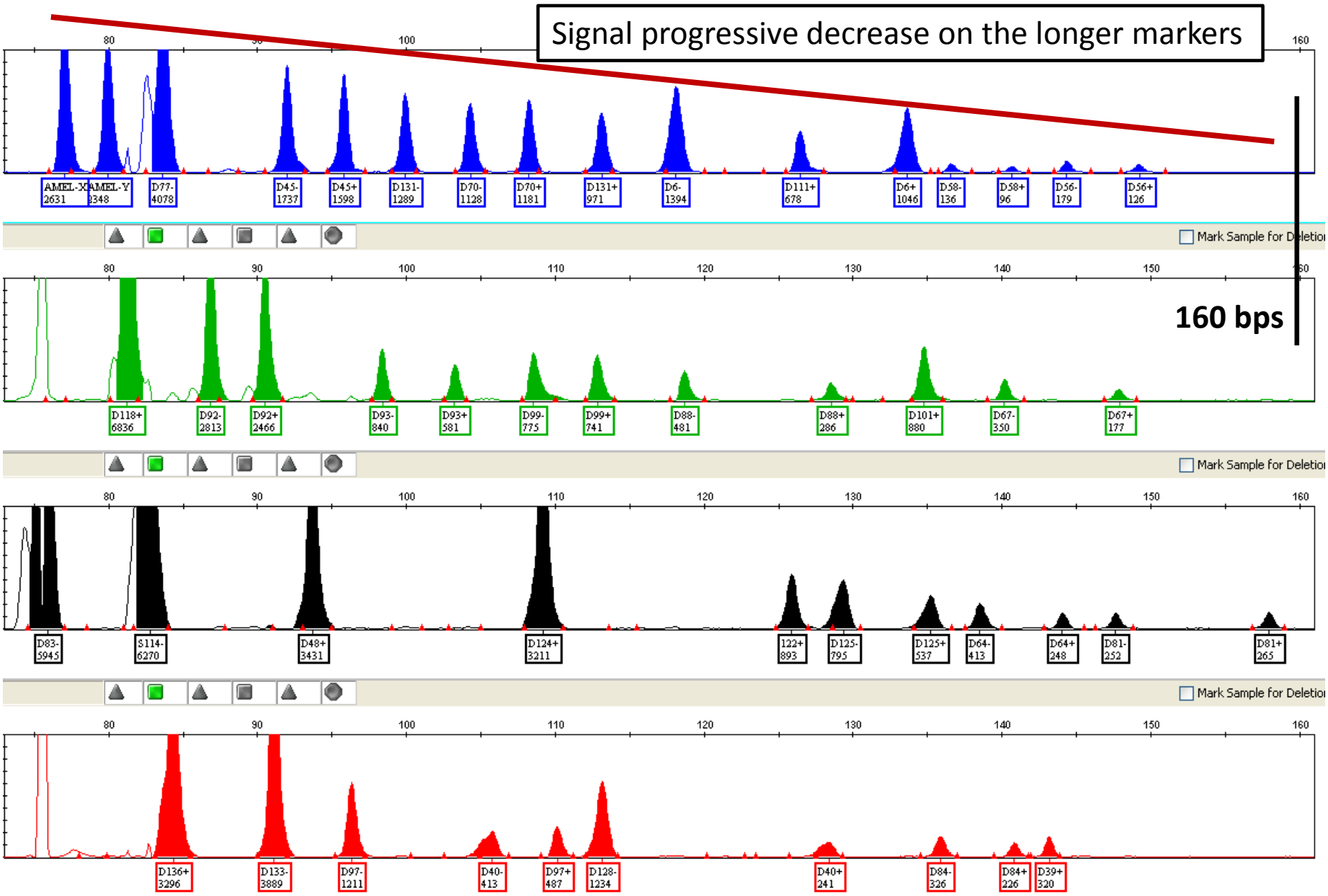


Signal progressive decrease on the longer markers

All profiles shown scaled to 2000 RFUs

DIPplex – 49 alleles detected

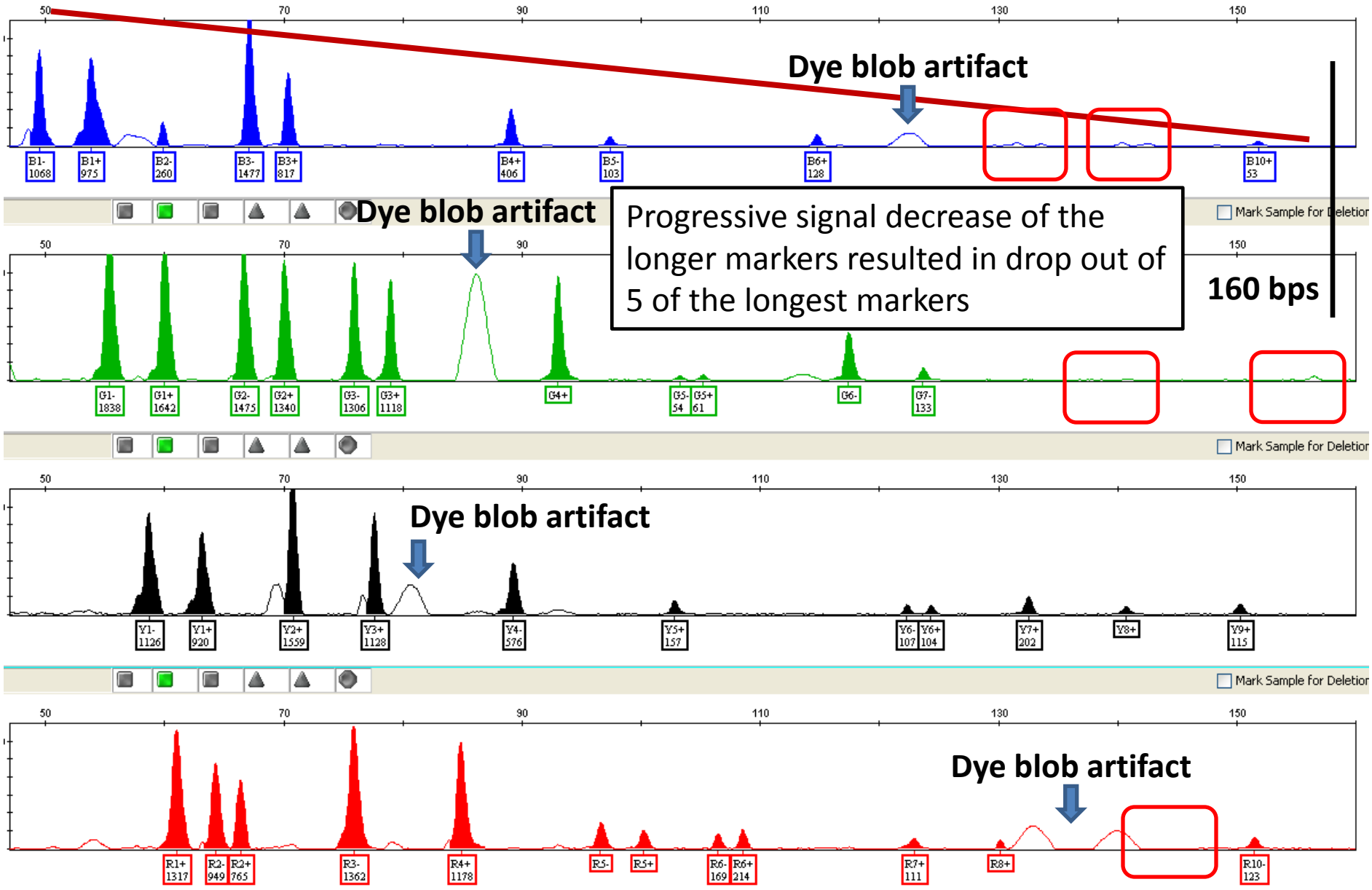
30 PCR cycles



All profiles shown scaled to 2000 RFUs

HID-38plex – 43 alleles detected

29 PCR cycles



Artificially Degraded DNA Assay

With the number of observed alleles on each kit, we obtained the following RMP values

Assay	Exp. Alleles	Obs. Alleles	Loci total	Amp. Loci	RMP
Identifiler	10	5	15	5	n/a
Minifiler	16	16	9	9	$2.89\ e^{-12}$
DIPplex	49	49	30	30	$4.77\ e^{-14}$
HID-38plex	43*	43*	38	33	$1.03\ e^{-14}$

** Based on surviving loci*

- We could assure that the application of short amplicon markers such as DIPplex and MiniFiler to challenging DNA samples would be of great interest for real casework.
- In case of limited amount of sample, InDel marker amplification should be considered in spite of other assays, such as Minifiler, unless core STRs are needed.
- For future sample preparation, increased shearing times could be tried in order to achieve a further level of fragmentation.

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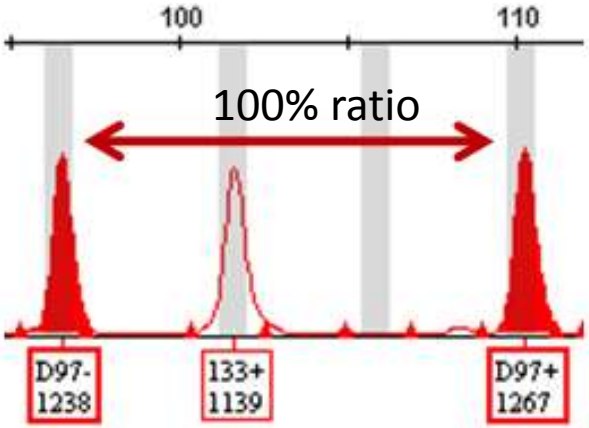
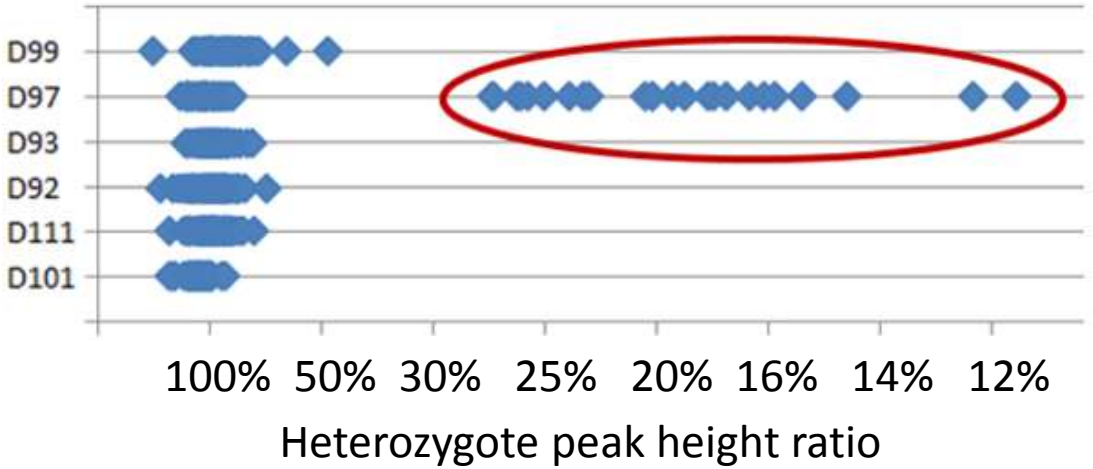
Sequencing of Previously Unreported Variation

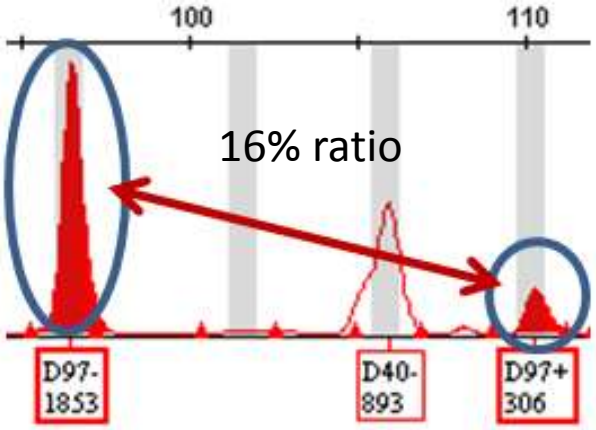
Consistent heterozygote peak imbalance

Balanced Heterozygote



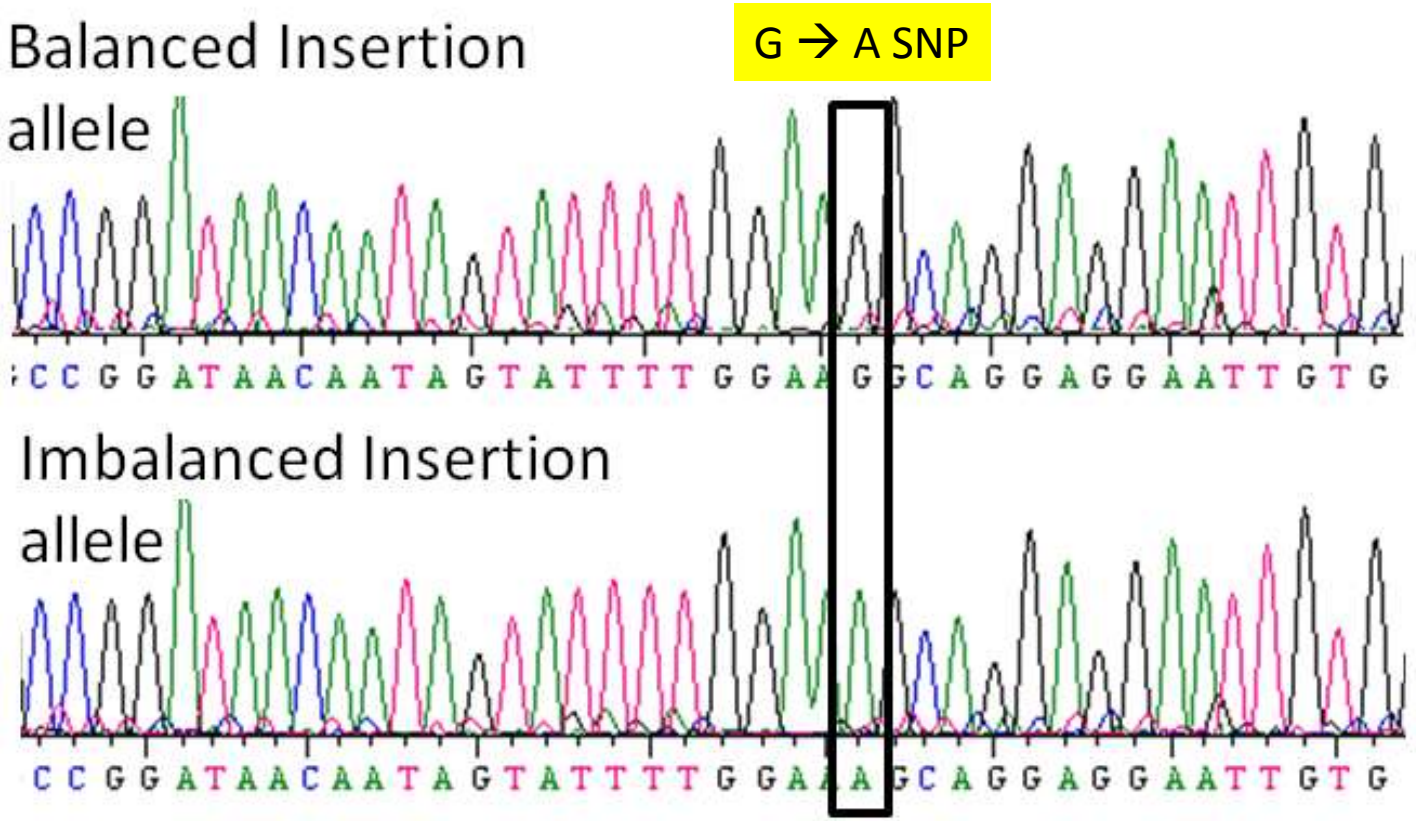


Imbalanced Heterozygote



This suggested the presence of a **SNP within the primer binding site** potentially disrupting primer annealing in samples carrying the minor allele.

D97- rs17238892 sequencing results



• A neighboring SNP (**G/A**), located 61 bp downstream from the main InDel site. This is a SNP referenced in the dbSNP database as **rs17245568**. The A allele of this SNP corresponds to the samples carrying the observed imbalance.

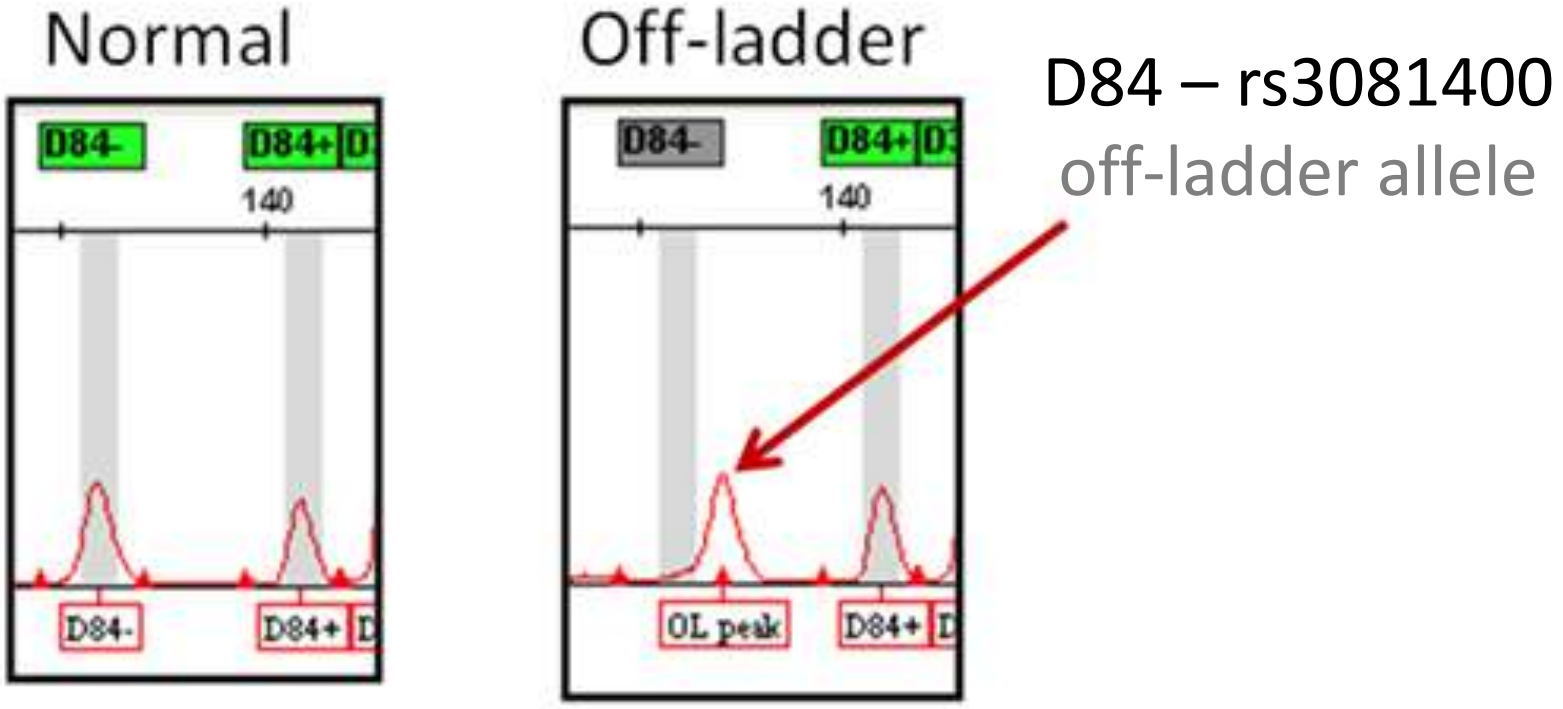
• We do not have the Qiagen PCR primer sequences. It is reasonable to assume that the G→A SNP 61 bases downstream from the insertion is the cause of the peak imbalance.

Sequencing of Previously Unreported Variation

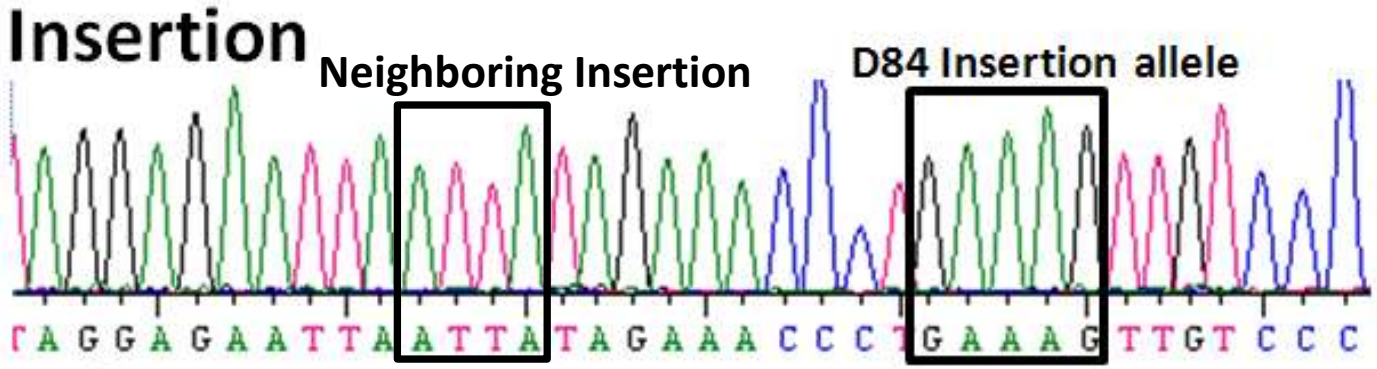
-A second feature would be the presence of a seemingly **third off-ladder allele** for two of the DIPplex markers (D99 and D84).
Two possible explanation for these features:

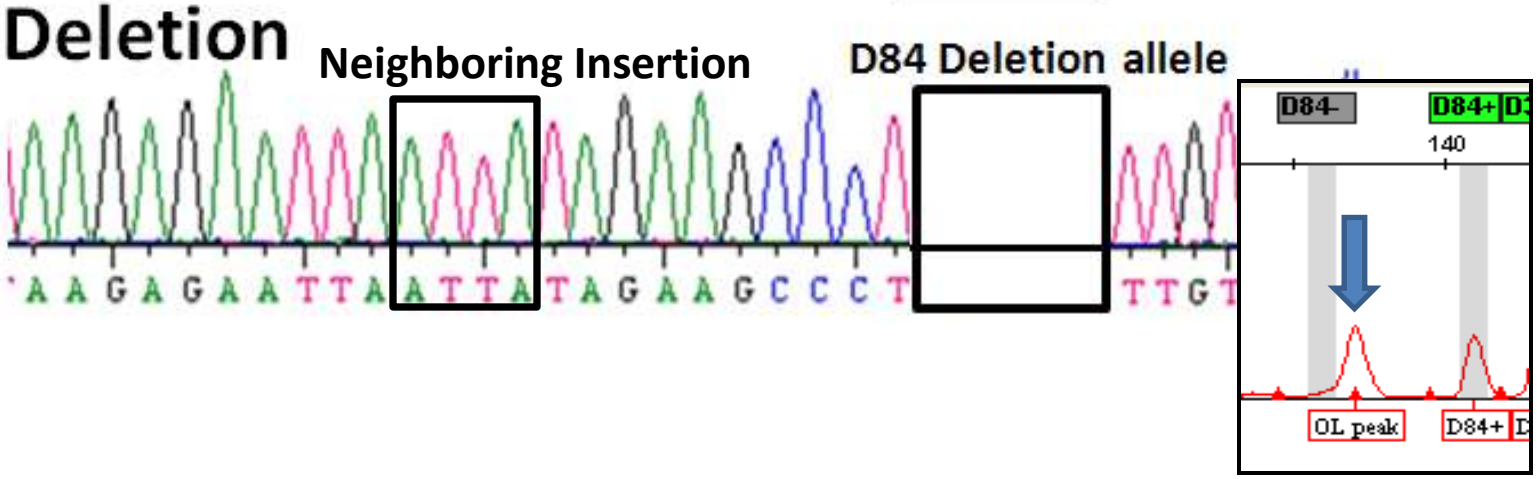
+A different size deletion/insertion allele at the locus

+An additional neighboring InDel site with a rare minor allele within the amplicon range.



D84 – rs3081400 off-ladder allele





Sequencing of Previously Unreported Variation

Observed frequency of the unreported variation

		U.S. Population		
Frequency	Caucasian	African	Hispanic	Asian
D97 imbalance	**0.044**	**0.22**	**0.062**	**0.06**
D83 imbalance	0	**0.08**	**0.015**	0
D99 OL allele	0	**0.0766**	**0.0156**	0
D84 OL allele	0	**0.0443**	0	0

- **We would suggest a reformulation of the reverse primer for the marker D97, as nearly as much as a quarter of the analyzed African-American samples displayed imbalance.**

- **This situation may lead, especially in degraded DNA samples, to the drop-out of the Insertion allele of this marker.**

- **The non-standard mobility variants observed in the Qiagen DIPplex InDel set have proven to be stable and due to a single characterized polymorphic variant.**

- **The characterization of such rarer mobility variants, far from being a hindrance, can further contribute to the informative power of InDel typing.**

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Conclusions

- Collected U.S. population data (n=712) on 68 InDel loci
  (In 2 multiplexes and 1.6 ng of total DNA)

- Demonstrated improved success rates with artificially degraded DNA compared to Identifiler STR typing.

- Characterized some unreported off-ladder alleles and imbalanced heterozygotes.

- InDels can be a supporting tool to STRs for challenging casework samples.

Acknowledgements

- **Qiagen** for providing access to DIPplex kits.
- **Margaret Kline and Becky Hill** from NIST for assistance with allele sequencing.
- **Jennifer McDaniel** from NIST for assistance with the COVARIS system.
- **Carla Santos** from USC for providing HID-38plex primer mix.

A final version of the slides will be uploaded to STRbase webpage http://www.cstl.nist.gov/strbase/ISHI2011-InDel.pdf