



Technology Transition Workshop | *Thomas Hall, Ph.D.*

Overview of the Ibis™ STR Assay

Outline

- **Ibis™ STR assay format**
 - Primer design
 - Database
 - Assay layout
 - Sample analysis
- **Comparison to traditional STR analysis**
 - Measures intrinsic property of product (mass)
 - Traditional analysis is indirect (migration in column)
 - No allelic ladder needed for Ibis™ assay
 - Novel alleles more precisely characterized
- **Polymorphic STR alleles**
- **Example of data for a sample**
- **Sensitivity**
- **Reproducibility**

Core CODIS Loci Targeted

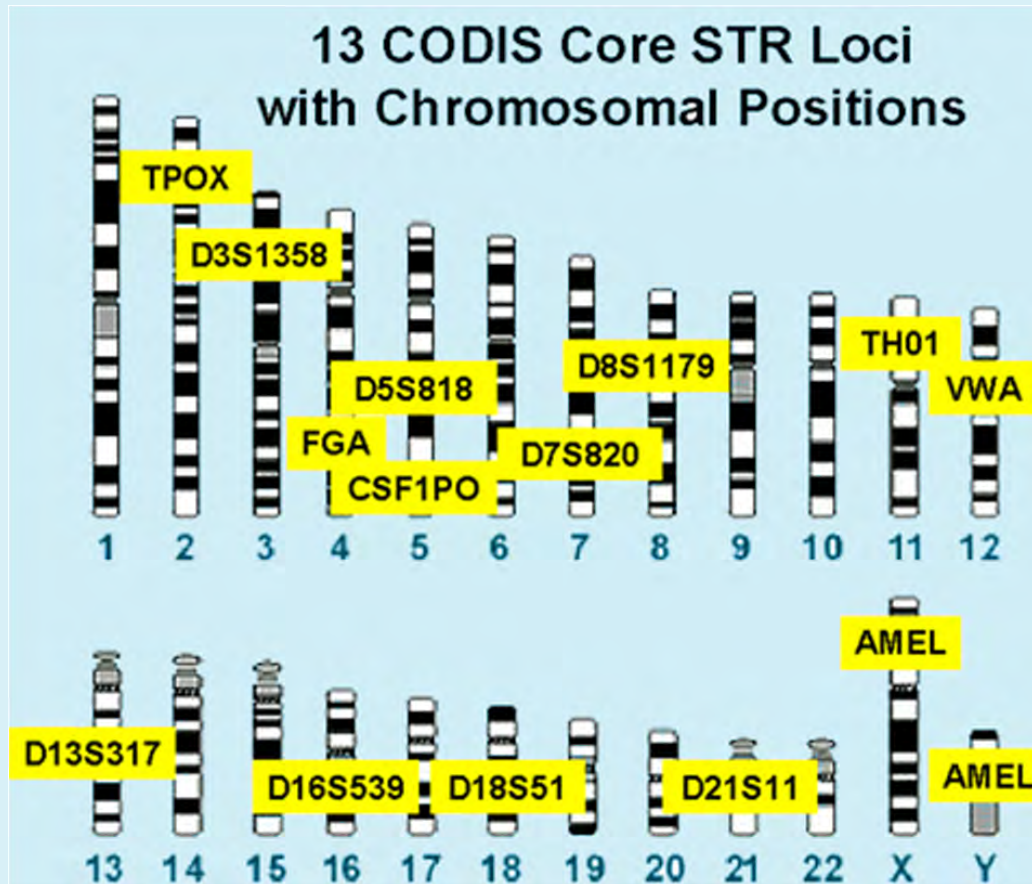


IMAGE COURTESY OF: <http://www.cstl.nist.gov/div831/strbase/fbicore.htm>

Primer Design

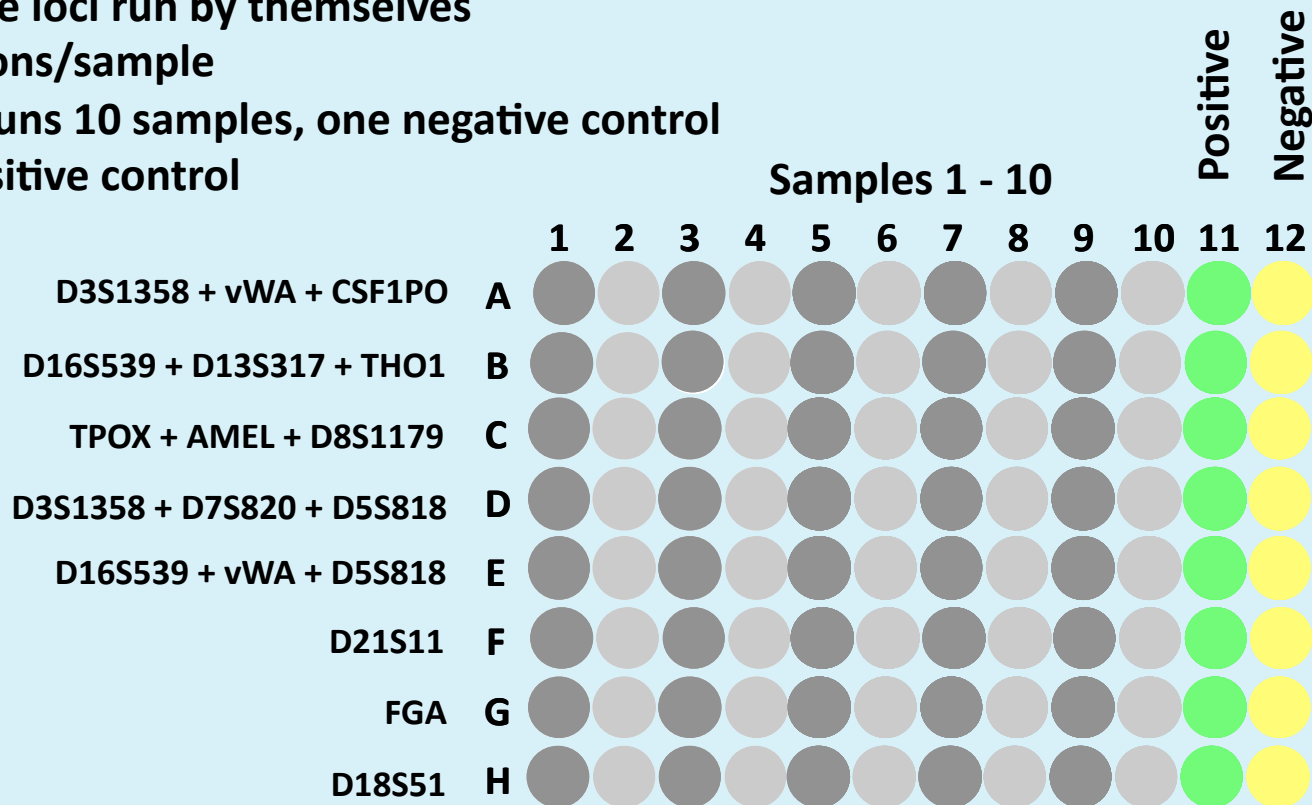
Example: Locus D13S317



- Primers placed close to repeat unit to minimize size
- Multiplexed products must resolve by mass

STR Assay Layout

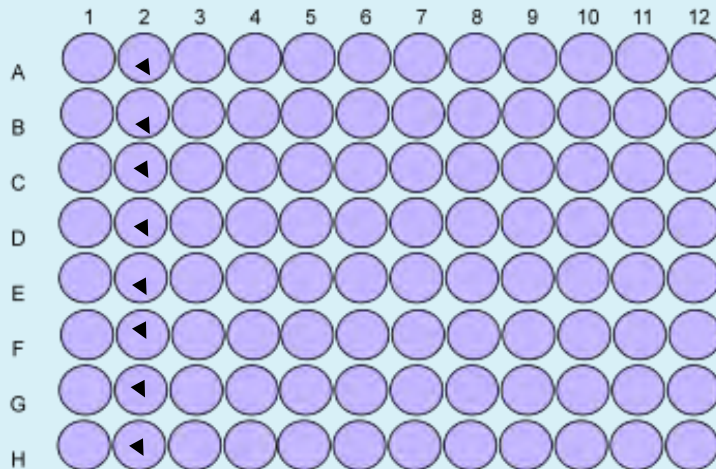
- Five semi-redundant 3-plexes
- Largest three loci run by themselves
- Eight reactions/sample
- Each plate runs 10 samples, one negative control and one positive control



Assay Plate Setup

	1	2	3	4	5	6	7	8	9	10	11	12
A		UT57318	WT51362	WA29594	JT51471	OT05897	PT84223	PT84232	GT37778	GT37900	TT51422	ZT80786
B	UT57300	WT51342	WT51373	WA29612	JT51499	OT05898	PT84224	PT84234	GT37812	GT37913	TT51435	ZT80815
C	UT57301	WT51343	WT51378	ZT81387	OT05888	OT05899	PT84225	PT84236	GT37828	JT52076	TT51483	ZT80826
D	UT57302	WT51345	WT51381	MT94859	OT05890	OT05901	PT84226	PT84239	ZT80932	OT07280	TT51511	ZT80863
E	UT57303	WT51354	WT51386	MT94866	OT05892	PT84214	PT84227	PT84240	GT37862	PT85612	TT51530	ZT80865
F	UT57310	WT51355	BC11352	MT94868	OT05893	PT84215	PT84228	PT84241	GT37864	PT85658	ZT80731	ZT80869
G	UT57312	WT51358	MT97172	MT94869	OT05894	PT84216	PT84230	PT84242	GT37869	TT51399	ZT80737	ZT80870
H	UT57317	WT51359	WA29584	MT94875	OT05896	PT84222	PT84231	PT84243	GT37888	TT51407	ZT80782	ZT80925

Add 5 µl template to each well of a pre-made plate and thermocycle



Each sample is distributed across one column of a pre-made assay plate

No Allelic Ladder Required

D13S317

A32 G11 C34 T57

A34 G11 C36 T61

40823.24 Da

42175.71 Da

43243.79 Da

44715.05 Da

40696.8

41549.7

42402.5

43255.3

44108.1

44108.1

Mass (Da)

Direct Allele Assignment

D13S317

A32 G11 C34 T57

A34 G11 C36 T61

40823.24 Da

42175.71 Da

43243.79 Da

44715.05 Da

40696.8 41549.7 42402.5 43255.3 44108.1 44108.1
 Mass (Da)

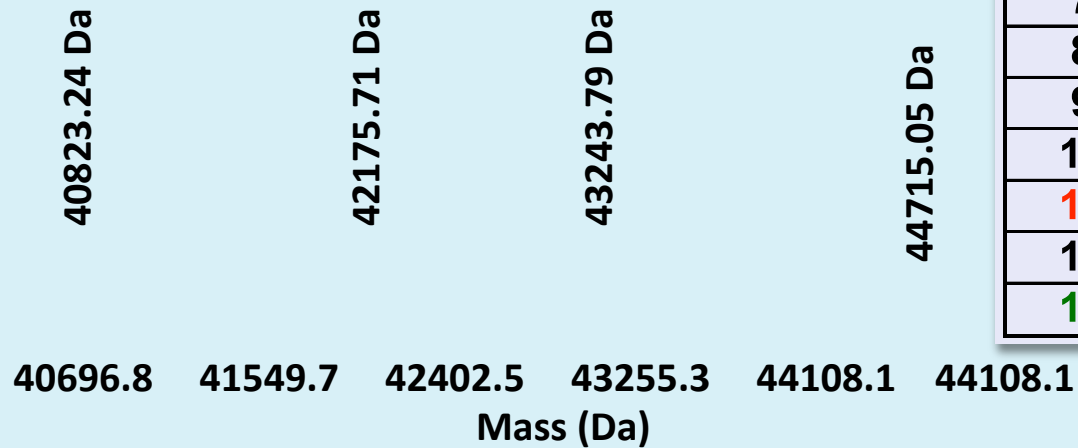
Allele	Length	A	G	C	T
5	110	26	11	28	45
6	114	27	11	29	47
7	118	28	11	30	49
8	122	29	11	31	51
9	126	30	11	32	53
10	130	31	11	33	55
11	134	32	11	34	57
12	138	33	11	35	59
13	142	34	11	36	61

Direct Allele Assignment

D13S317

Allele 11
A32 G11 C34 T57

Allele 13
A34 G11 C36 T61



Allele	Length	A	G	C	T
5	110	26	11	28	45
6	114	27	11	29	47
7	118	28	11	30	49
8	122	29	11	31	51
9	126	30	11	32	53
10	130	31	11	33	55
11	134	32	11	34	57
12	138	33	11	35	59
13	142	34	11	36	61

Direct Allele Assignment

vWA

A33 G16 C32 T58

A34 G16 C33 T60

42488.66 Da

43492.56 Da

43696.74 Da

44761.64 Da

40696.8

41549.7

42402.5

43255.3

44108.1

44108.1

Mass (Da)

Direct Allele Assignment

vWA

A33 G16 C32 T58

A34 G16 C33 T60

Allele	Length	A	G	C	T
12	119	28	16	27	48
13	123	29	16	29	49
14	127	30	16	28	53
15	131	31	16	30	54
16	135	32	16	31	56
17	139	33	16	32	58
18	143	34	16	33	60
19	147	35	16	34	62
20	151	36	16	35	64
21	155	37	16	36	66
22	159	38	16	37	68
23	163	39	16	38	70
24	167	40	16	39	72
25	171	41	16	40	74

42488.66 Da

43492.56 Da

43696.74 Da

44761.64 Da

42402.5

43255.3

44108.1

44108.1

Mass (Da)

Direct Allele Assignment

vWA

Allele 17

A33 G16 C32 T58

Allele 18

A34 G16 C33 T60

Allele	Length	A	G	C	T
12	119	28	16	27	48
13	123	29	16	29	49
14	127	30	16	28	53
15	131	31	16	30	54
16	135	32	16	31	56
17	139	33	16	32	58
18	143	34	16	33	60
19	147	35	16	34	62
20	151	36	16	35	64
21	155	37	16	36	66
22	159	38	16	37	68
23	163	39	16	38	70
24	167	40	16	39	72
25	171	41	16	40	74

42488.66 Da

43492.56 Da

43696.74 Da

44761.64 Da

42402.5

43255.3

44108.1

44108.1

Mass (Da)

Automated Data Processing

Software provides tools to view and QC data

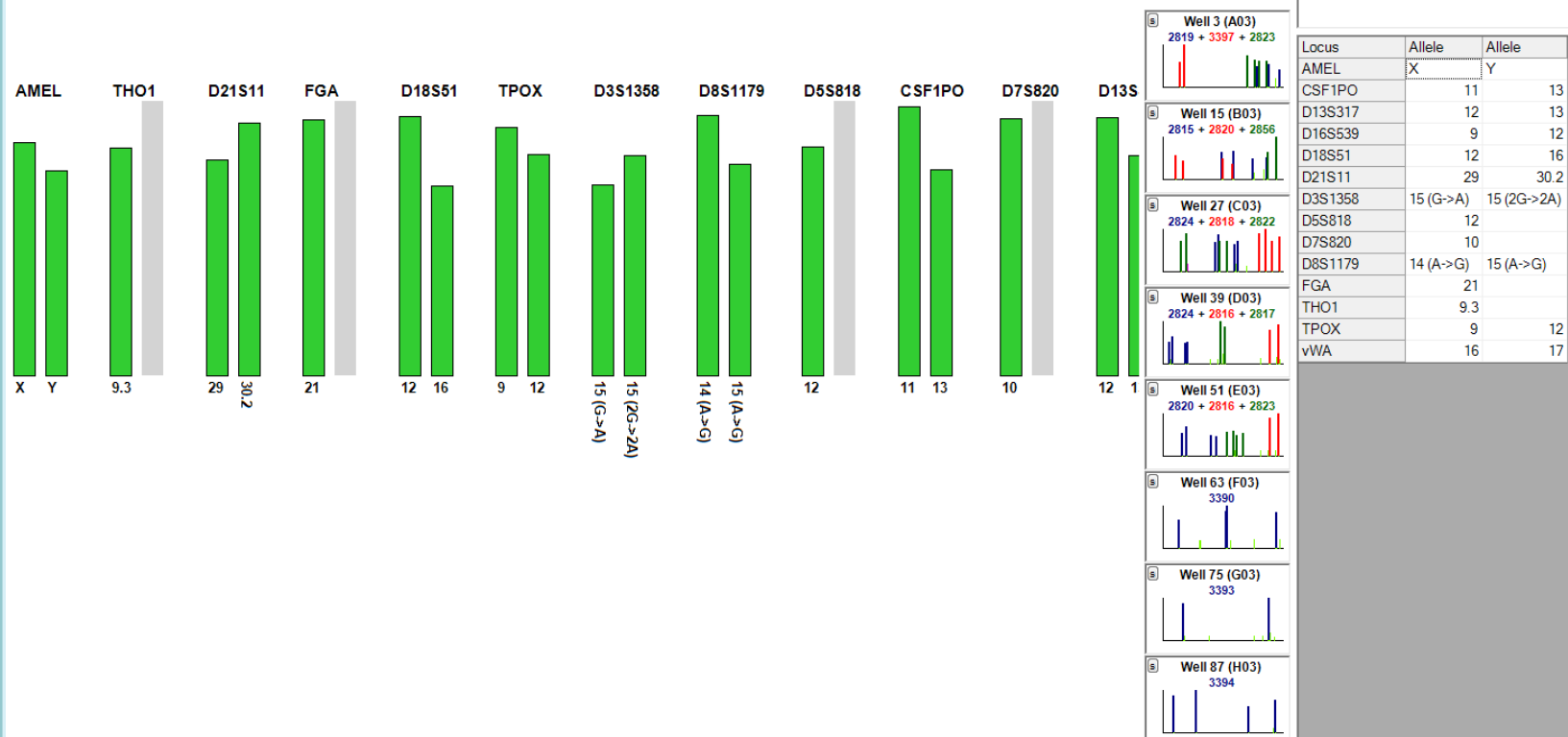
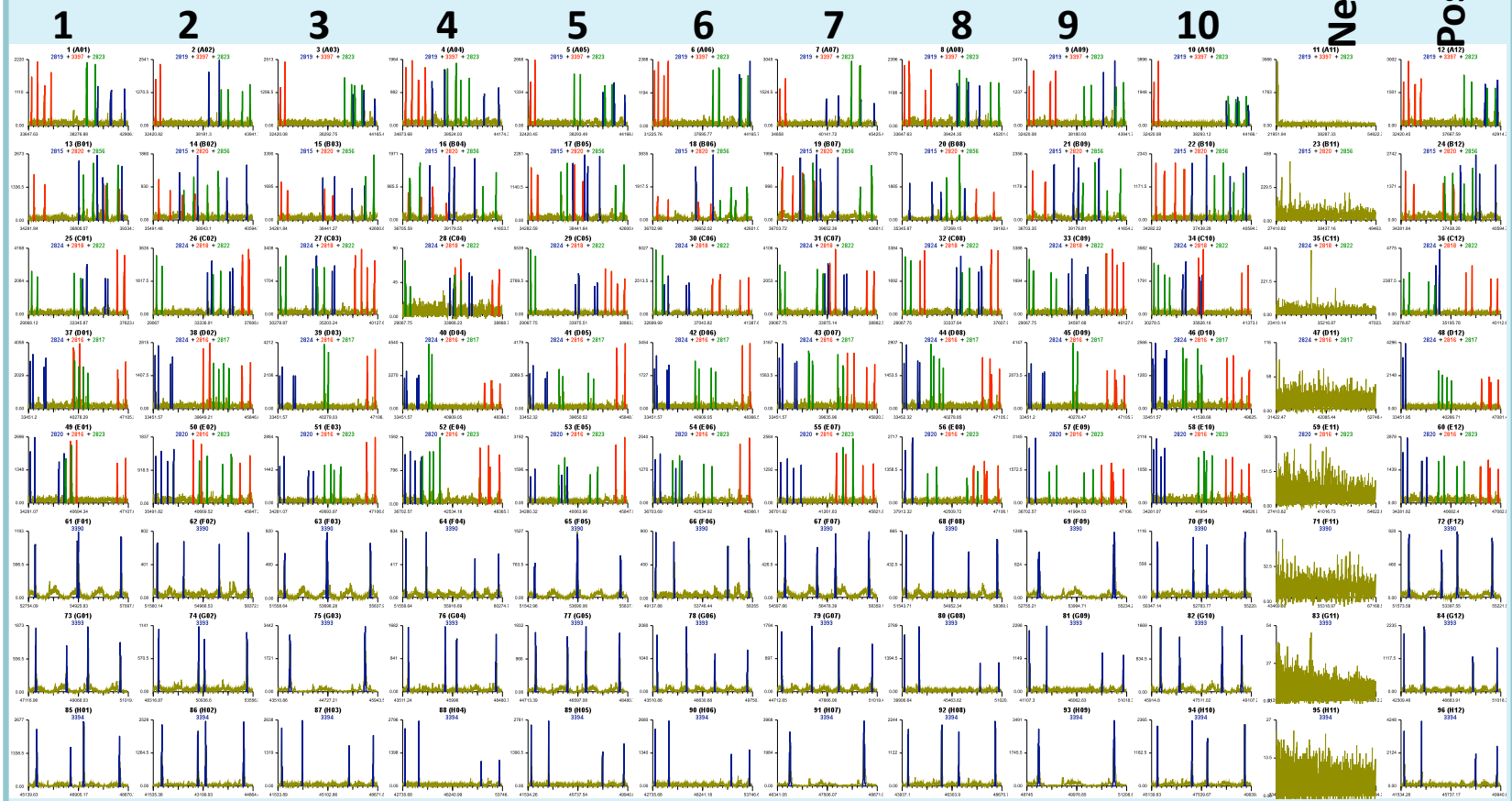


Plate-Based Analysis



12 samples analyzed at a time

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Plate-Based Analysis

Allele call summary for a plate

Base allele calls only
 Show both homozygous alleles
 J. Planz allele nomenclature

Sample	AMEL	CSF1PO	D13S317	D16S539	D18S51	D21S11	D3S1358	D5S818	D7S820	D8S1179	FGA	TH01	TPOX	vWA
<input checked="" type="checkbox"/> NIST-GT37869	X, Y	10, 11	11, 12	9, 12	15, 16	30, 31.2	16S1, 17S1	7, 12	10, 11	13, 13S2	24, 25	6, 7	8, 11	15, 15S1
<input checked="" type="checkbox"/> NIST-TT51399	X, Y	10, 12	10S9, 10S9	10, 11	12, 13	29, 32, 2S1	15S1.2, 15S1	7, 11	11, 12S10	13S2S5, 13S2	25, 27	6, 7	8, 12	16, 18
<input checked="" type="checkbox"/> NIST-ZT80737	X, Y	11, 13	12, 13	9, 12	12, 16	29, 30.2	15S1.2, 15S1	12, 12	10, 10	14S2, 15S2	21, 21	9.3, 9.3	9, 12	16, 17
<input checked="" type="checkbox"/> NIST-ZT80870	X, Y	11, 12	9, 13S9	11, 12	13, 20	29, 34	17, 18	12S8, 13	8, 8	11, 14S2	21, 23	6, 9	8, 12	15S1, 16
<input checked="" type="checkbox"/> NIST-UT57317	X, Y	11, 12	12S9, 13	9, 12	12, 17	29S1, 30.2	15S1.2, 15S1	11, 11	8, 11	13S2, 14	22, 23	6, 9.3	8, 8	14S2S4.2, 17
<input checked="" type="checkbox"/> NIST-WT51359	X, Y	12, 12	13, 13	11, 13	13, 20	27S2, 32.2	14S1, 15S1	13, 13	10, 12	14S2, 16S2	21, 24	9, 9.3	11, 11	15S1, 18S1
<input checked="" type="checkbox"/> NIST-WA29584	X, Y	11, 12	11, 14	11, 12	16, 16	31.2, 32.2	17, 17S1	9S8, 11S8	8, 11S10	11, 14	22, 25	7, 9.3	8, 11	18, 18
<input checked="" type="checkbox"/> NIST-MT94875	X, Y	9, 10	11S9, 12S9	12, 12	14, 16	29S1, 32.2	16S1, 18	11, 12	8, 9	8, 13S2	18, 25	6, 6	8, 12	16, 19
<input checked="" type="checkbox"/> NIST-OT05896	X, Y	11, 12	12, 12	11, 11	18, 18	30, 30S2	15S1.2, 17S1	11, 12	10, 10	14S2, 15S2	19, 25	8, 9	8, 10	15, 18
<input checked="" type="checkbox"/> NIST-PT84222	X, Y	10, 12	12, 13	9, 10	15, 17	28, 30S2	15S1.2, 15S1.2	12S8, 14	9, 11	12S2, 16S2.2	23, 23.2	7, 8	9, 10	17, 18
<input type="checkbox"/> WATERCTRL	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data
<input type="checkbox"/> N31774Q2	X, X	11, 12	12, 12S9	9, 11	12, 17	29S2, 30S2	15S1.2, 16S1.2	11, 12S8	9, 10	13S2, 15S2.2	20, 25	6, 7	9, 11	15, 17

Comparison to Standard Method

- **Industry standard is electrophoretic mobility**
 - **Most common instruments (e.g. ABI 3100 Genetic Analyzer) use capillary electrophoresis (CE)**
 - **1, 4, 16, 48 and 96 parallel capillary machines available**
 - **Gives size of allele PCR products**
 - **Requires allelic ladder with each run (or set of about 10 sequential runs)**
 - **ABI recommends about 25 minutes per sample per channel**
 - **Highly multiplexed (all CODIS loci plus in one reaction)**
 - **Large dynamic product size range for separating multiplexed products**

Comparison to Standard Method

- **Ibis™ technology measures product mass**
 - Gives base composition of products
 - Expands the number of alleles at several loci with polymorphic alleles (several loci have alleles with SNPs)
 - No allelic ladder required
 - Mass is intrinsic property of the product and requires no relative reference for assignment
 - 96-well format
 - Current assay = 8 wells per sample → 12 samples per plate
 - Currently about 7.5 minutes/sample (55 sec./well or 90 min./plate)
 - PLEX-ID™ will run at about 30 sec./well (about 4 min./sample)
 - Comparable to 4-capillary genetic analyzer for sample throughput
 - Multiplexing limited by spectral congestion
 - Product sizes need to be short (<250 bp for STRs)

Polymorphic STR Alleles

- **Not all alleles contain only uniform repeats**
 - Some alleles have SNPs in repeat units
 - Some alleles have SNPs in flanking regions
- **Mass spectrometry differentiates alleles of the same length that have sequence polymorphisms**
- **Allele base of several loci is substantially expanded by recognition of polymorphic alleles**

Some Alleles Have SNPs

Locus

D5S818

A52 G33 C10 T46 *

A56 G34 C10 T49 *

43372.49

44544.23

45818.42

47048.52

43090.15 43553.57 44017 44480.43 44943.86 45407.28 45870.71 46334.14 46797.57 47261 47724.42
Mass (Da)

* Masses reflect +A product and amplification with ^{13}C -enriched dGTP

Some Alleles Have SNPs

Locus

D5S818

A52 G33 C10 T46 *

A56 G34 C10 T49 *

Allele	Length	A	G	C	T
7	129	46	30	10	43
8	133	48	31	10	44
9	137	50	32	10	45
10	141	52	33	10	46
11	145	54	34	10	47
12	149	56	35	10	48
13	153	58	36	10	49
14	157	60	37	10	50
15	161	62	38	10	51

44943.86 45407.28 45870.71 46334.14 46797.57 47261 47724.42
Mass (Da)

45818.42

47048.52

* Masses reflect +A product and amplification with ¹³C-enriched dGTP

Some Alleles Have SNPs

Locus

D5S818

Allele 10

A52 G33 C10 T46 *

???

A56 G34 C10 T49 *

Allele	Length	A	G	C	T
7	129	46	30	10	43
8	133	48	31	10	44
9	137	50	32	10	45
10	141	52	33	10	46
11	145	54	34	10	47
12	149	56	35	10	48
13	153	58	36	10	49
14	157	60	37	10	50
15	161	62	38	10	51

45818.42

47048.52

44943.86 45407.28 45870.71 46334.14 46797.57 47261 47724.42
Mass (Da)

* Masses reflect +A product and amplification with ¹³C-enriched dGTP

Some Alleles Have SNPs

Locus

D5S818

Allele 10

A52 G33 C10 T46 *

Allele 12 with a G→T SNP

A56 G34 C10 T49 *

Allele	Length	A	G	C	T
7	129	46	30	10	43
8	133	48	31	10	44
9	137	50	32	10	45
10	141	52	33	10	46
11	145	54	34	10	47
12	149	56	35	10	48
12 (G→T)	149	56	34	10	49
13	153	58	36	10	49
14	157	60	37	10	50
15	161	62	38	10	51

45818.42

47048.52

4943.86 45407.28 45870.71 46334.14 46797.57 47261 47724.42
Mass (Da)

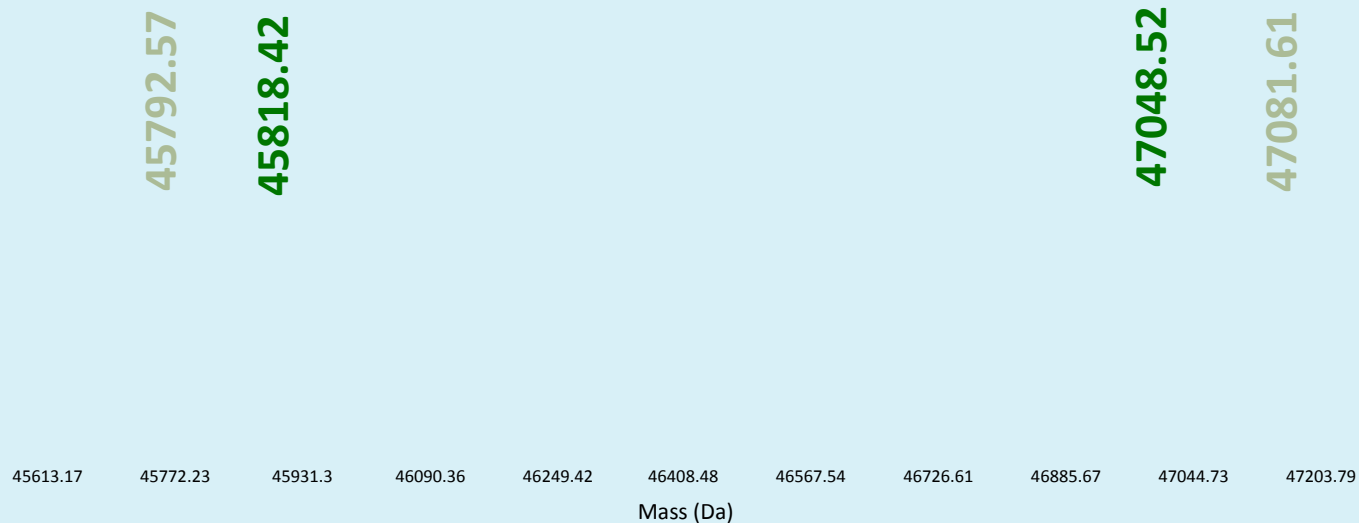
* Masses reflect +A product and amplification with ¹³C-enriched dGTP

SNPs are Accurately Assigned

Locus
D5S818

Allele 12 has very different masses

Allele 12 with a G→T SNP
A56 G34 C10 T49 *



* Masses reflect +A product and amplification with ^{13}C -enriched dGTP

Same-Length Heterozygotes

Locus

D5S818

Allele 12 A56 **G35** C10 **T48** *

Allele 12 (G→T) A56 **G34** C10 **T49** *

45794.49

45817.38

Sample 89409 from UNTHSC

47048.11

47081.84

45722.32 45871.95 46021.58 46171.21 46320.84 46470.46 46620.09 46769.72 46919.35 47068.98 47218.6

Mass (Da)

* Masses reflect +A product and amplification with ¹³C-enriched dGTP

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Several Additional Alleles Seen

Locus shown
is D5S818

Each SNP-annotated
allele has been
observed

Allele	Length	A	G	C	T
7	129	46	30	10	43
8	133	48	31	10	44
8 (G->T)	133	48	30	10	45
9	137	50	32	10	45
9 (A->C)	137	49	32	11	45
9 (G->T)	137	50	31	10	46
10	141	52	33	10	46
10 (G->T)	141	52	32	10	47
11	145	54	34	10	47
11 (G->T)	145	54	33	10	48
12	149	56	35	10	48
12 (G->T)	149	56	34	10	49
13	153	58	36	10	49
13 (G->C)	153	58	35	11	49
13 (G->T)	153	58	35	10	50
14	157	60	37	10	50
14 (G->C)	157	60	36	11	50
14 (G->T)	157	60	36	10	51
15	161	62	38	10	51
15 (G->C)	161	62	37	11	51

Polymorphisms in 688 Samples

- 95 reference samples from J. Butler (NIST)
- 593 reference samples from J. Planz (UNTHSC)
- Polymorphisms observed in 11 of 13 loci
- Same-length heterozygous genotypes seen in nine loci

Locus	Number of Alleles with SNPs	% of alleles with one or more SNPs	% of samples heterozygous with same length alleles
D3S1358	1037	75.4	9.3
D8S1179	918	66.7	5.7
D13S317	431	31.3	7.6
D21S11	374	27.2	3.3
vWA	356	25.9	5.4
D5S818	330	24.0	11.2
D7S820	125	9.1	2.5
FGA	30	2.2	0.1
D18S51	23	1.7	0.4
D16S539	5	0.4	0.0
CSF1PO	2	0.1	0.0

SNPs Expand Allele Space

- Additional alleles observed beyond standard length-based alleles
- For all polymorphic alleles, non-polymorphic allele has also been observed (it is not simply an allele-labeling artifact)
- From 688 samples, additional alleles have been noted in 11 of 13 loci

Locus	Number of additional alleles
CSF1PO	2
D13S317	7
D16S539	3
D18S51	10
D21S11	24
D3S1358	18
D5S818	10
D7S820	7
D8S1179	15
FGA	13
vWA	23

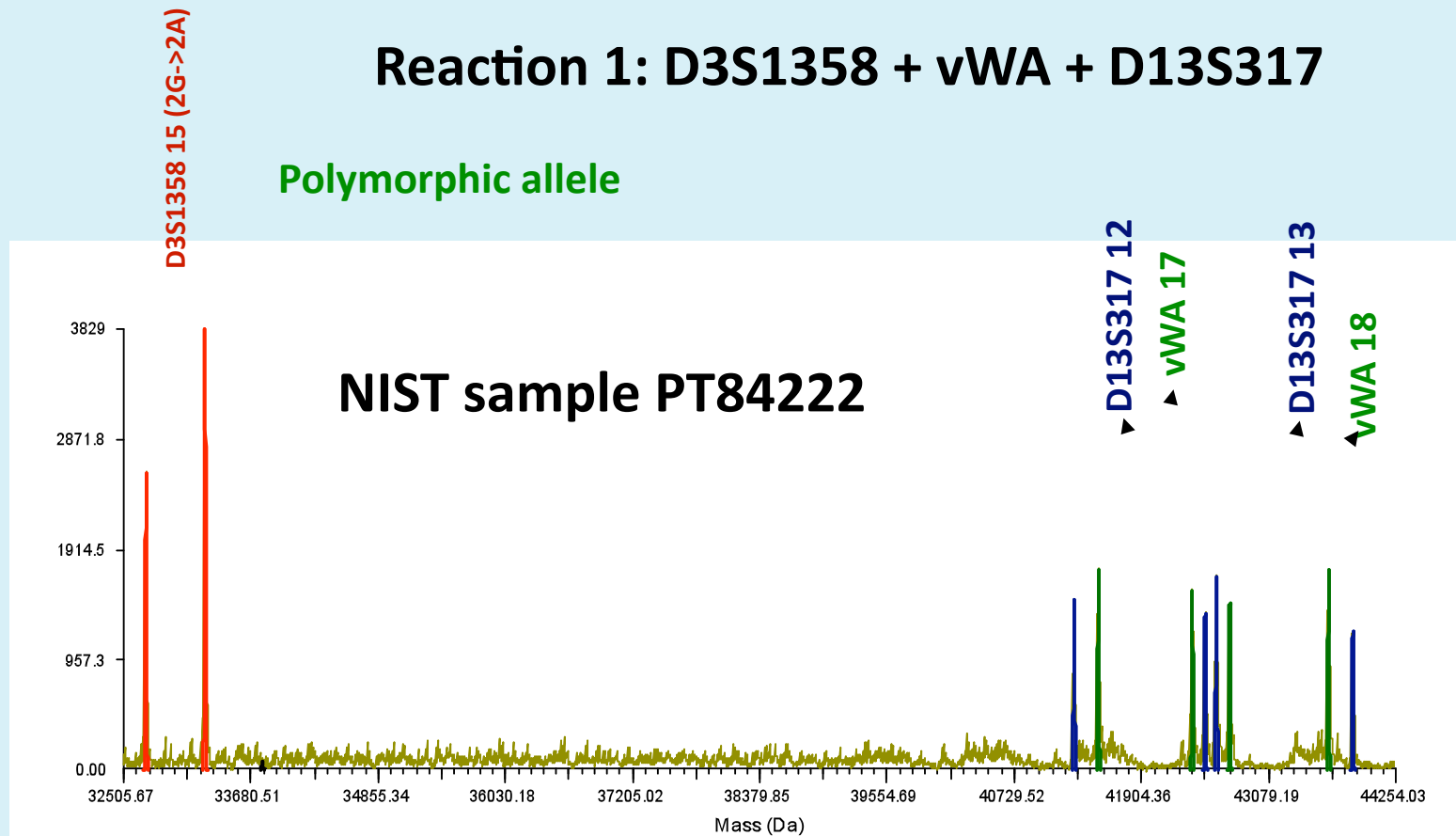


Table shows the number of discrete alleles observed beyond those predicted based upon published allele sequences and/or published repeat structure

Example of STR MS Data

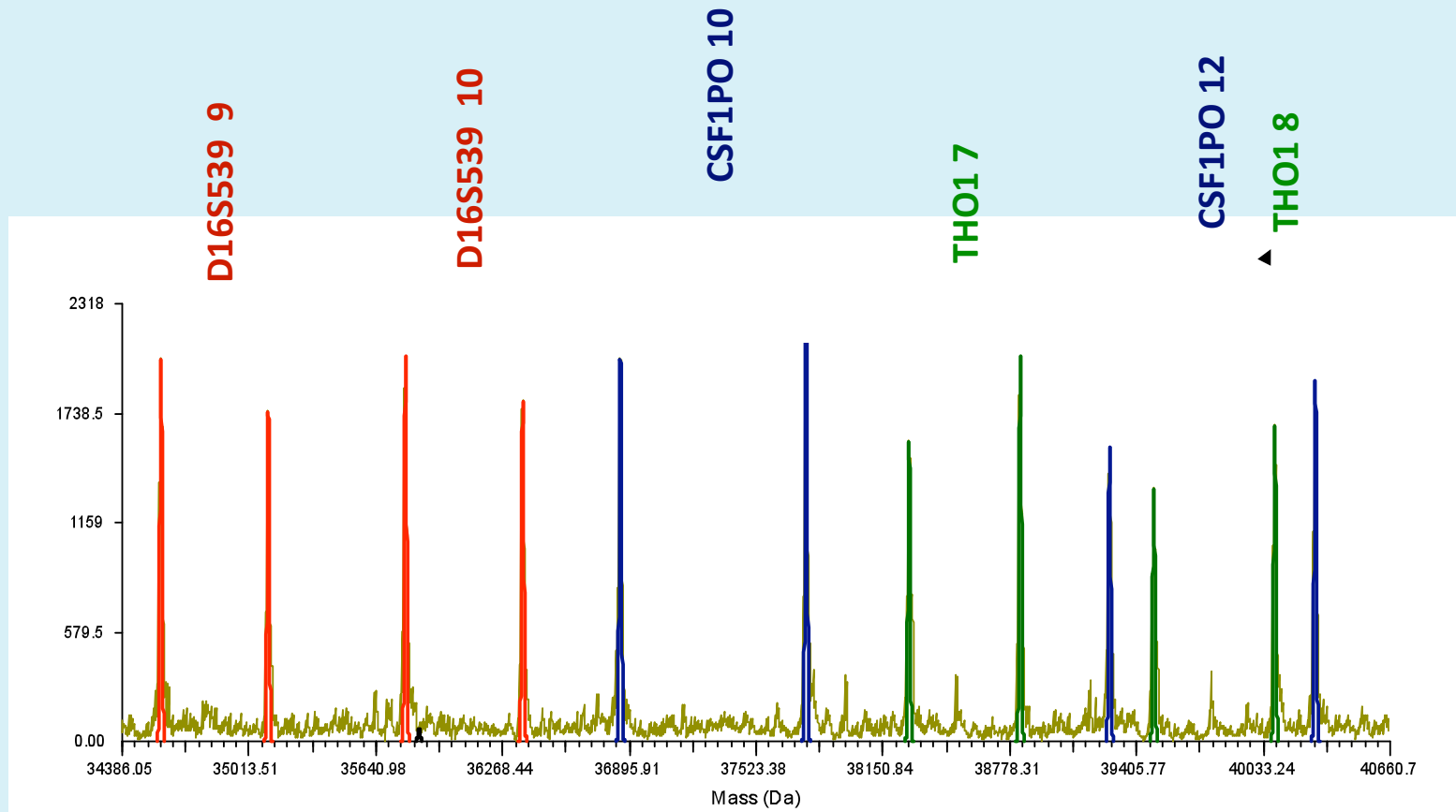
Reaction 1: D3S1358 + vWA + D13S317

Polymorphic allele



Reaction 2

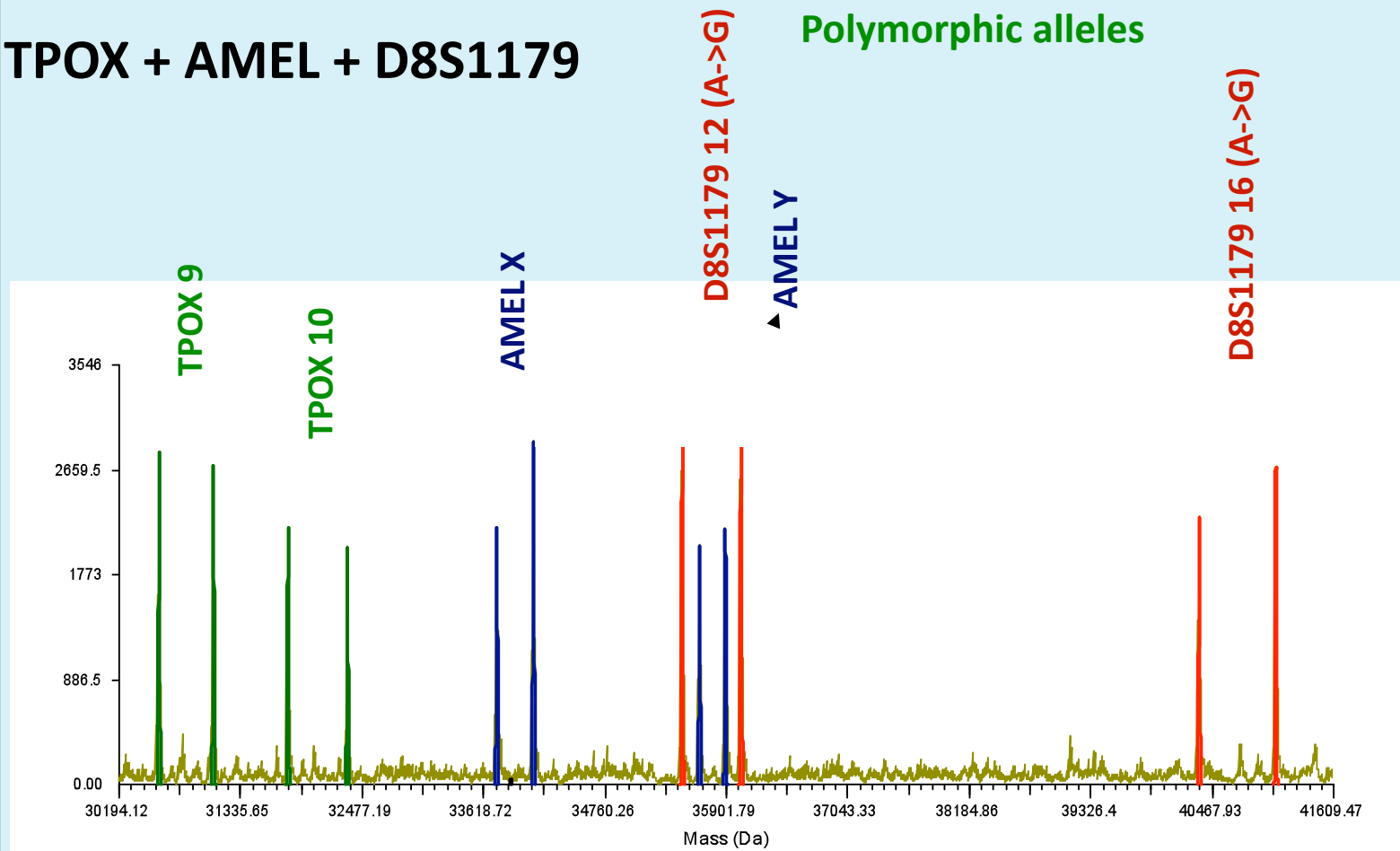
D16S539 + CSF1PO + THO1



Reaction 3

TPOX + AMEL + D8S1179

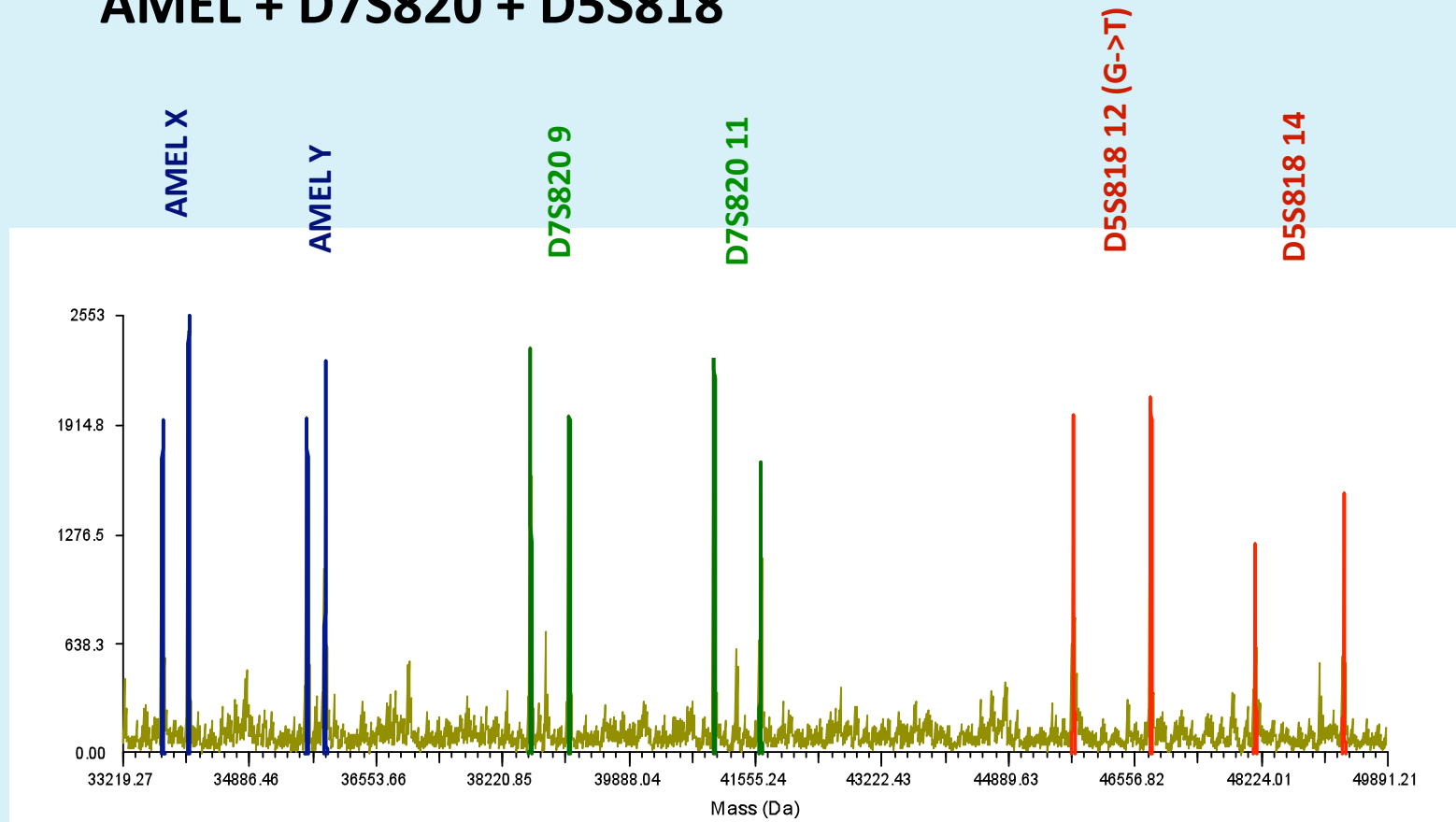
Polymorphic alleles



Reaction 4

AMEL + D7S820 + D5S818

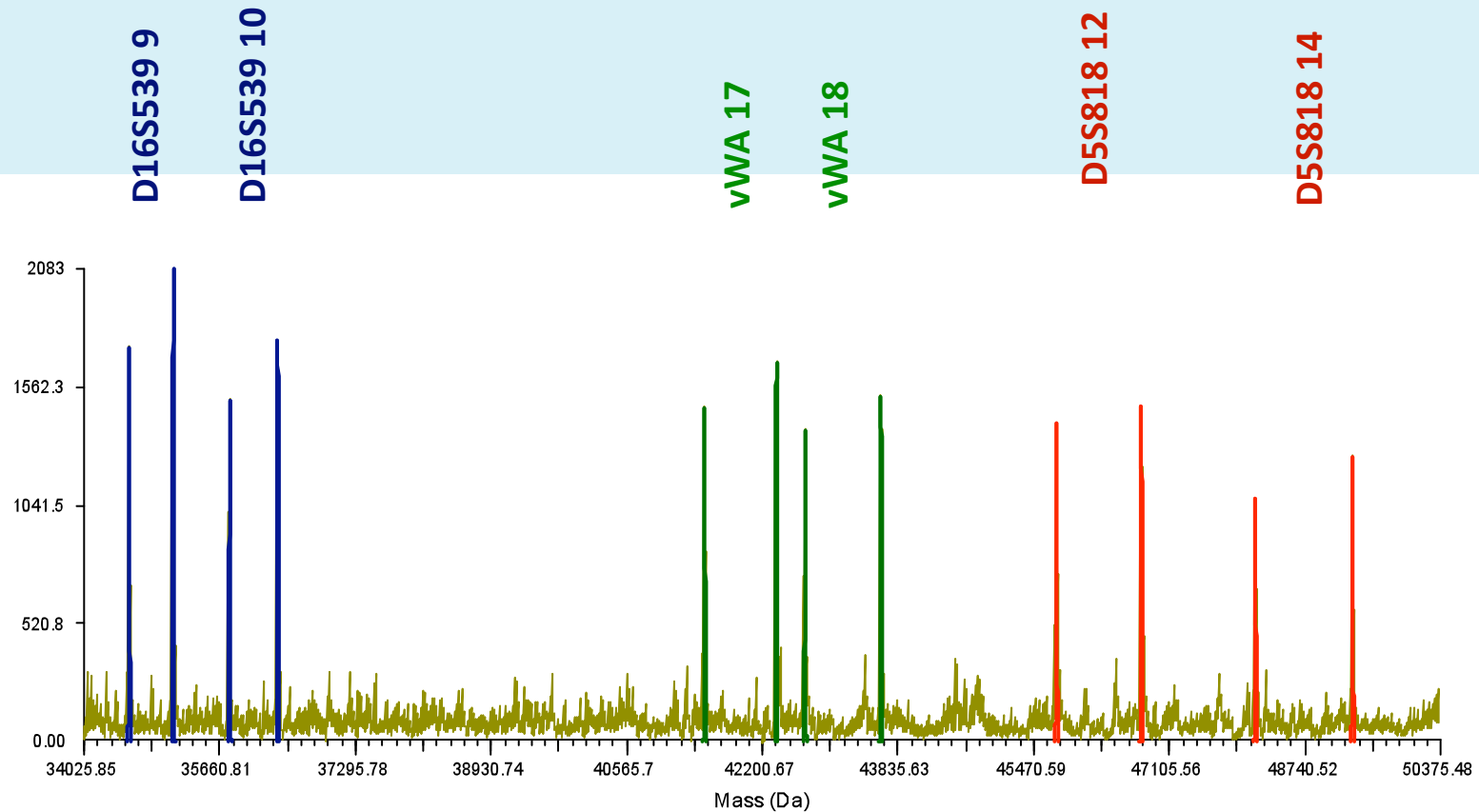
Polymorphic allele



Reaction 5

D16S539 + vWA + D5S818

Polymorphic allele



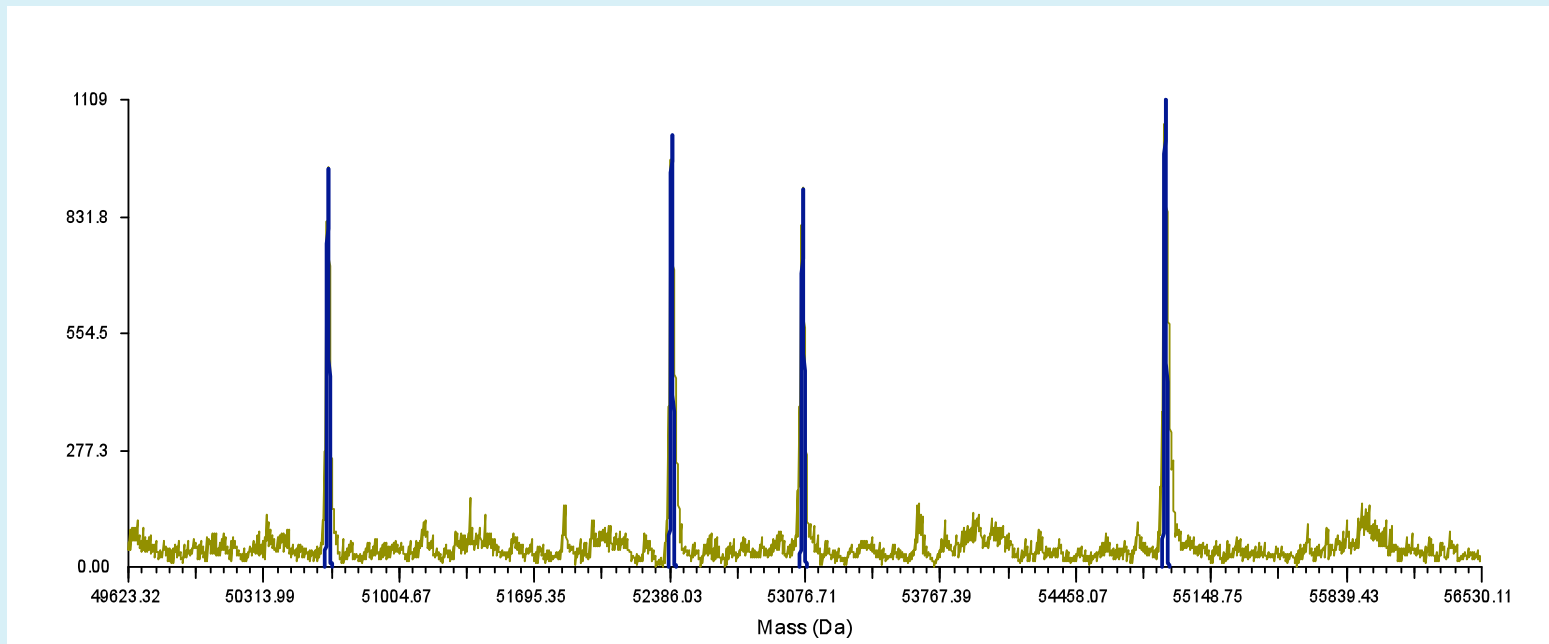
Reaction 6

D21S11

D21S11 28

D21S11 30 (G->A)

Polymorphic allele

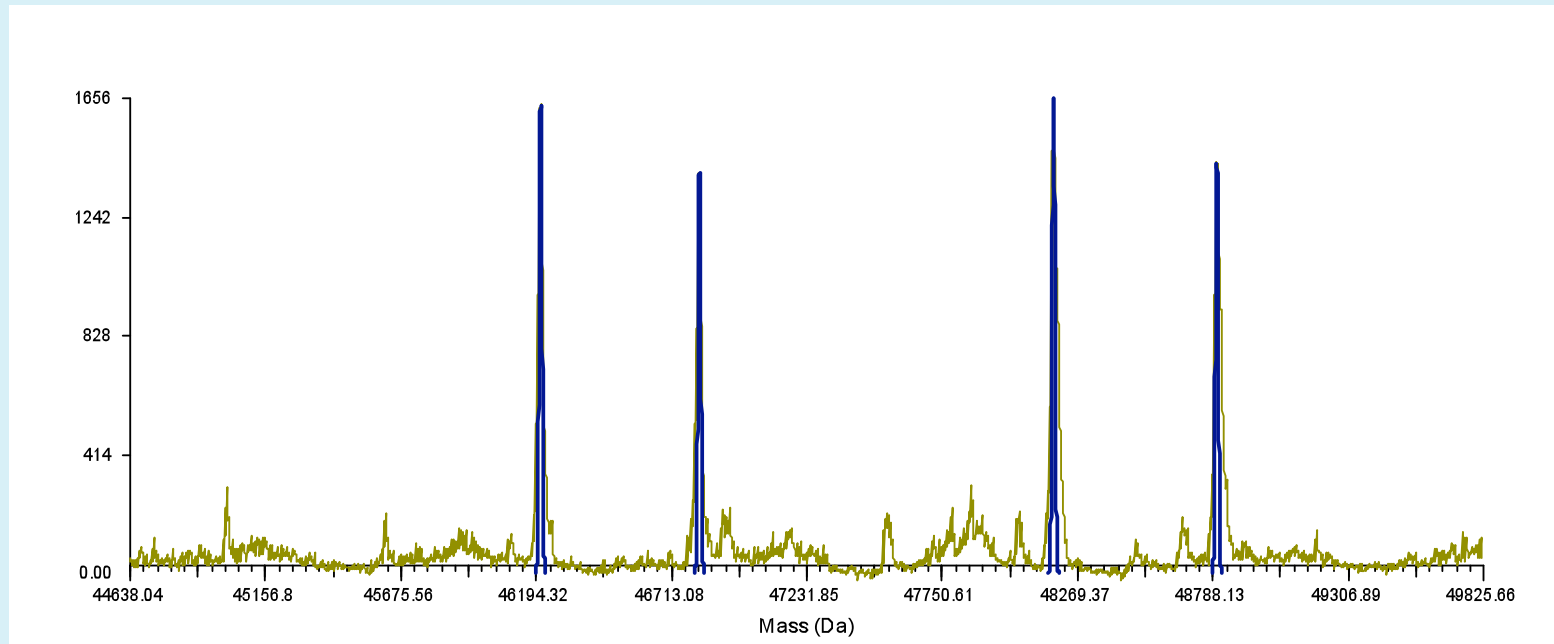


Reaction 7

FGA

FGA 23

FGA 23.2

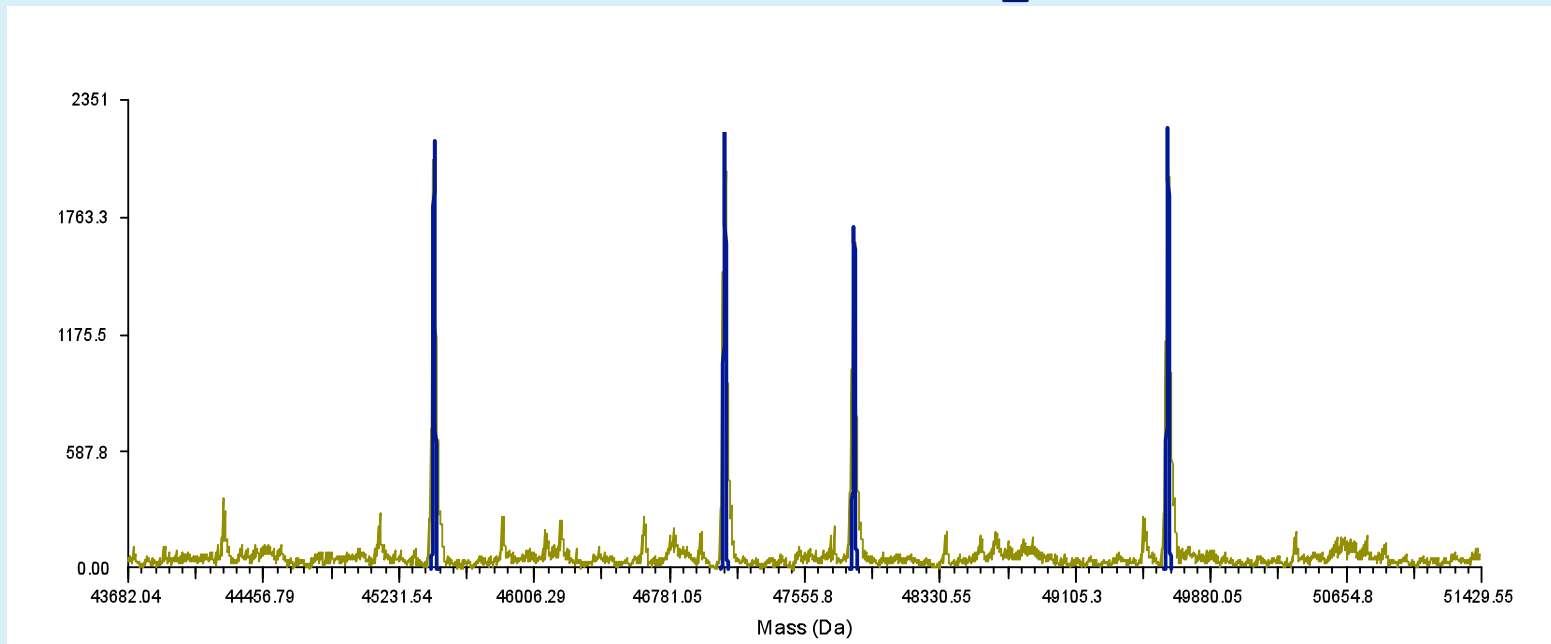


Reaction 8

D18S51

D18S51 15

D18S51 17

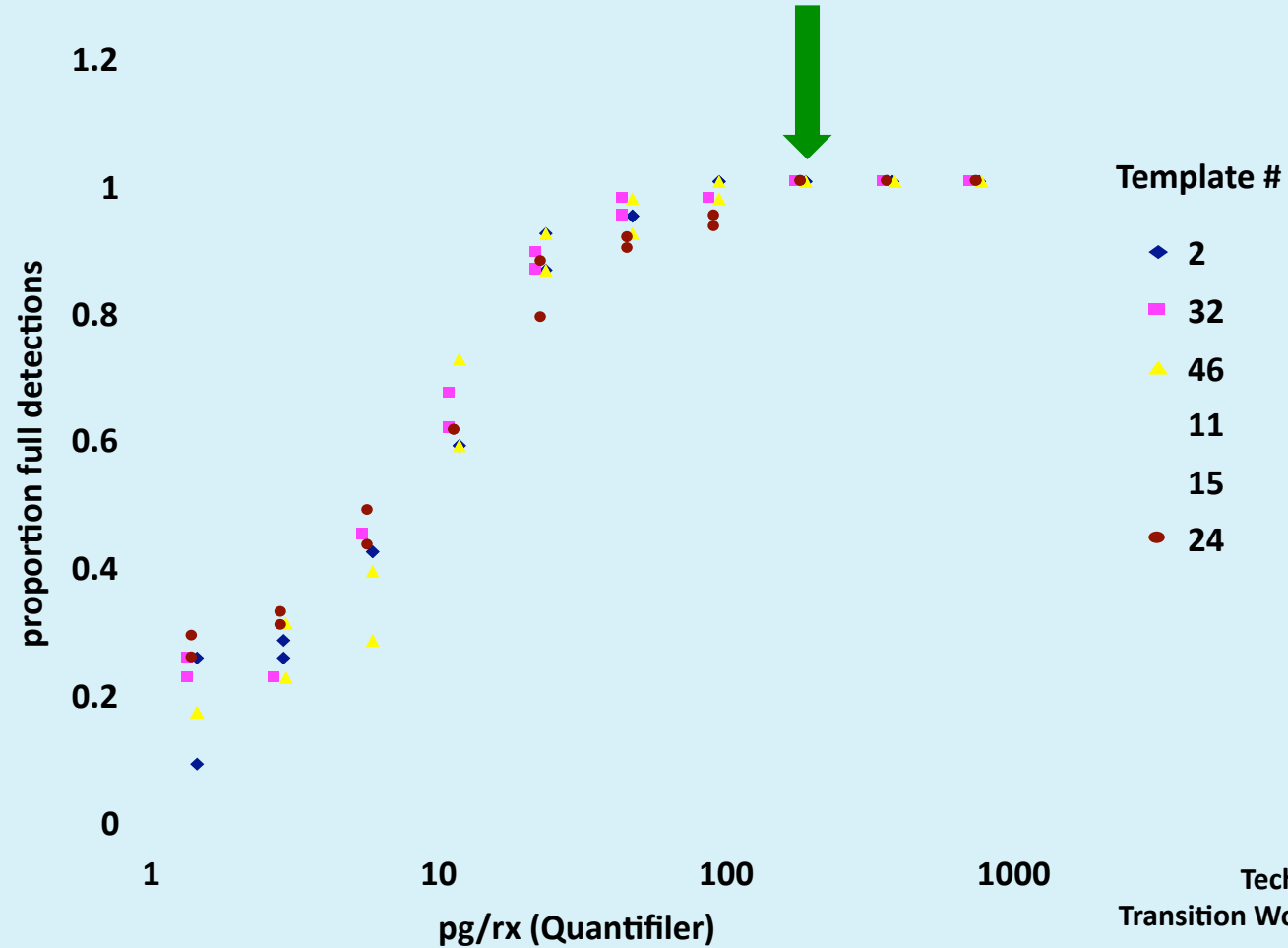


Sensitivity

- **Six templates examined in DTE**
 - DNA isolated from blood
 - Quantified by Quantifiler® and by A_{260}
 - 1 ng/reaction to <1 pg/reaction
 - Each DTE series run in duplicate
- **All 12 replicates produced full profiles at 125 pg/reaction**
 - 1 ng total for 8 reactions
- **Several replicates produced full profiles at 62.5 pg/reaction**

Sensitivity

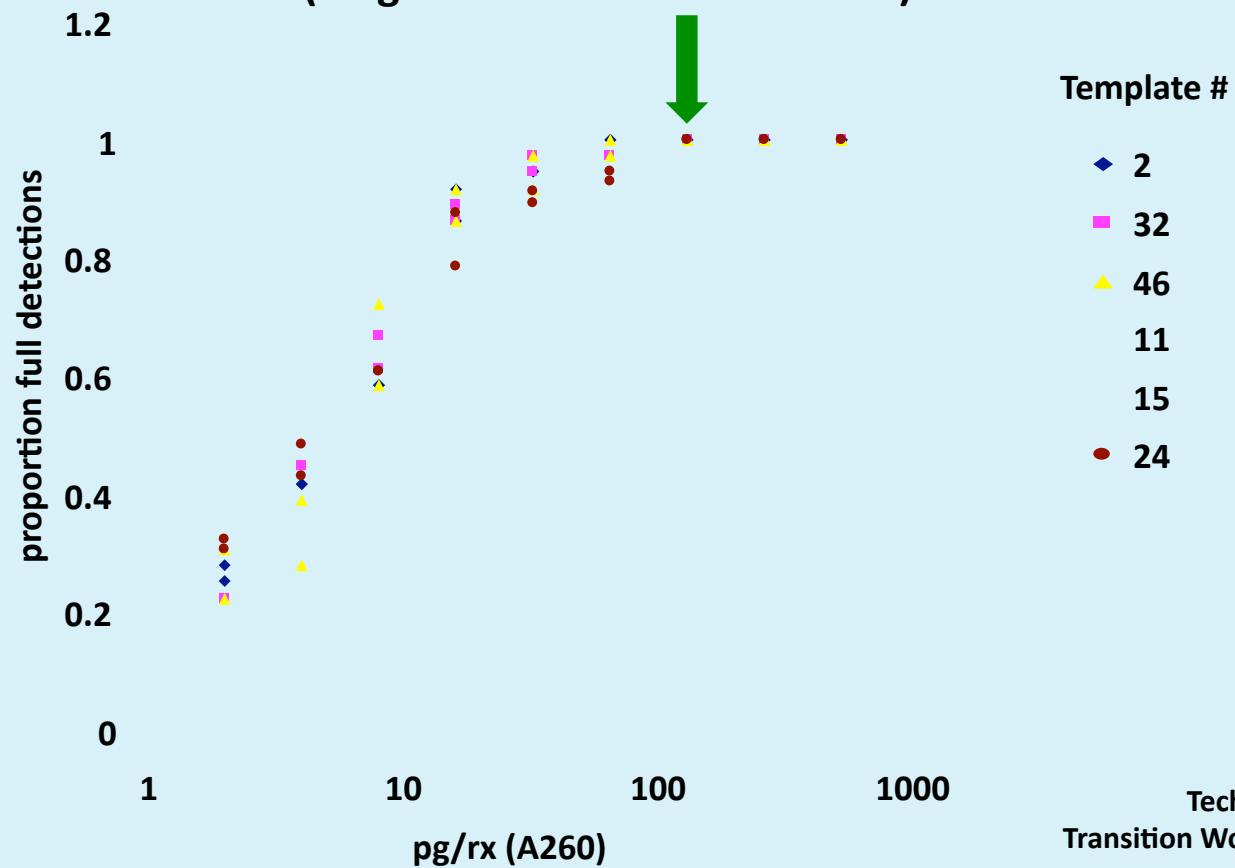
All templates gave full profiles at 125 pg/reaction
(1 ng total DNA for 8 reactions)



Sensitivity

Slight difference in quantification with A_{260} (about 20% less on average)

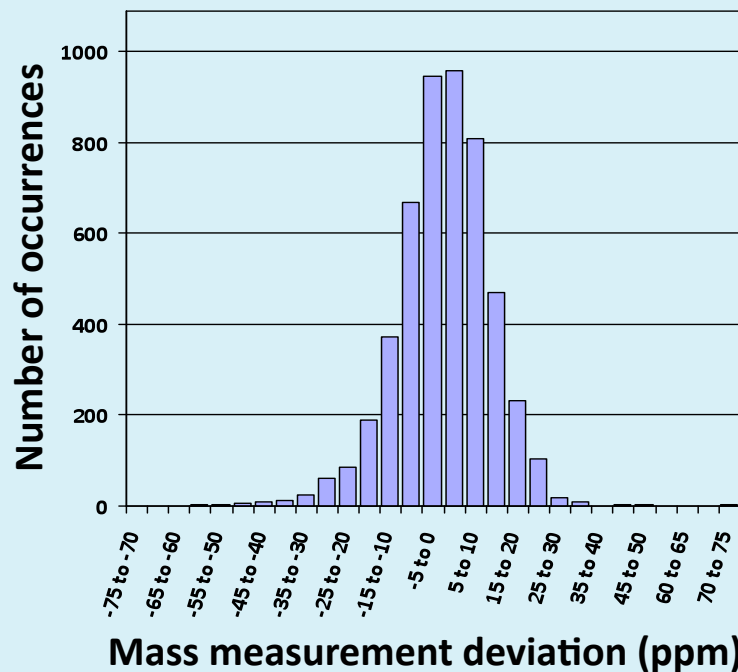
All templates gave full profiles at 125 pg/reaction
(1 ng total DNA for 8 reactions)



Precision and Accuracy

- 75 runs of 53 templates
- 2495 double-stranded product assignments
- 4990 mass measurements

Distribution of mass measurement deviations for 4990 measurements



- Average deviation magnitude = 8.5 ± 7.2 ppm
- Average deviation = 0.15 ± 11.2 Daltons (errors are centered on zero)
- >95% of all assignments are within ± 25 ppm

Summary

- **Core CODIS loci covered**
- **12 samples per plate**
- **About 4 minutes per sample, fully automated post-PCR**
- **No allelic ladder required**
- **Polymorphic alleles discovered with high precision**
 - **A benefit in analysis of related samples (e.g. paternity, missing persons, pedigree analysis, kinship-based searching)**
- **Polymorphic alleles are common in six loci (> 20%)**
- **Sensitivity per reaction about 125 pg/reaction**
 - **Ibis™ assay uses eight reactions per sample, so overall requires a little more DNA than current kits (> = 1 ng/sample)**

Questions?

Contact Information

Thomas Hall

Ibis Biosciences division of Abbott Molecular

760-476-3375

thall@ibisbio.com

Note: *All images and charts courtesy of Tom Hall, Ph.D.
unless otherwise noted.*