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

Overview of the Ibis™ Y-STR, mtDNA and SNP Assays

Assays

- **Ibis™ Y-STR Assay format**
- **Ibis™ mtDNA Assay format**
- **Ibis™ SNP Assay format**

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Y-STR Assay Format

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Y-STR Markers

- **Core minimum haplotype markers + recommended loci DYS437, DYS438 and DYS439**

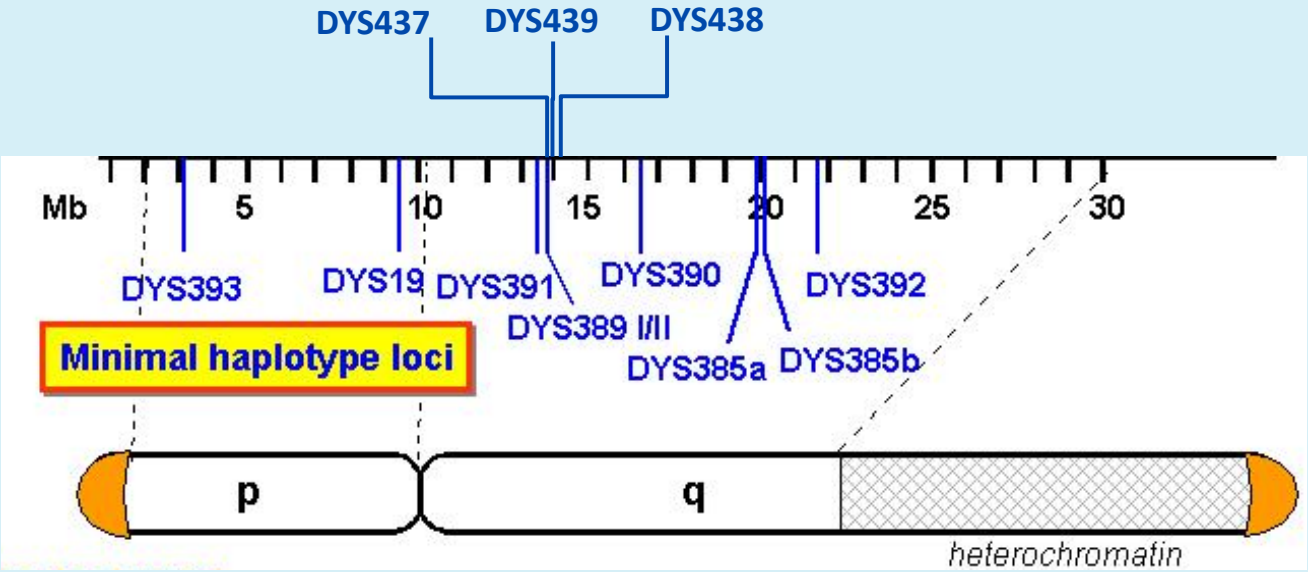

Image adapted from:
http://www.cstl.nist.gov/biotech/strbase/ystrpos1.htm

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Additional Y-STR Loci Being Considered

- **DYS456**
- **DYS458**
- **DYS448**
- **DYS635**
- **Y-GATA-H4**

Included in various tested assay configurations

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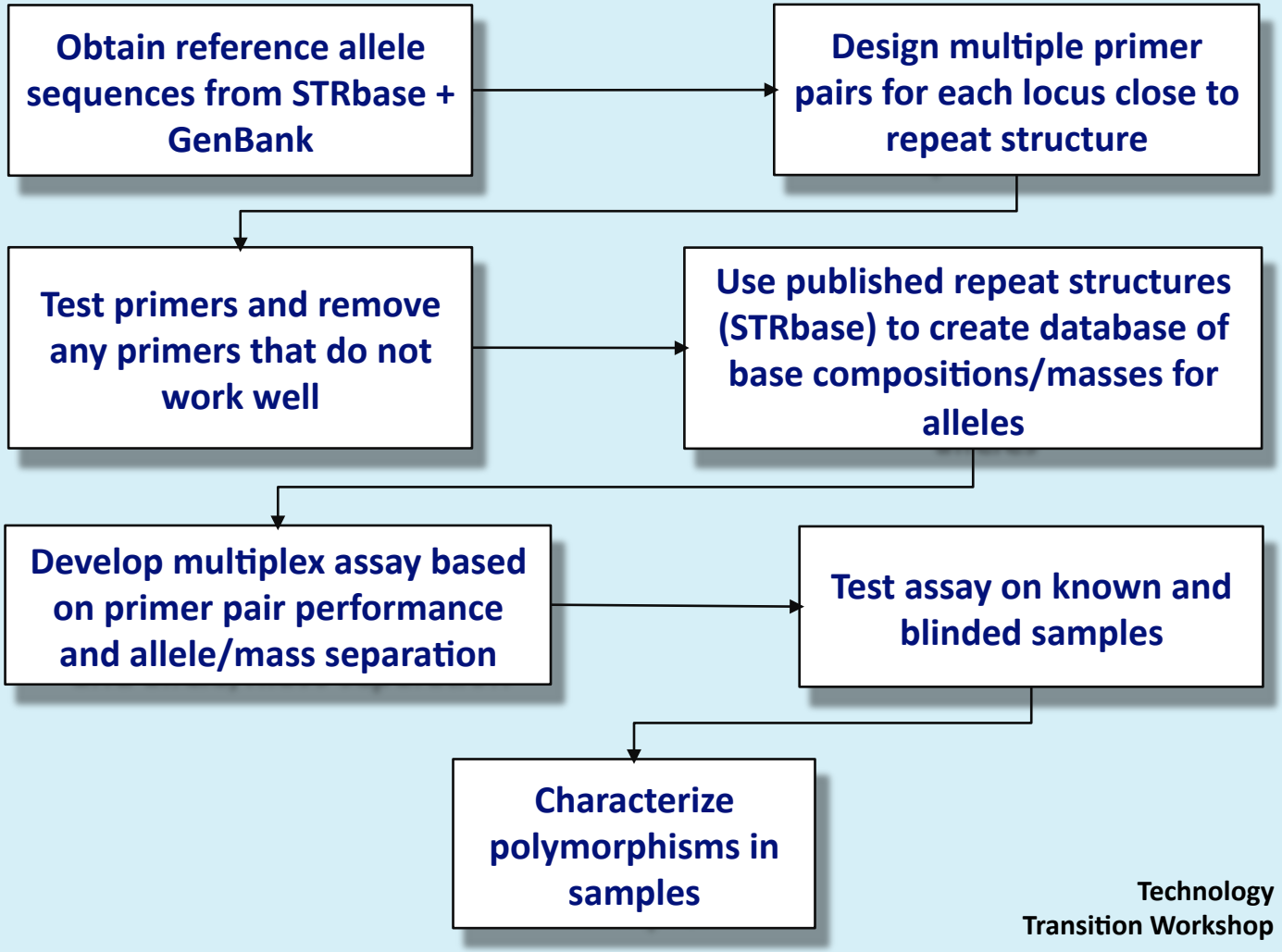
Approach to Y-STR Assay Development



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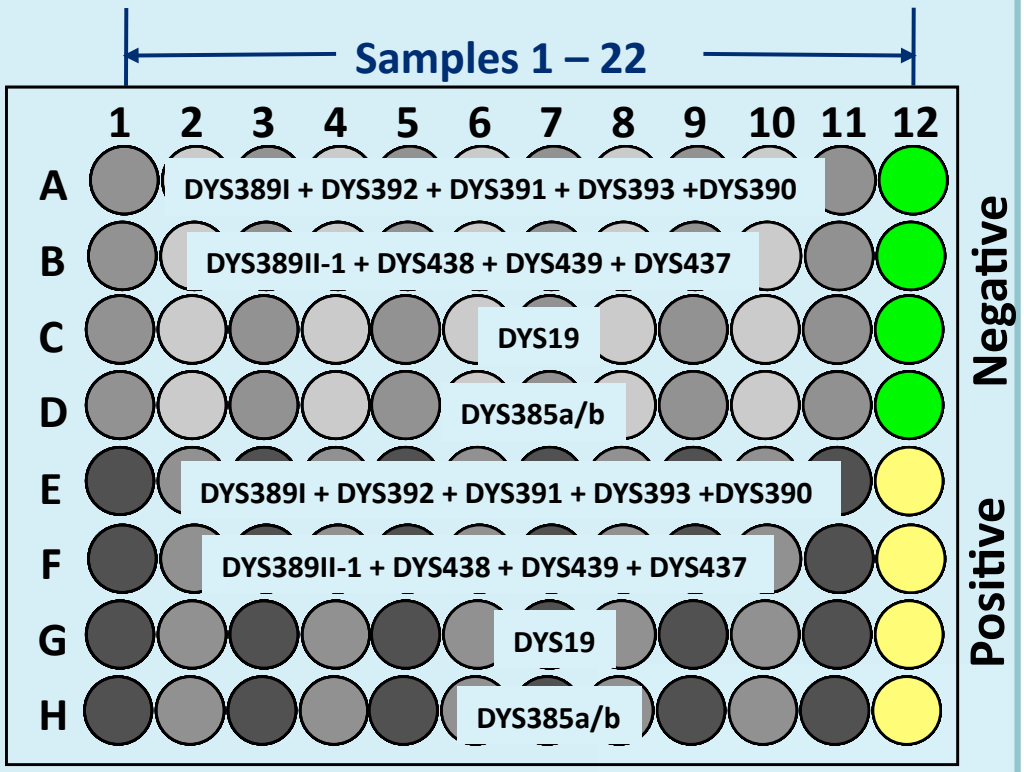
Original Y-STR Assay Layout

Reaction	Primer pair	Locus	Conc (nM)
Multiplex 1	4586	DYS389I	160
	4597	DYS392	160
	4594	DYS391	160
	4602	DYS393	160
	4591	DYS390	160
Multiplex 2	4587	DYS389II-1	200
	4611	DYS438	200
	4615	DYS439	200
	4608	DYS437	200
Single-plex 1	4579	DYS19	250
Single-plex 2	4582	DYS385a/b	250

- 24 samples per plate
- 5-plex, 4-plex and 2 single-plex reactions



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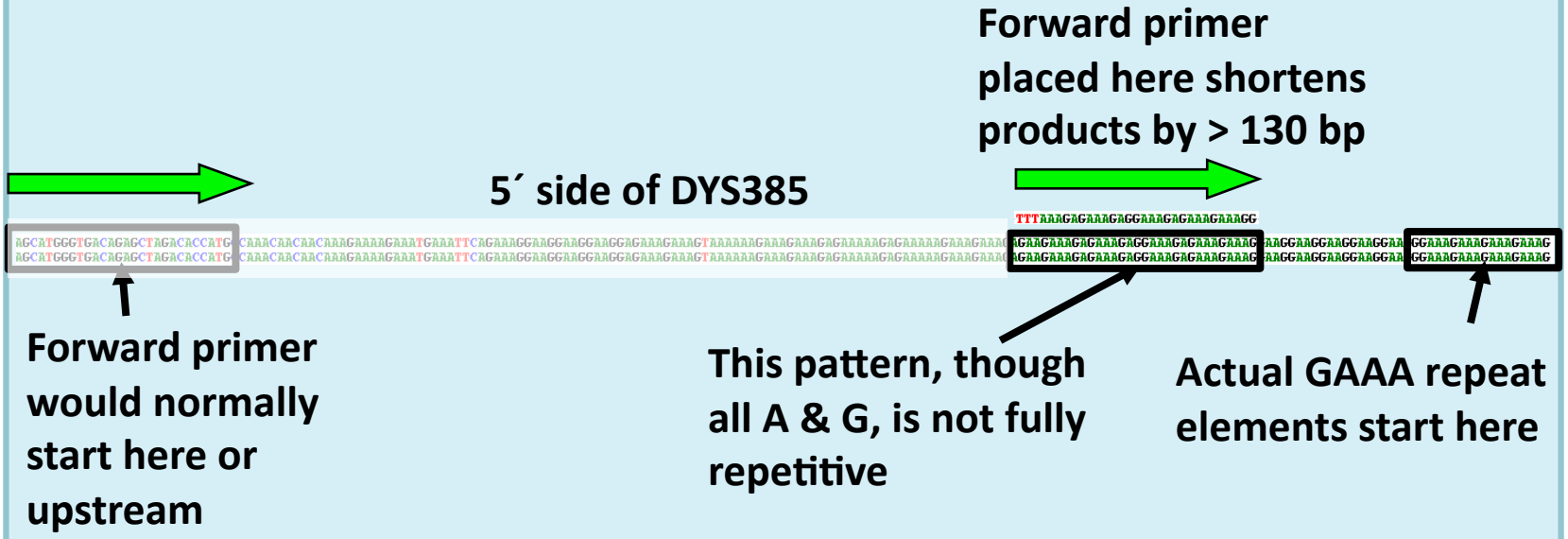
Reducing the Size of DYS385a/b

- DYS385a/b has a large product size range
- Shortest primer pair in STRbase has range of 241 – 324 bp
- Our system does best at < 200 bp



- This priming strategy has been tested with 126 samples and confirmed by standard typing

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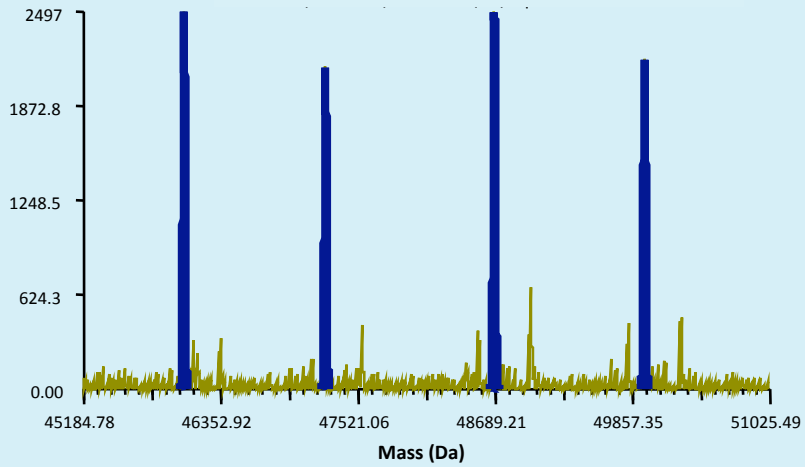
Reducing the Size of DYS385a/b

- **Two blood-derived DNAs tested at 1 ng each**

072109C

DYS385a/b
14: [A93 G46 C5 T13] (157 bp)

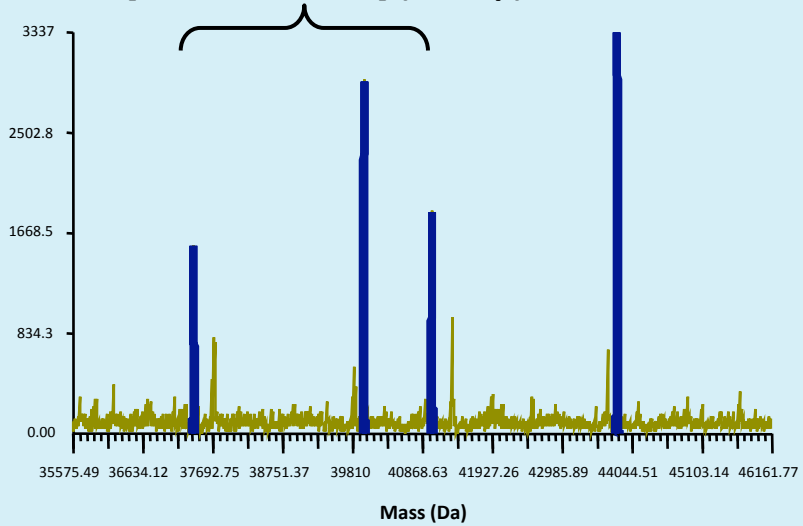
DYS385a/b
13: [A90 G45 C5 T13] (153 bp)



SC35495

DYS385a/b
14: [A93 G46 C5 T13] (157 bp)

DYS385a/b
11: [A84 G43 C5 T13] (145 bp)



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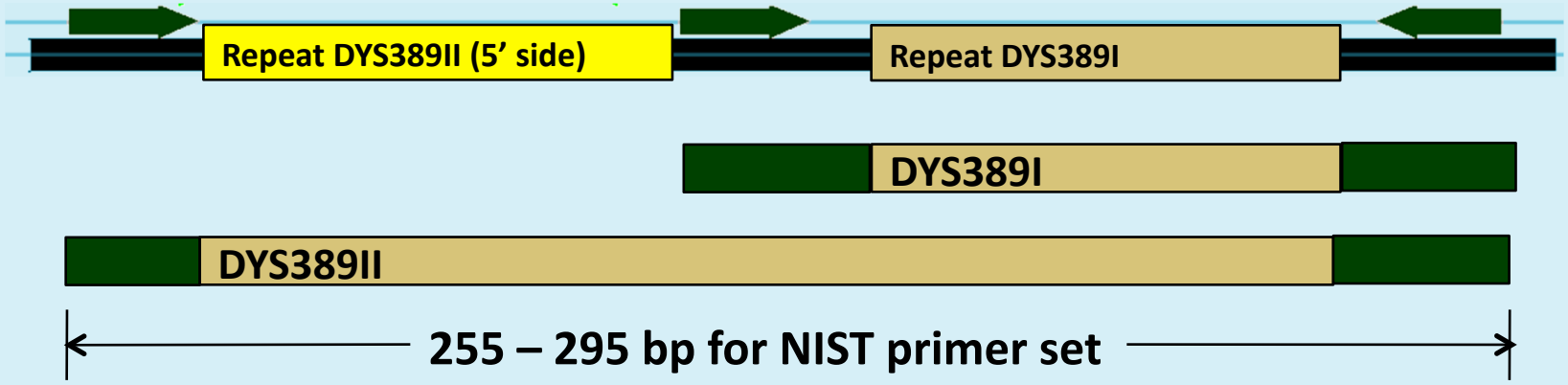
Splitting DYS389I/II into Separate Products

- DYS389 has a duplicated forward primer binding region
- The duplicated primer target spans one repeat region
- The reverse primer and second forward primer span DYS389I
- Two products are generated with one primer pair



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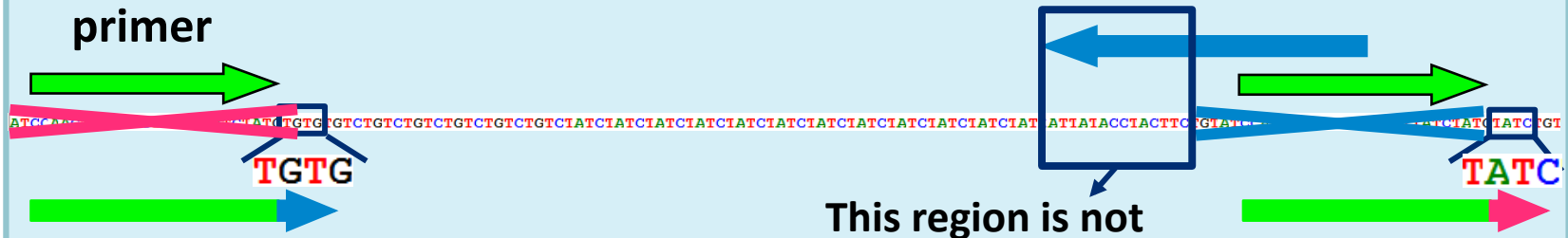
Splitting DYS389I/II into Separate Products

- DYS389 has a duplicated forward primer binding region
- There is a 4-base difference that can be exploited
- Modified upstream primer won't prime downstream site
- Modified downstream primer won't prime upstream site
- A reverse primer can be made to pair with the upstream forward primer



- This priming strategy has been tested with 126 samples and confirmed by standard typing
- DYS389II = DYS389I + DYS389II-1
  - When AB has DYS389I = 13 and DYS389II = 29, Ibis™ gets DYS389II-1 = 16

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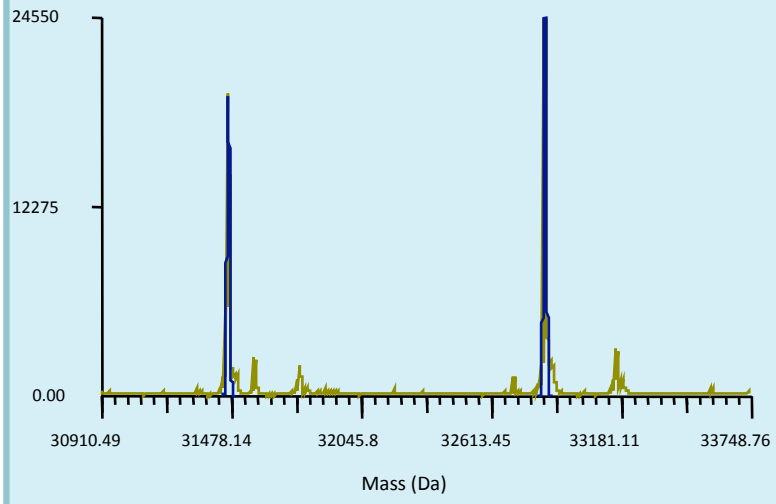
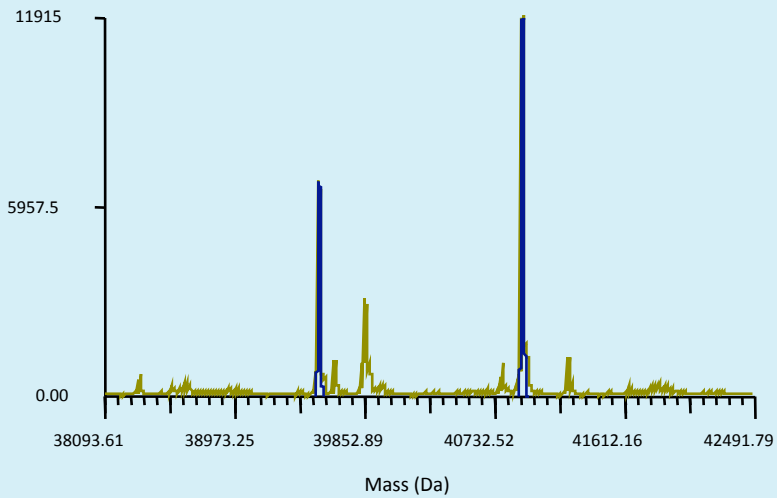
Splitting DYS389I/II into Separate Products

DYS389II = 13 + 16 = 29

DYS389I 13



DYS389II-1 16



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Y-STR Assay Testing

- 95 male population samples from NIST (John Butler) tested
  - 13 loci: 92 samples had previous Y-STR truth data
- 36 male samples at Ibis™ – 16 loci – 34 also typed with Y-Filer
- All loci were concordant

Locus	Number of alleles observed with SNP(s)	Samples tested	%
DYS635	14	36	38.9
DYS389II-1	42	131	32.1
DYS437	28	131	21.4
DYS390	10	131	7.6
DYS385a/b	5	131	3.8
DYS458	1	36	2.8
DYS393	2	131	1.5
DYS438	2	131	1.5
DYS391	1	131	0.8
DYS19	0	131	0.0
DYS389I	0	131	0.0
DYS392	0	131	0.0
DYS439	0	131	0.0
DYS448	0	36	0.0
DYS456	0	36	0.0
Y-GATA-H4	0	36	0.0

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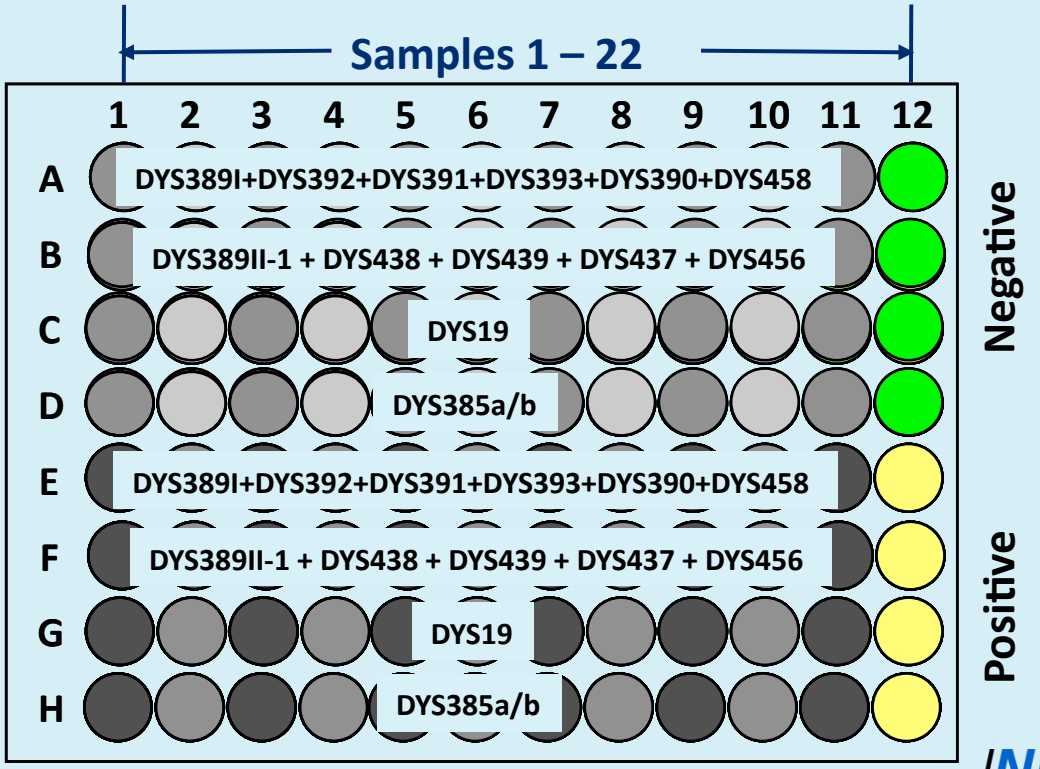
Updated Y-STR Assay Layout

Reaction	Primer pair	Locus
Multiplex 1	4586	DYS389I
	4597	DYS392
	4594	DYS391
	4601	DYS393
	4591	DYS390
	4924	DYS458
Multiplex 2	4587	DYS389II-1
	4611	DYS438
	4615	DYS439
	4608	DYS437
	4929	DYS456
Single-plex 1	4579	DYS19
Single-plex 2	4692	DYS385a/b

- Still 24 samples per plate
- DYS456 and DYS458 have been added
- 6-plex, 5-plex and 2 single-plex reactions



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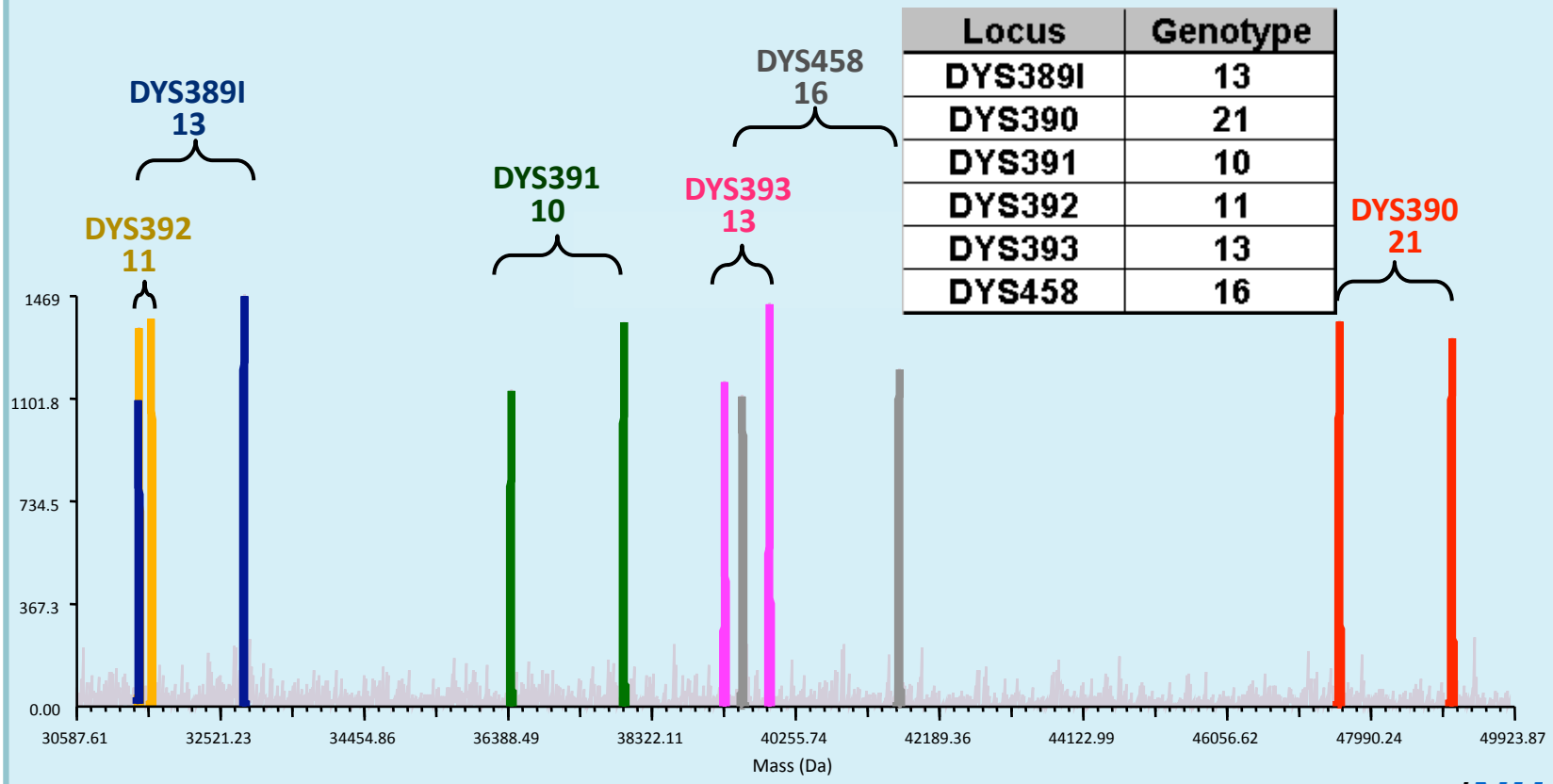
6-plex Reaction 1: Sample N31773



Locus	Genotype
DYS389I	13
DYS390	21
DYS391	10
DYS392	11
DYS393	13
DYS458	16

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5-plex Reaction 2: Sample N31773

Locus	Genotype
DYS389I	13
DYS389II-1	19 (A->G)
DYS389II	32 (A->G)
DYS390	21
DYS391	10
DYS392	11
DYS393	13
DYS437	14 (C->T)
DYS438	11
DYS439	11
DYS456	15
DYS458	16

+

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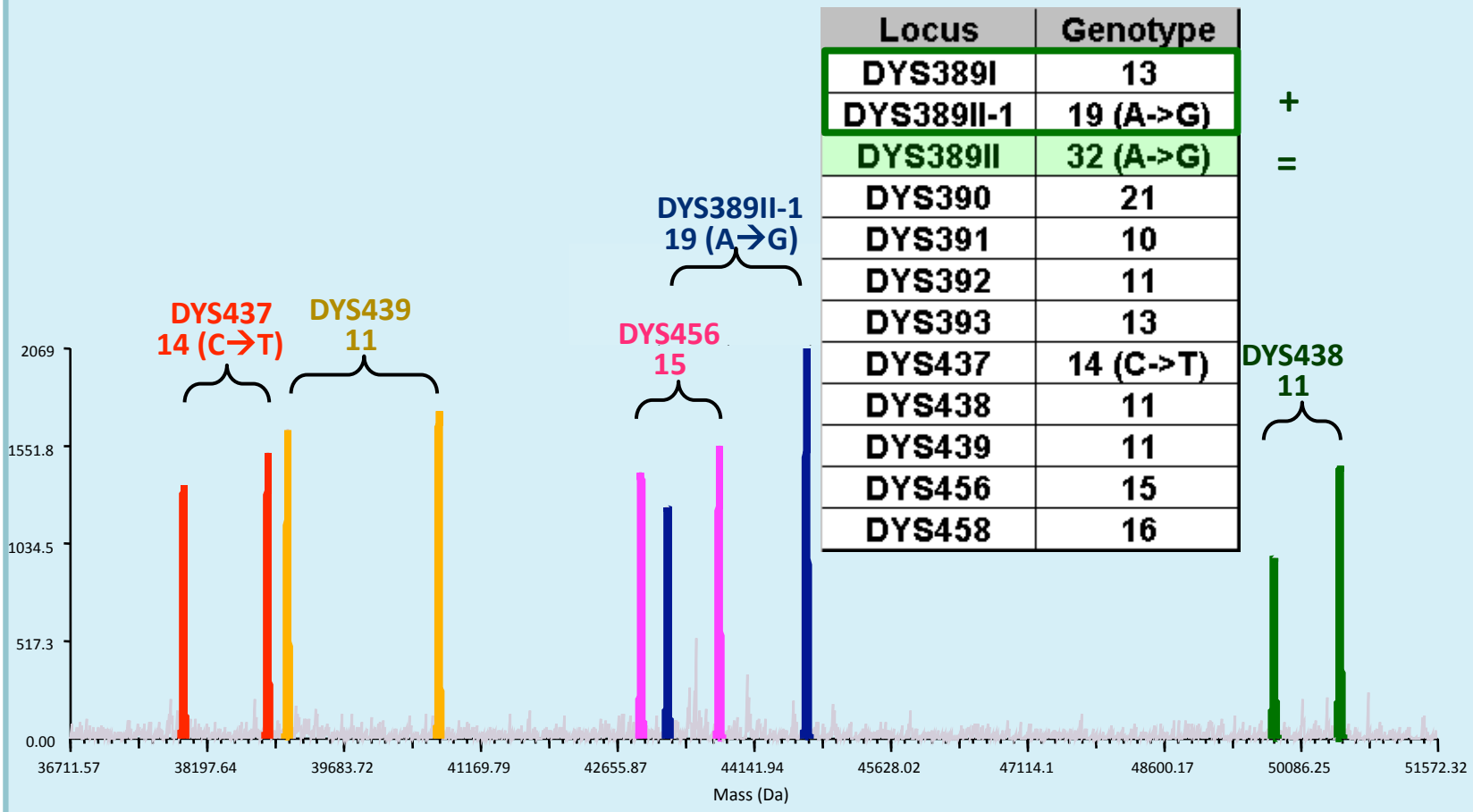

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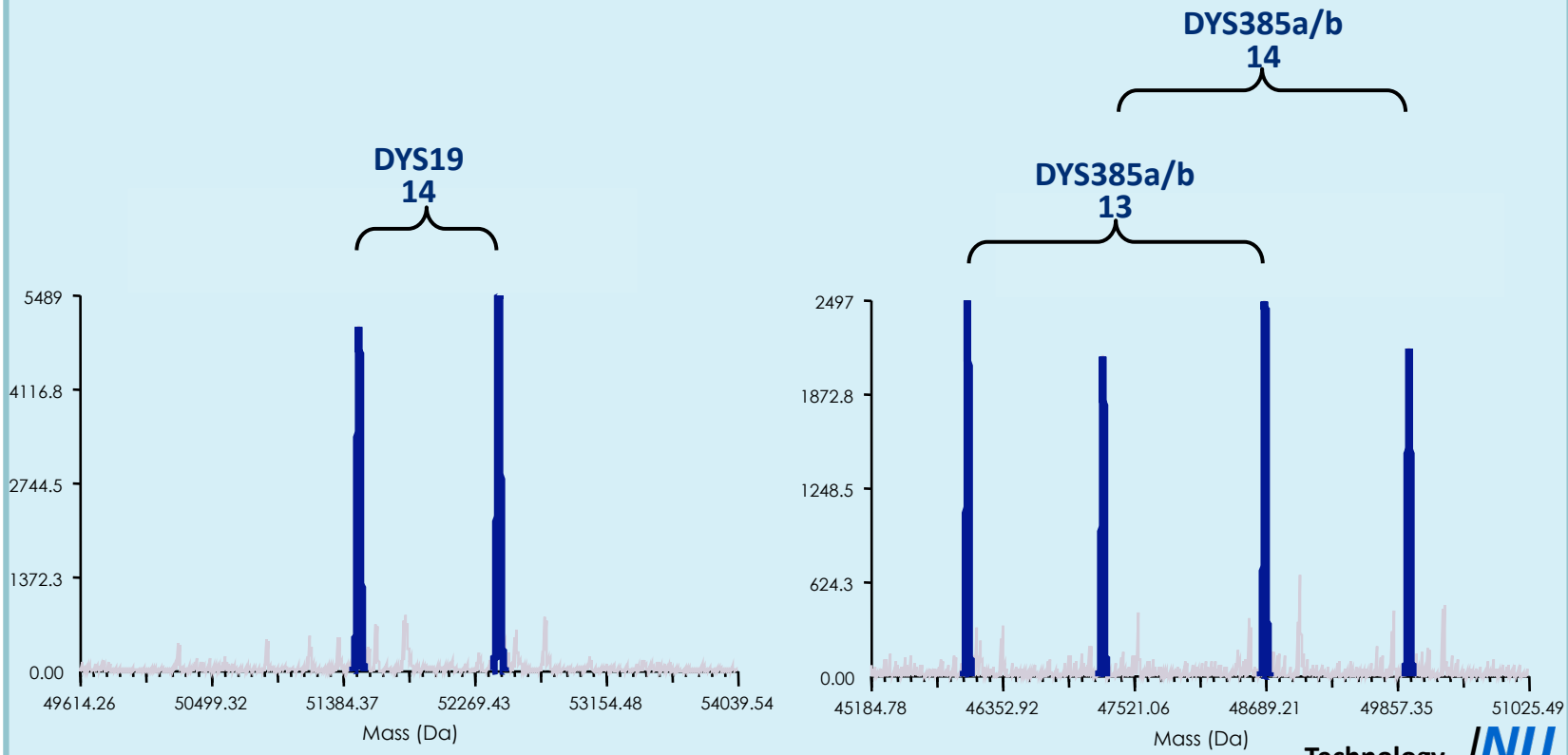
Single Reactions 3 and 4: Sample N31773



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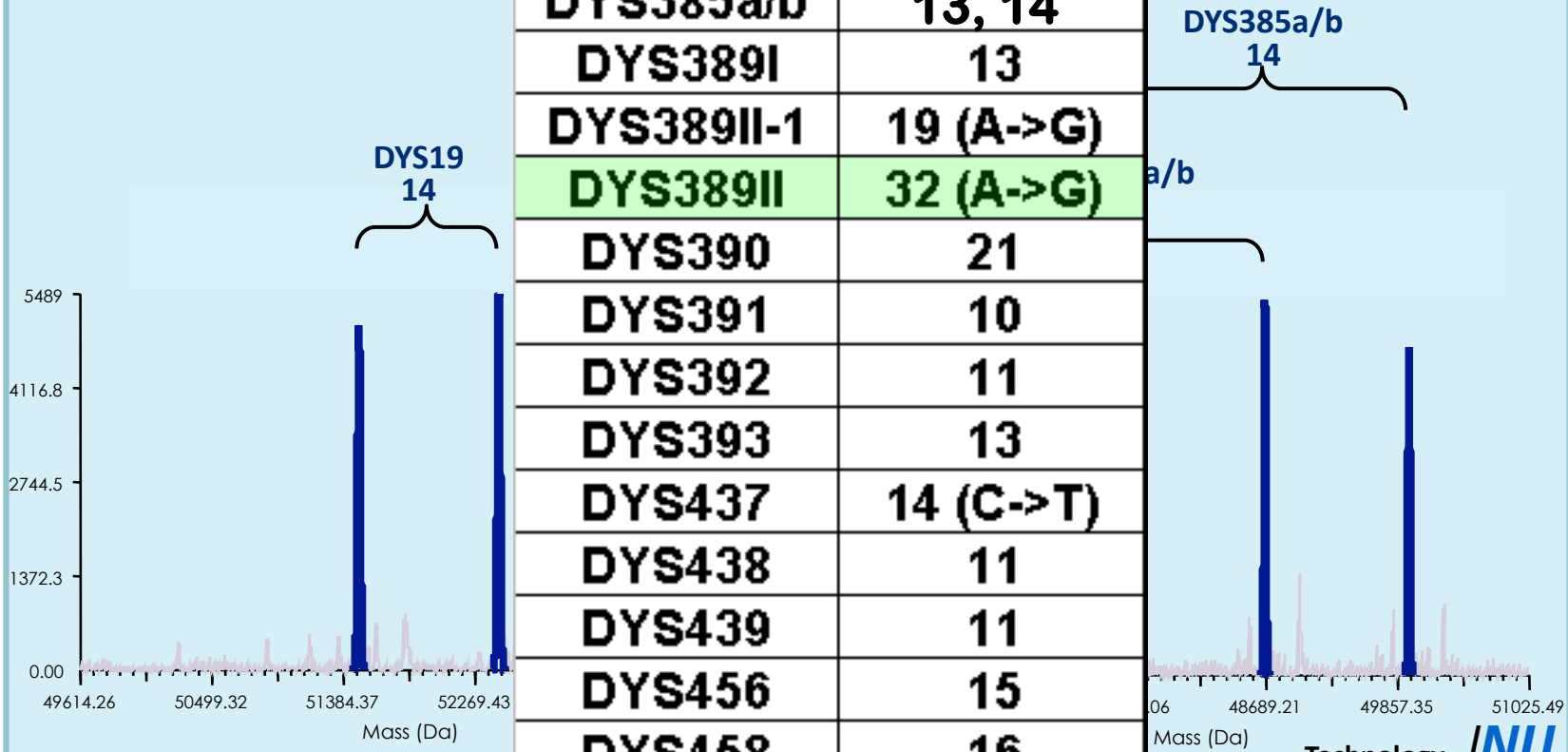
Single Reactions 3 and 4: Sample N31773

Locus	Genotype
DYS19	14
DYS385a/b	13, 14
DYS389I	13
DYS389II-1	19 (A->G)
DYS389II	32 (A->G)
DYS390	21
DYS391	10
DYS392	11
DYS393	13
DYS437	14 (C->T)
DYS438	11
DYS439	11
DYS456	15
DYS458	16



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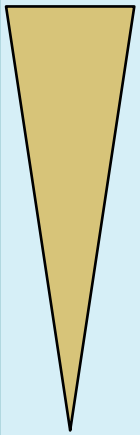
Sensitivity

Template 072109C

- **Dilutions from 1 ng down to 7.8 pg per reaction produced full profiles down to 62.5 pg/reaction (250 pg for four reactions/sample)**



62.5 pg

Template Quantity (pg)	DYS19	DYS385a/b	DYS389I	DYS389II-1	DYS390	DYS391	DYS392	DYS393	DYS437	DYS438	DYS439	DYS456	DYS458
1000	14	13, 14	12	17	23	10	11	14	16	10	11	14	16
	14	13, 14	12	17	23	10	11	14	16	10	11	14	16
500	14	13, 14	12	17	23	10	11	14	16	10	11	14	16
	14	13, 14	12	17	23	10	11	14	16	10	11	14	16
250	14	13, 14	12	17	23	10	11	14	16	10	11	14	16
	14	13, 14	12	17	23	10	11	14	16	10	11	14	16
125	14	13, 14	12	17	23	10	11	14	16	10	11	14	16
	14	13, 14	12	17	23	10	11	14	16	10	11	14	16
	14	13, 14	12	17	23	10	11	14	16	10	11	14	16
	14	13, 14	12	17	23	10	11	14	16	10	11	14	16
	14	13, 14	12	17	23	---	11	12, 14	16	10	11	14	16
	14	13, 14	12	17	23	10	11	12, 14	14, 16	10	11	14	16
15.6	14	13, 14	12	17	23	10	11	14	16	10	11	14	16
	14	13, 14	12	17	23	10	11	14	16	10	11	13, 14, 15	16
7.8	14	13, 14	---	16, 17	23	10	11	14	16	10	11	15	16
	14	13, 14	---	16, 17	23	10	---	14	---	10	---	14	16
Negative	---	---	---	---	---	---	---	---	---	---	---	---	---
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Species Specificity

- **Human blood-derived DNA sample was tested in triplicate in the presence of a 10-fold excess of exogenous DNA**
- **1 ng of human DNA per reaction**
- **10 ng exogenous DNA**
  - **Dog (male American Eskimo – buccal swab)**
  - **Cat (male long-hair, buccal swab)**
  - **Candida albicans (yeast)**
  - **Aspergillus oryzae (environmental filamentous fungus)**
  - **Escherichia coli (gram negative bacterium)**
  - **Staphylococcus aureus (gram positive bacterium)**
- **All tests with exogenous DNA gave a full profile**

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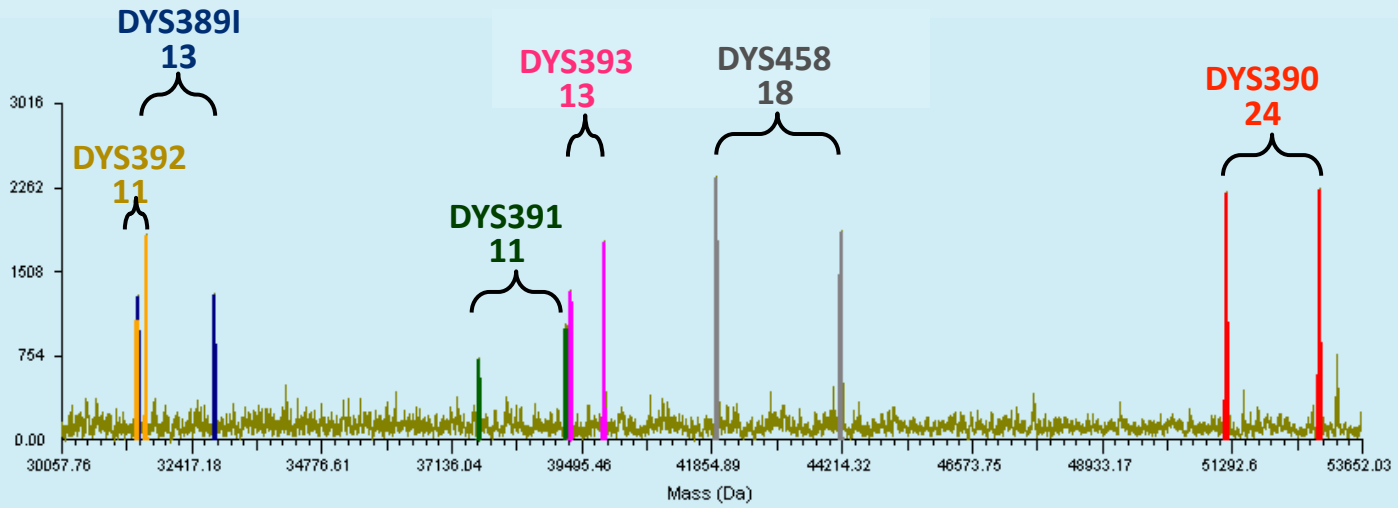
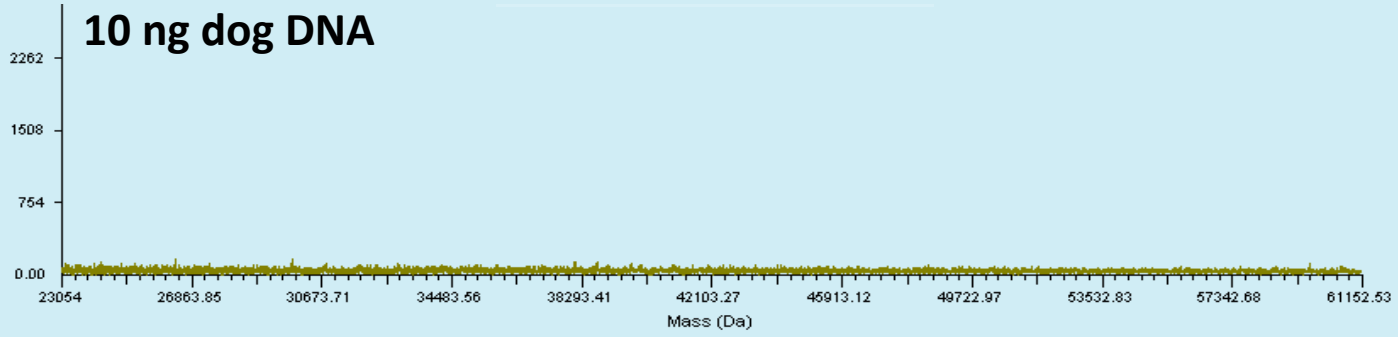
Species Specificity – 6-plex Reaction 1

1 ng human DNA + 10 ng dog DNA





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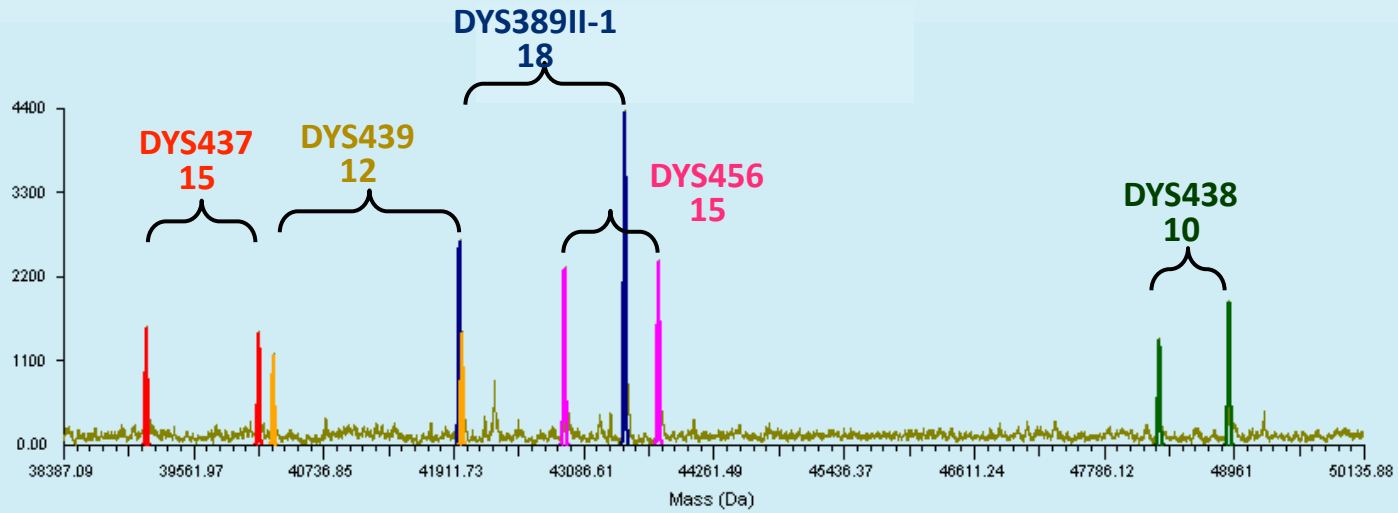
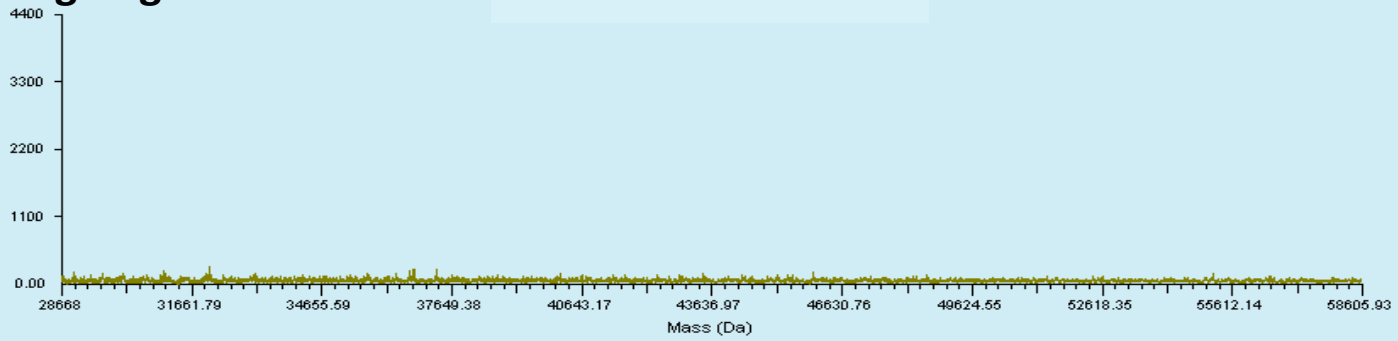
Species Specificity – 5-plex Reaction 2

1 ng human DNA + 10 ng dog DNA



10 ng dog DNA



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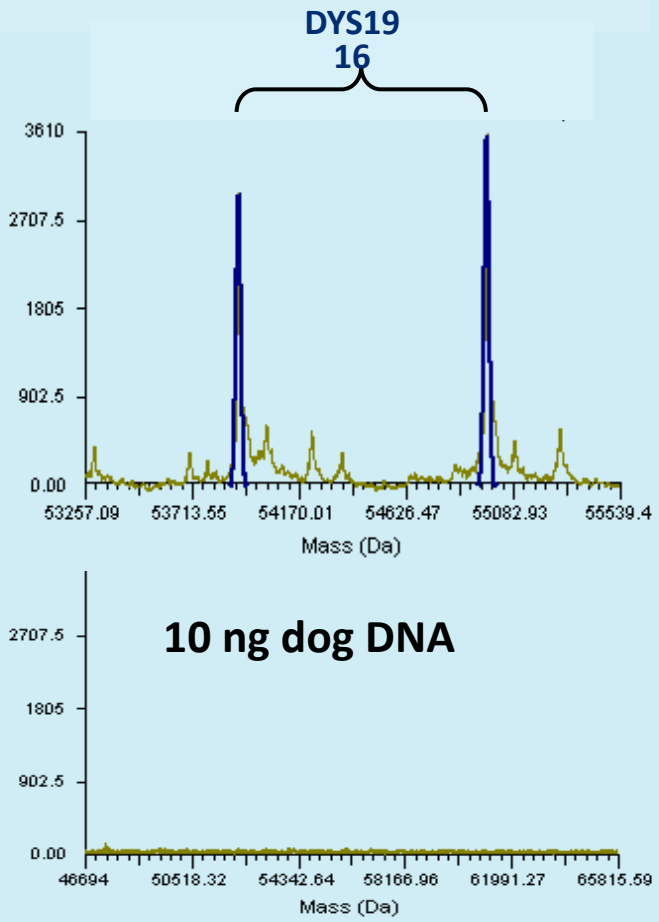
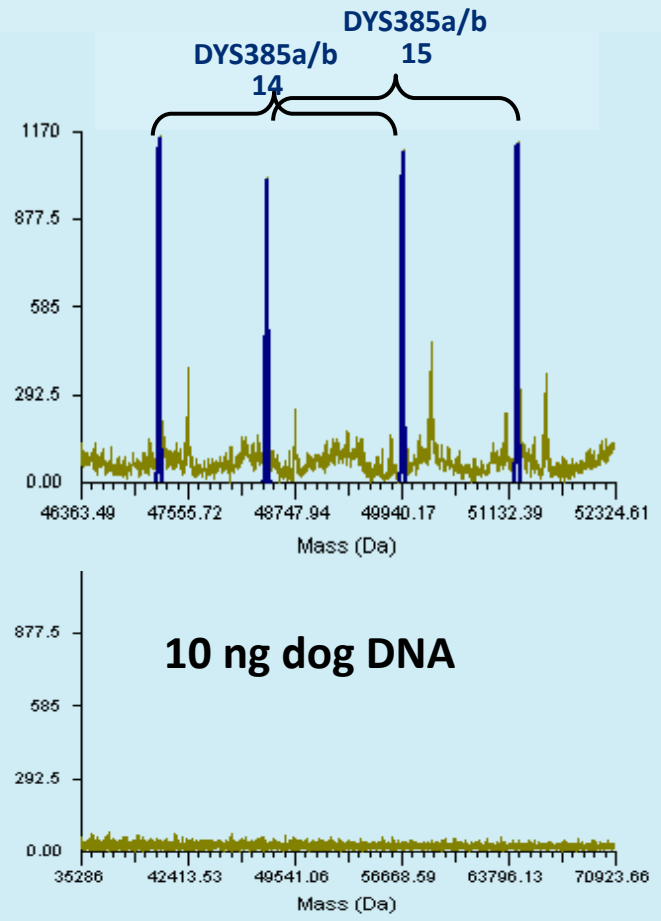
Species Specificity – Single Reactions 3 and 4

1 ng human DNA + 10 ng dog DNA





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Species Specificity

Sample	DYS19	DYS385a/b	DYS389I	DYS389II-1	DYS390	DYS391	DYS392	DYS393	DYS437	DYS438	DYS439	DYS456	DYS458
Dog + Human DNA	16	14, 15	13	18	24	11	11	13	15	10	12	15	18
	16	14, 15	13	18	24	11	11	13	15	10	12	15	18
	16	14, 15	13	18	24	11	11	13	15	10	12	15	18
Cat + Human DNA	16	14, 15	13	18	24	11	11	13	15	10	12	15	18
	16	14, 15	13	18	24	11	11	13	15	10	12	15	18
	16	14, 15	13	18	24	11	11	13	15	10	12	15	18
Staphylococcus aureus + Human DNA	16	14, 15	13	18	24	11	11	13	15	10	12	15	18
	16	14, 15	13	18	24	11	11	13	15	10	12	15	18
	16	14, 15	13	18	24	11	11	13	15	10	12	15	18
Escherichia coli + Human DNA	16	14, 15	13	18	24	11	11	13	15	10	12	15	18
	16	14, 15	13	18	24	11	11	13	15	10	12	15	18
	16	14, 15	13	18	24	11	11	13	15	10	12	15	18
Candida albicans + Human DNA	16	14, 15	13	18	24	11	11	13	15	10	12	15	18
	16	14, 15	13	18	24	11	11	13	15	10	12	15	18
	16	14, 15	13	18	24	11	11	13	15	10	12	15	18
Aspergillus oryzae + Human DNA	16	14, 15	13	18	24	11	11	13	15	10	12	15	18
	16	14, 15	13	18	24	11	11	13	15	10	12	15	18
	16	14, 15	13	18	24	11	11	13	15	10	12	15	18
Human DNA alone	16	14, 15	13	18	24	11	11	13	15	10	12	15	18
	16	14, 15	13	18	24	11	11	13	15	10	12	15	18

Exogenous DNAs did not interfere with full profile detection

Cat alone (10 ng/reaction)	---	---	---	---	---	---	---	---	---	---	---	---	---
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Staphylococcus aureus alone (10 ng/reaction)	---	---	---	---	---	---	---	---	---	---	---	---	---
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Escherichia coli alone (10 ng/reaction)	---	---	---	---	---	---	---	---	---	---	---	---	---
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Candida albicans alone (10 ng/reaction)	---	---	---	---	---	---	---	---	---	---	---	---	---
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Aspergillus oryzae alone (10 ng/reaction)	---	---	---	---	---	---	---	---	---	---	---	---	---
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Water	---	---	---	---	---	---	---	---	---	---	---	---	---
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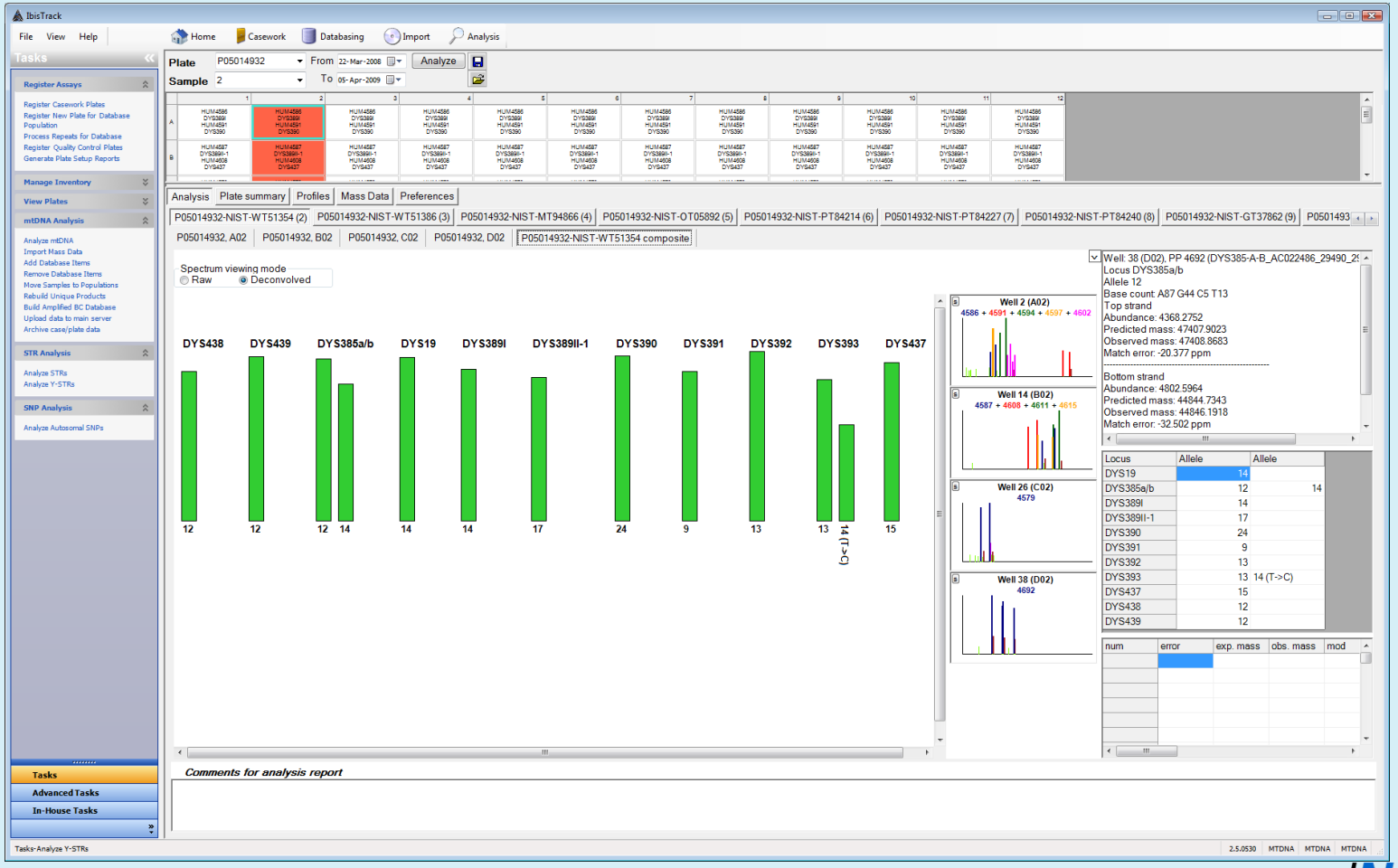
Software and Database in Place for Y-STRs



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mtDNA Assay Format

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Outline

- **Ibis™ mtDNA Assay format**
- **Base composition analysis of mtDNA samples**
  - **Data processing and analysis**
- **Information content relative to sequencing**
- **Heteroplasmy detection**
- **Sensitivity**
- **Reproducibility**

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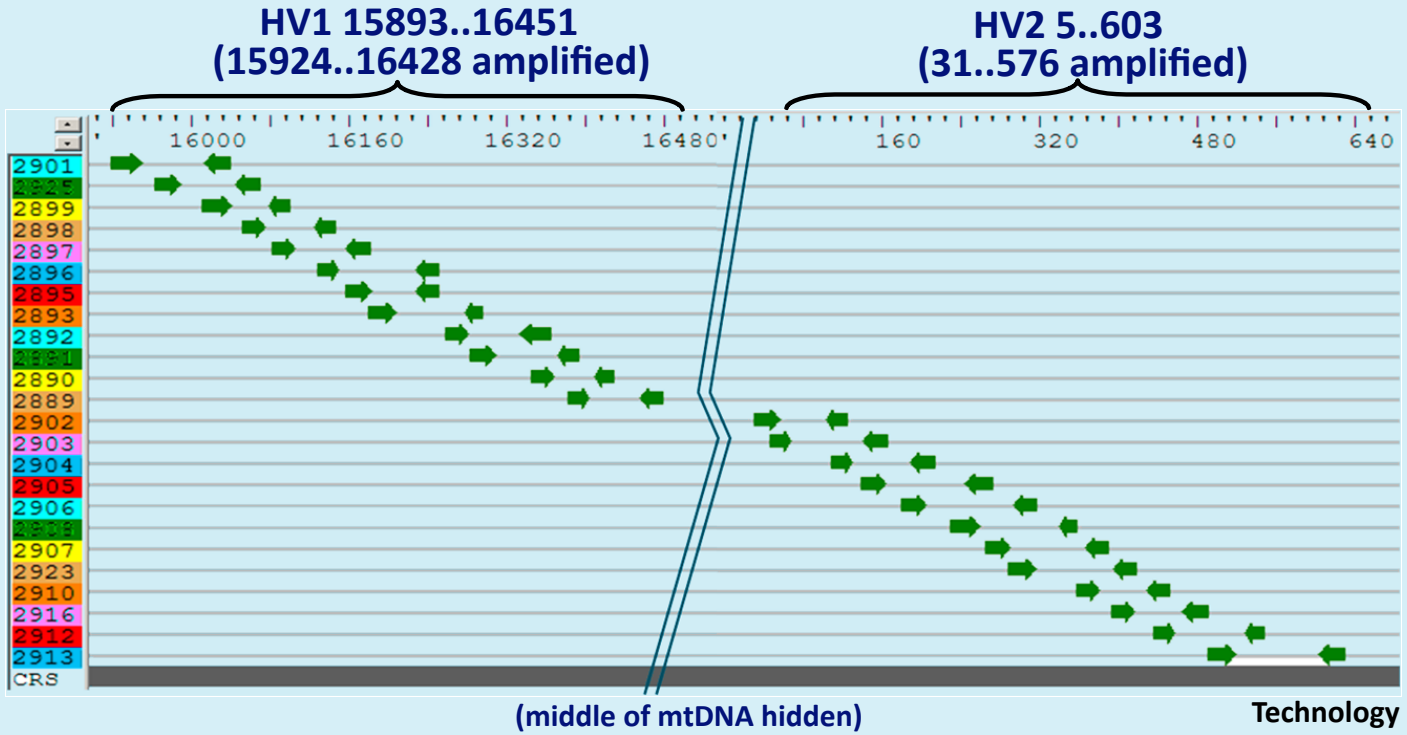
mtDNA Tiling Assay Format

- 24 primer pairs cover amplified coordinates HV1 15924..16428 and HV2 31..576
- Target most highly-conserved positions on 3′ ends of primers
- Grouped into eight triplexed sets (colored grouping) by maximum spatial separation and suitable mass separation of products



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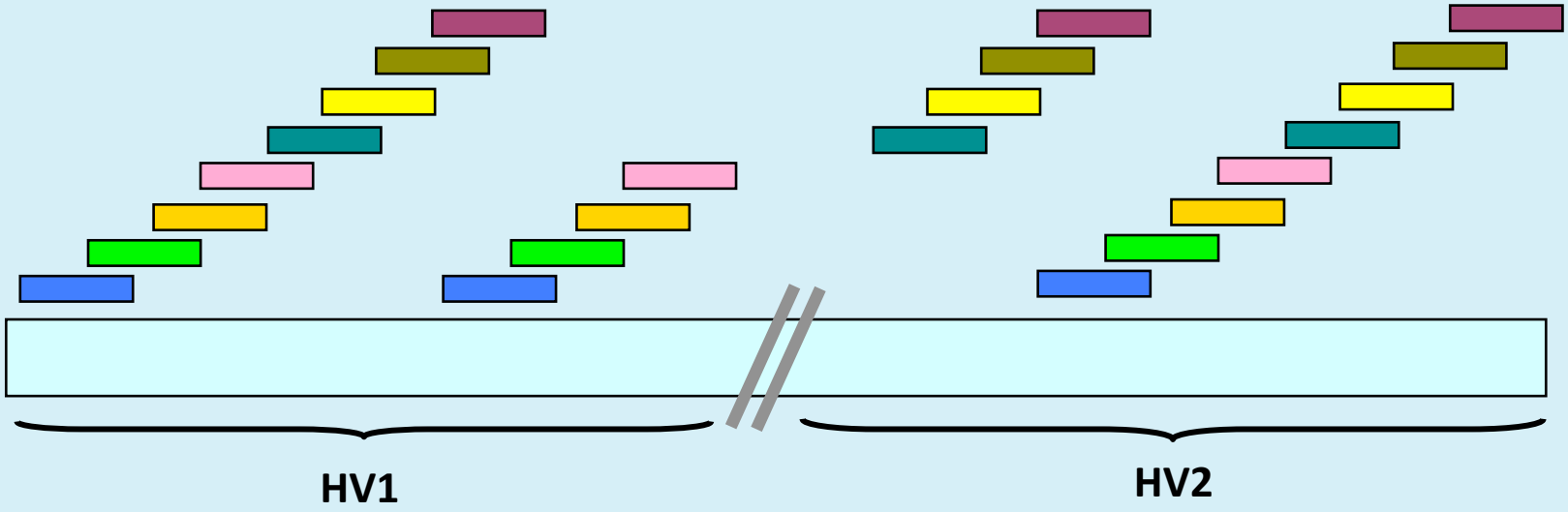
mtDNA Tiling Assay Format



- Primers grouped to maximize target site separation
- PCR reactions performed with short extension cycle (five seconds)
- Product masses resolve from each other in triplex groupings
- Product sizes range from 85 to 140 bp; all but three are <150 bp
- Relative primer pair concentrations in triplexes have been adjusted to favor simultaneous amplification of all products

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Base Composition Analysis

1. PCR
2. Desalting
3. ESI-TOF mass spectrometry
4. Raw spectrum processing/deconvolution
5. Base composition assignment/profile development

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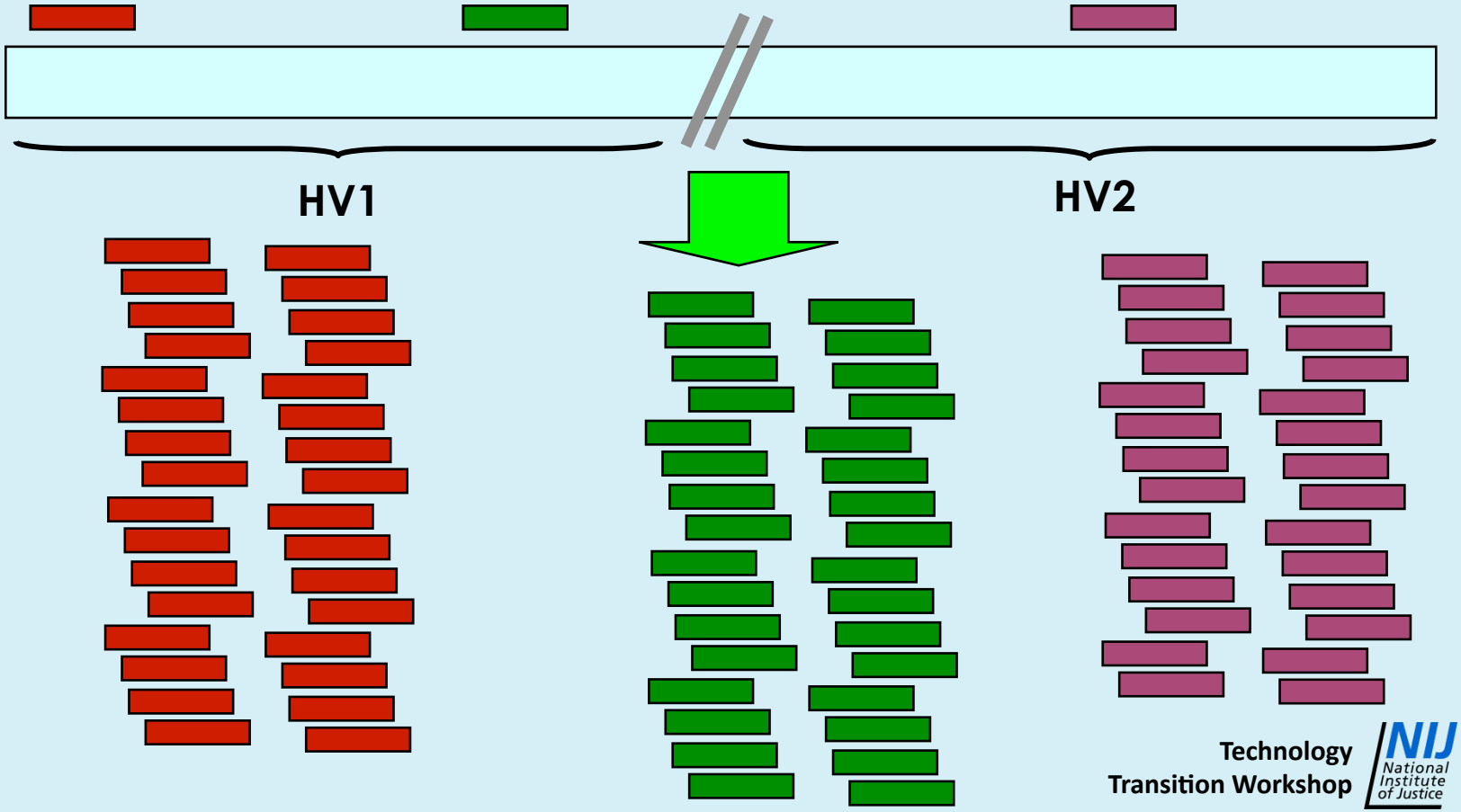
PCR: Three Primer Pairs Per Reaction



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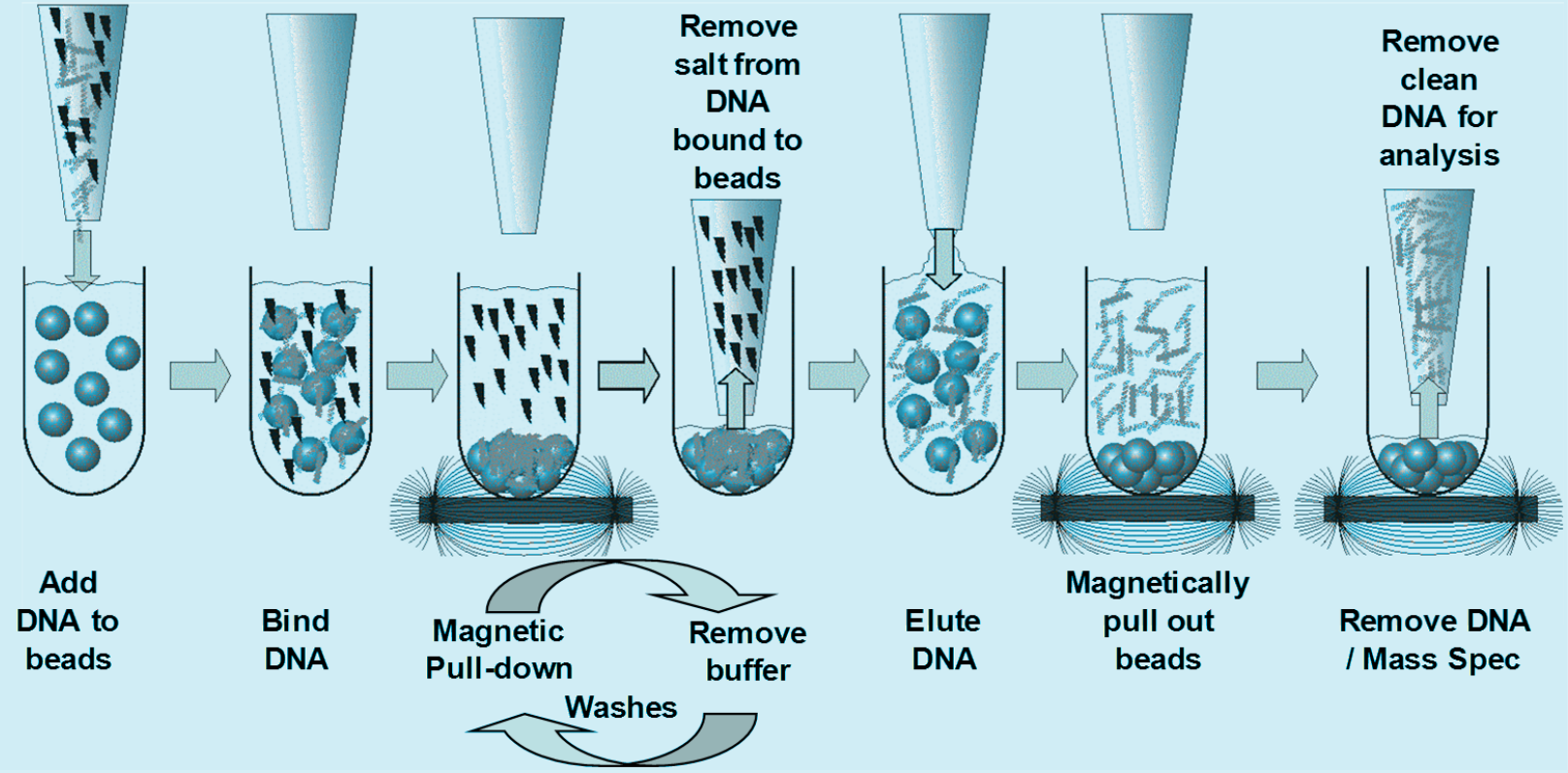
Desalting of PCR Reactions

Magnetic bead anion exchange



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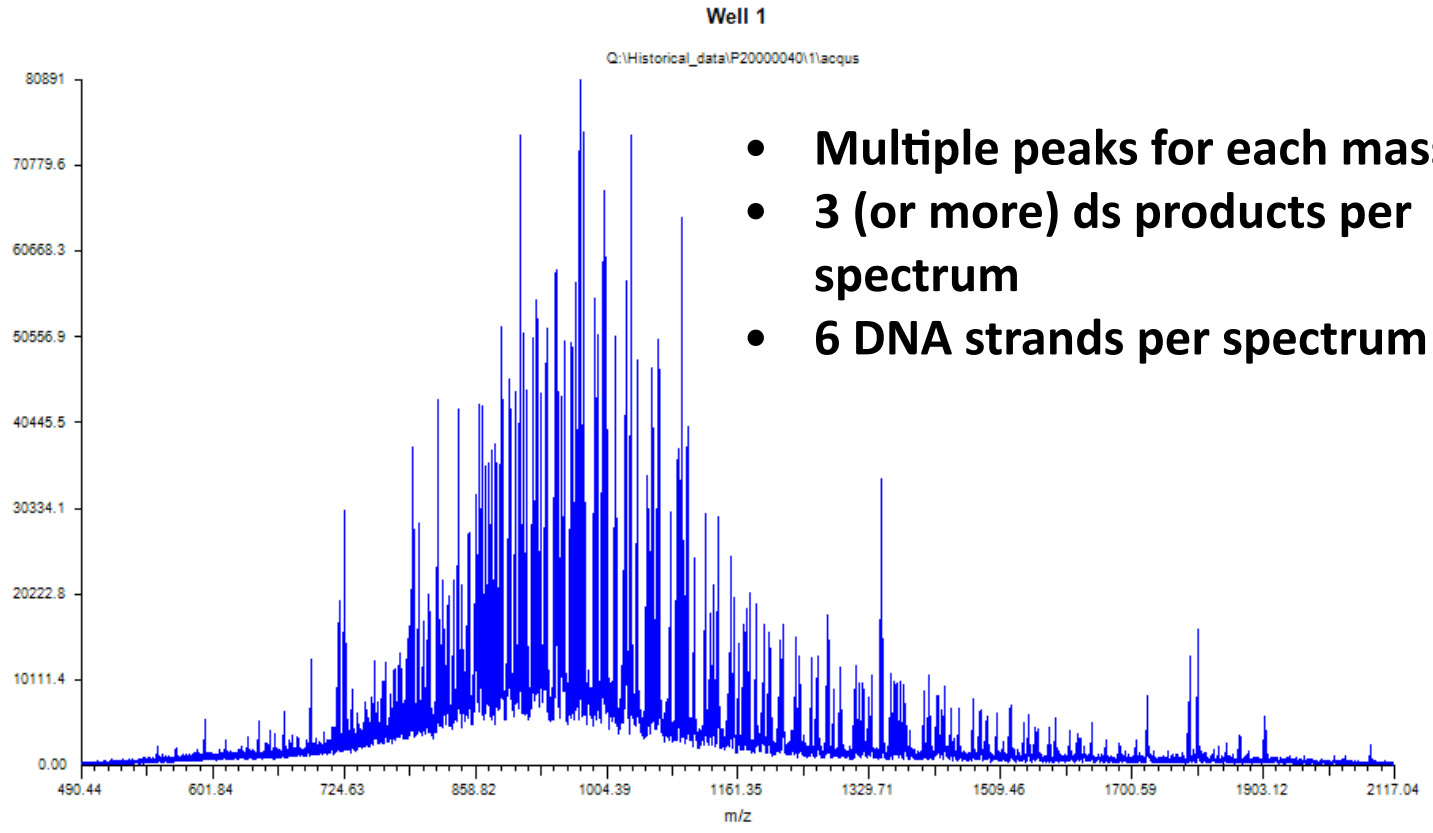
ESI-TOF Mass Spectromery

- **Three primer pairs per reaction**
- **Complex raw spectrum**



- **Multiple peaks for each mass**
- **3 (or more) ds products per spectrum**
- **6 DNA strands per spectrum**

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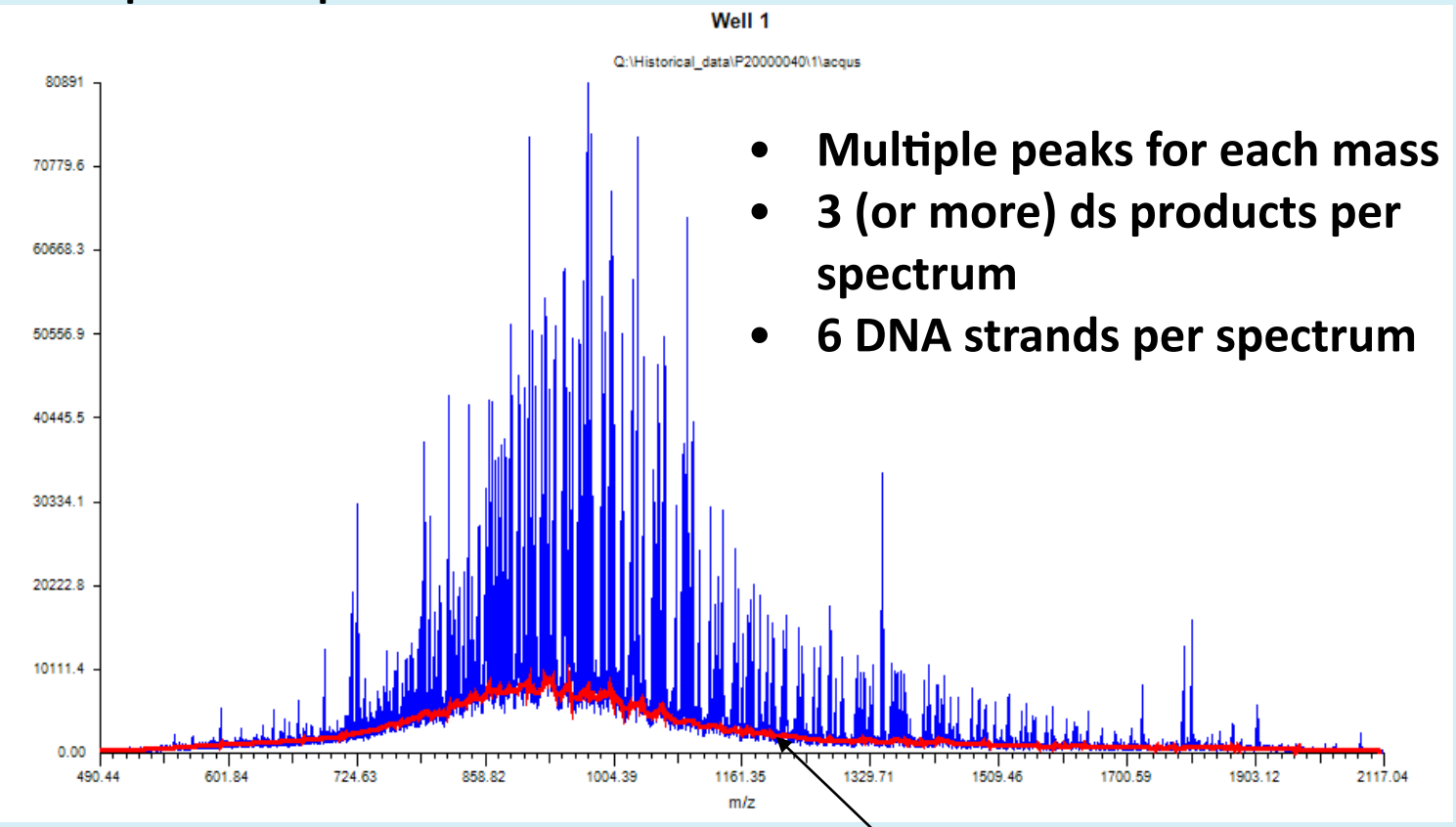
ESI-TOF Mass Spectromery

- **Three primer pairs per reaction**
- **Complex raw spectrum**



- **Multiple peaks for each mass**
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- **6 DNA strands per spectrum**

Noise baseline

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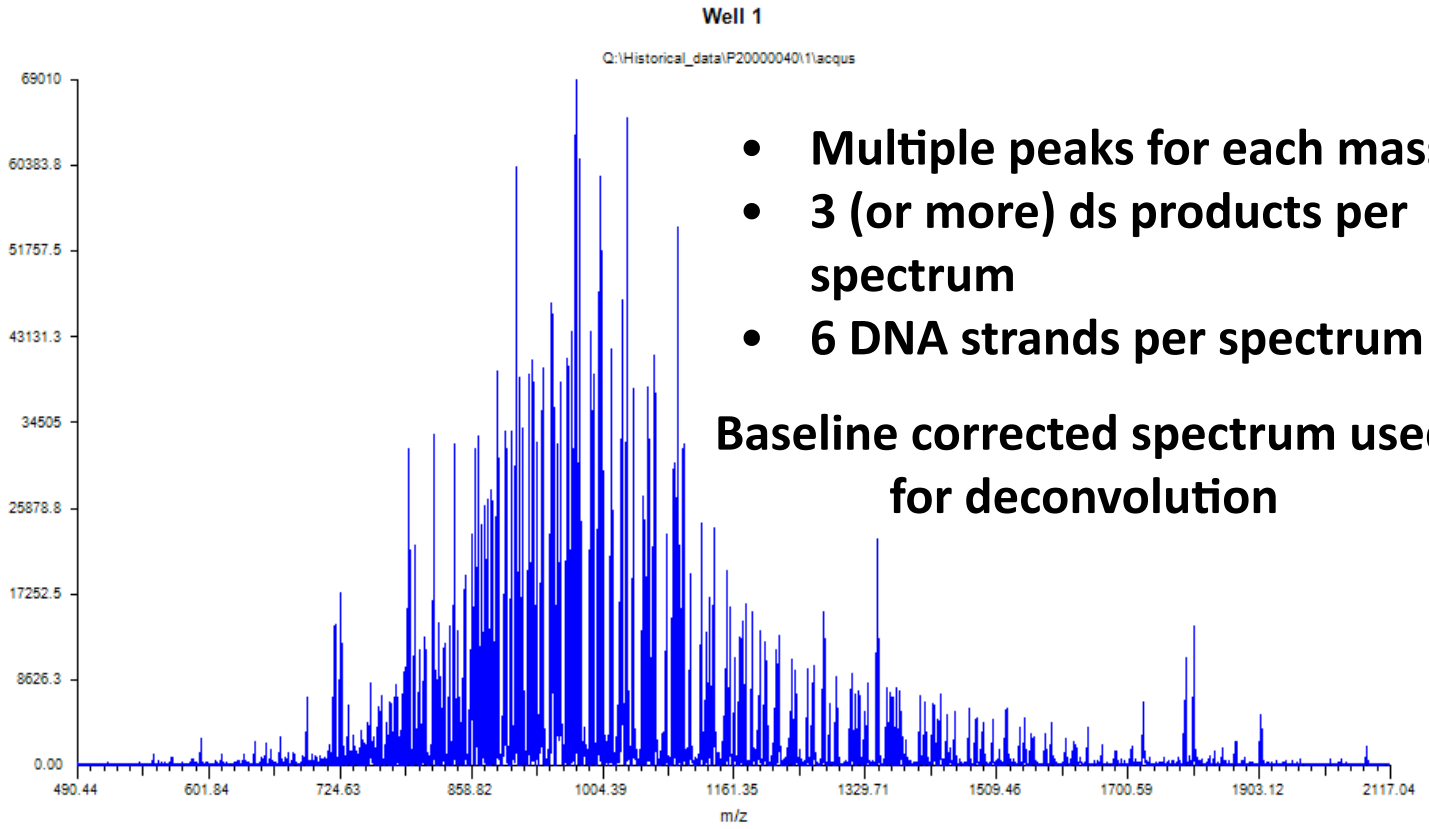
ESI-TOF Mass Spectromery

- **Three primer pairs per reaction**
- **Complex raw spectrum**



- **Multiple peaks for each mass**
- **3 (or more) ds products per spectrum**
- **6 DNA strands per spectrum**

Baseline corrected spectrum used for deconvolution

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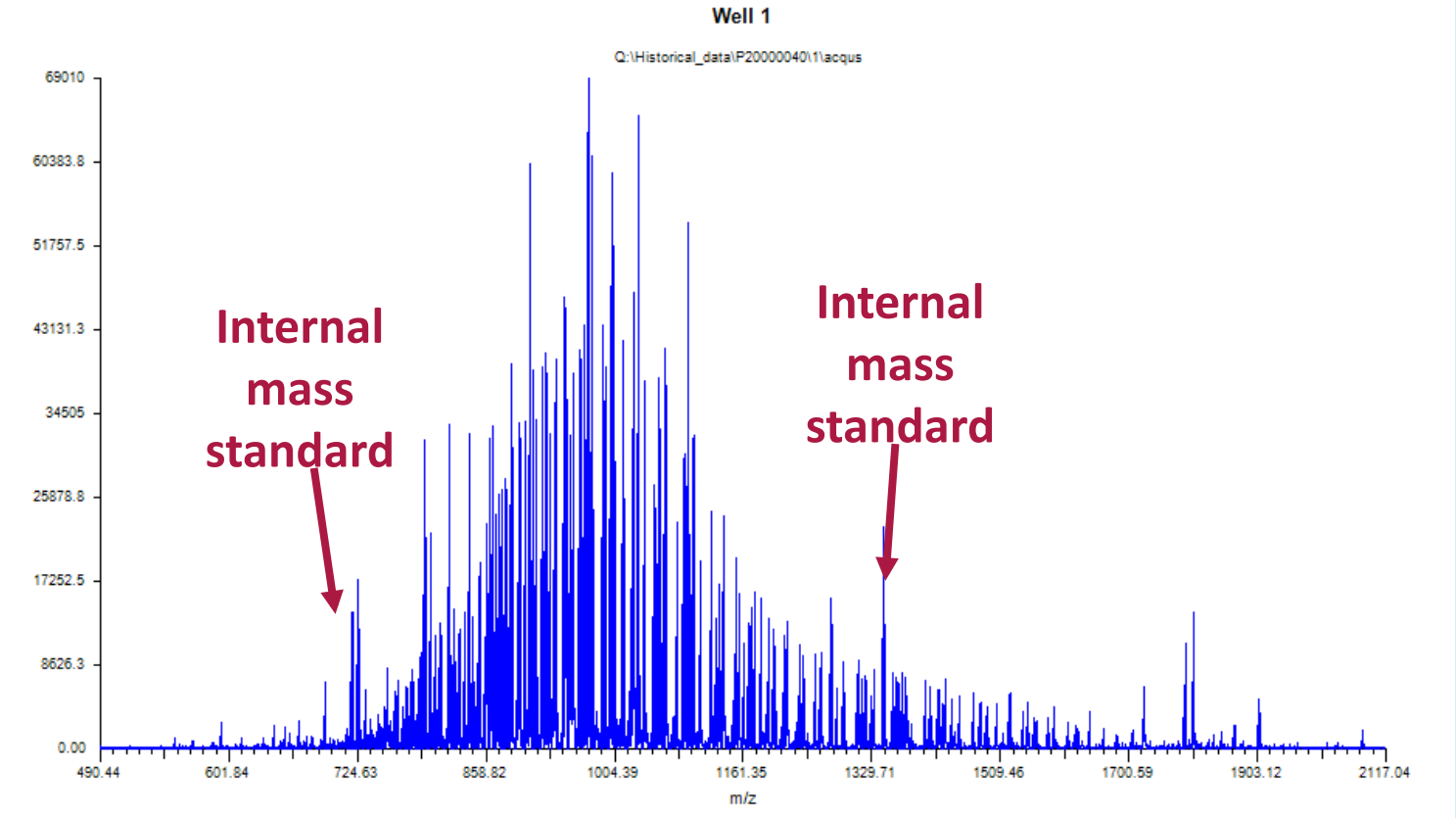
Raw Spectrum Processing

- **Internal mass standards bracket the spectrum for accurate calibration of the measurements before deconvolution**



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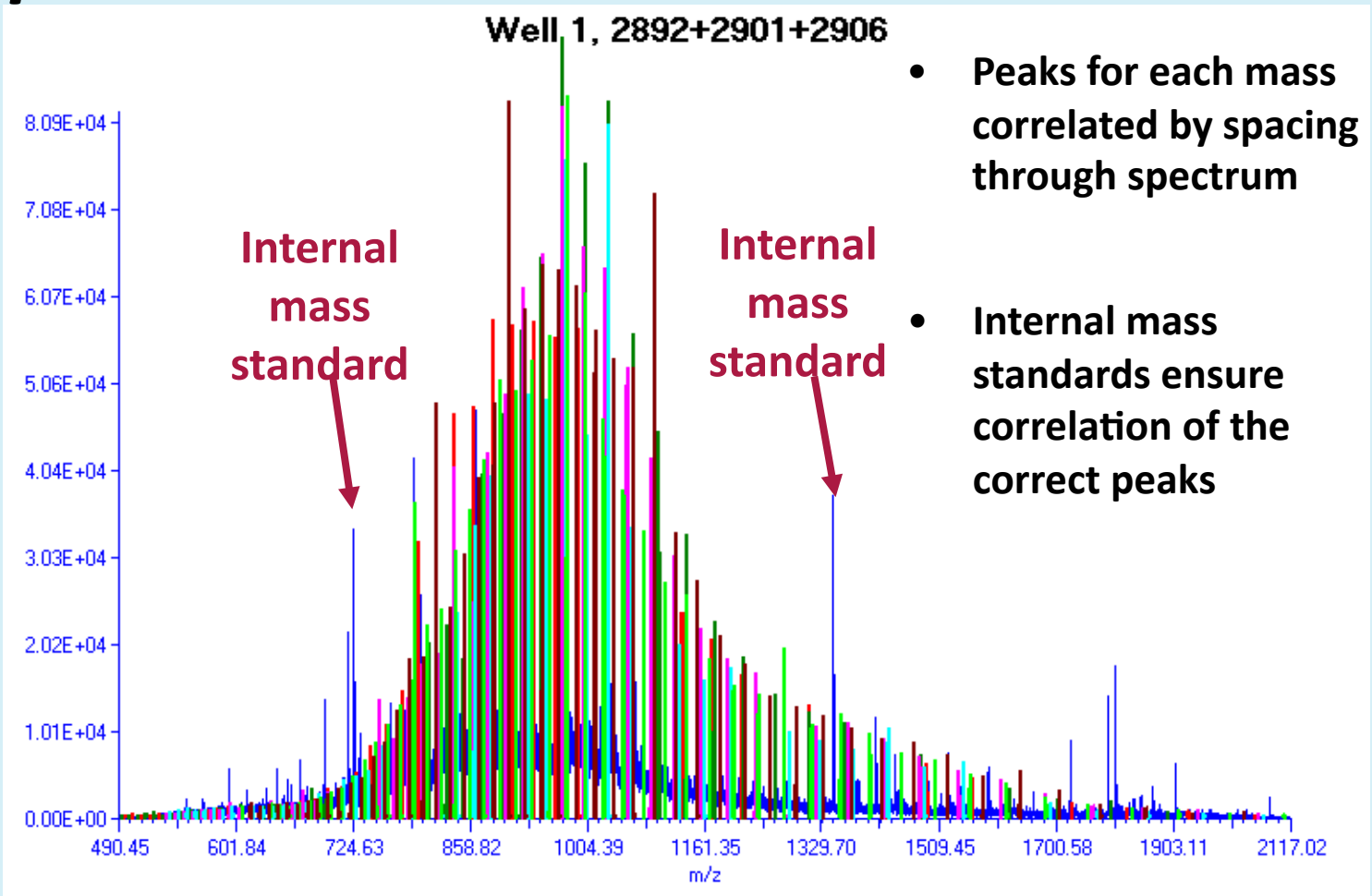
Spectral Deconvolution



- Peaks for each mass correlated by spacing through spectrum
- Internal mass standards ensure correlation of the correct peaks

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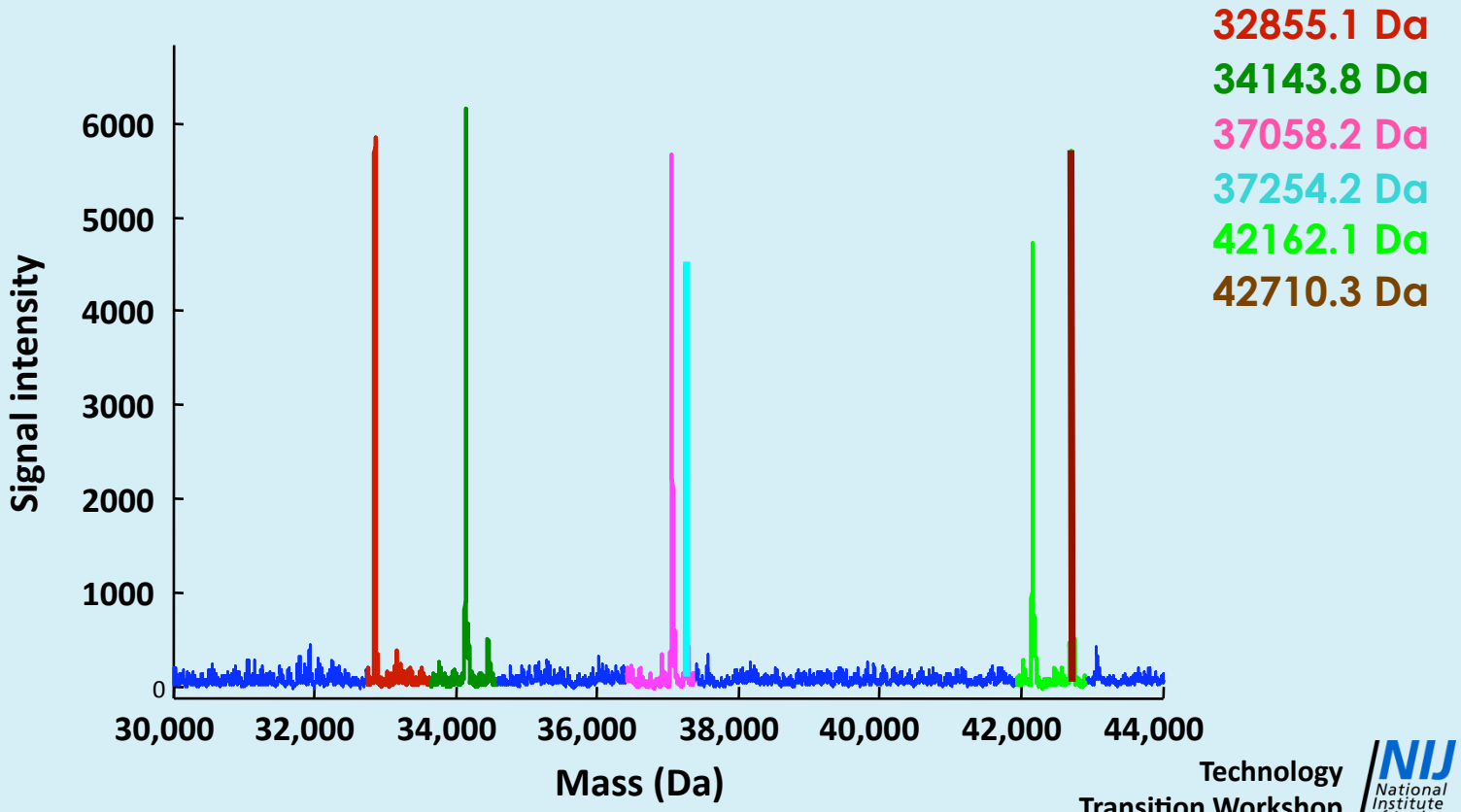
Deconvolution to Masses

- **Deconvolution results in one final measurement per molecular species**



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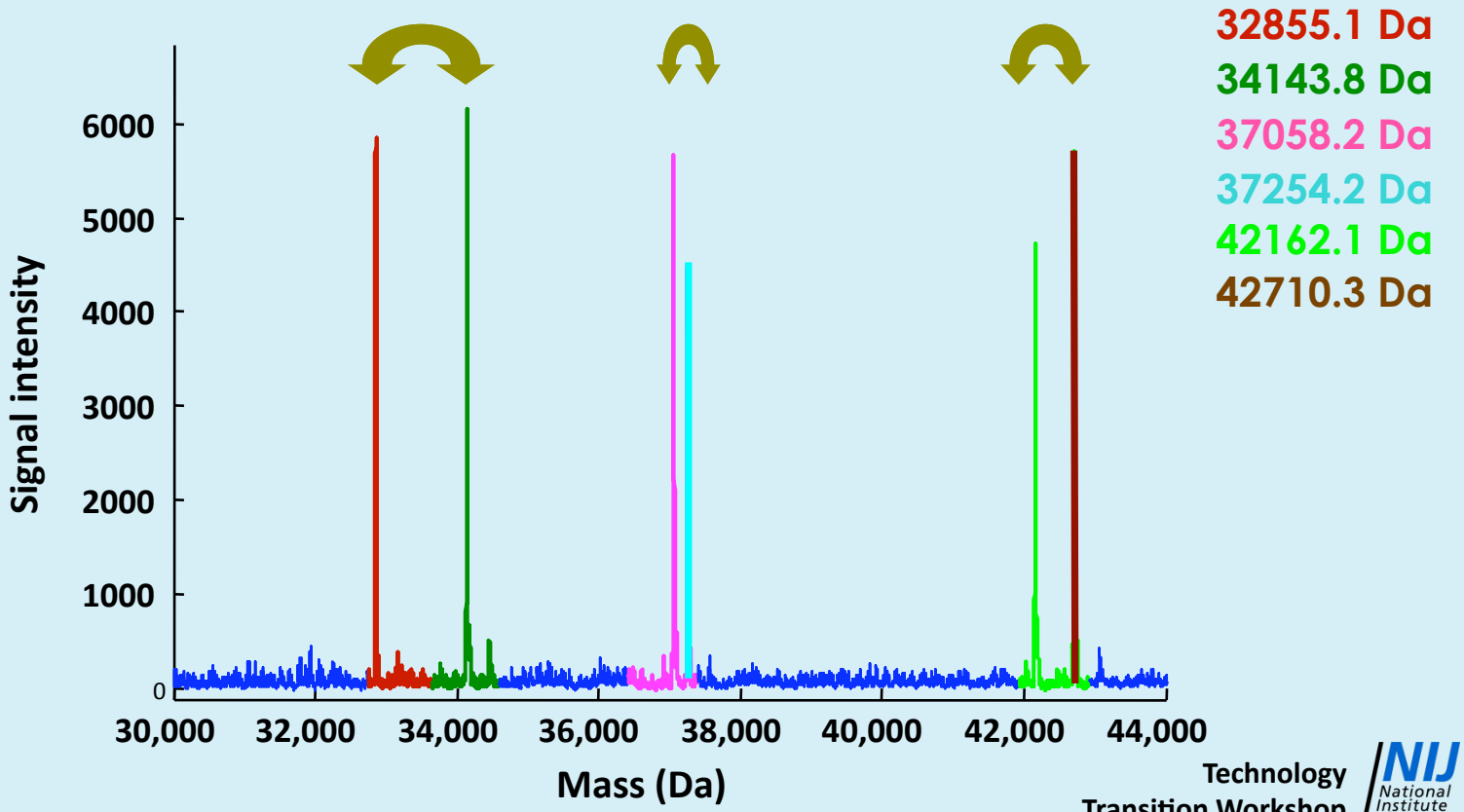
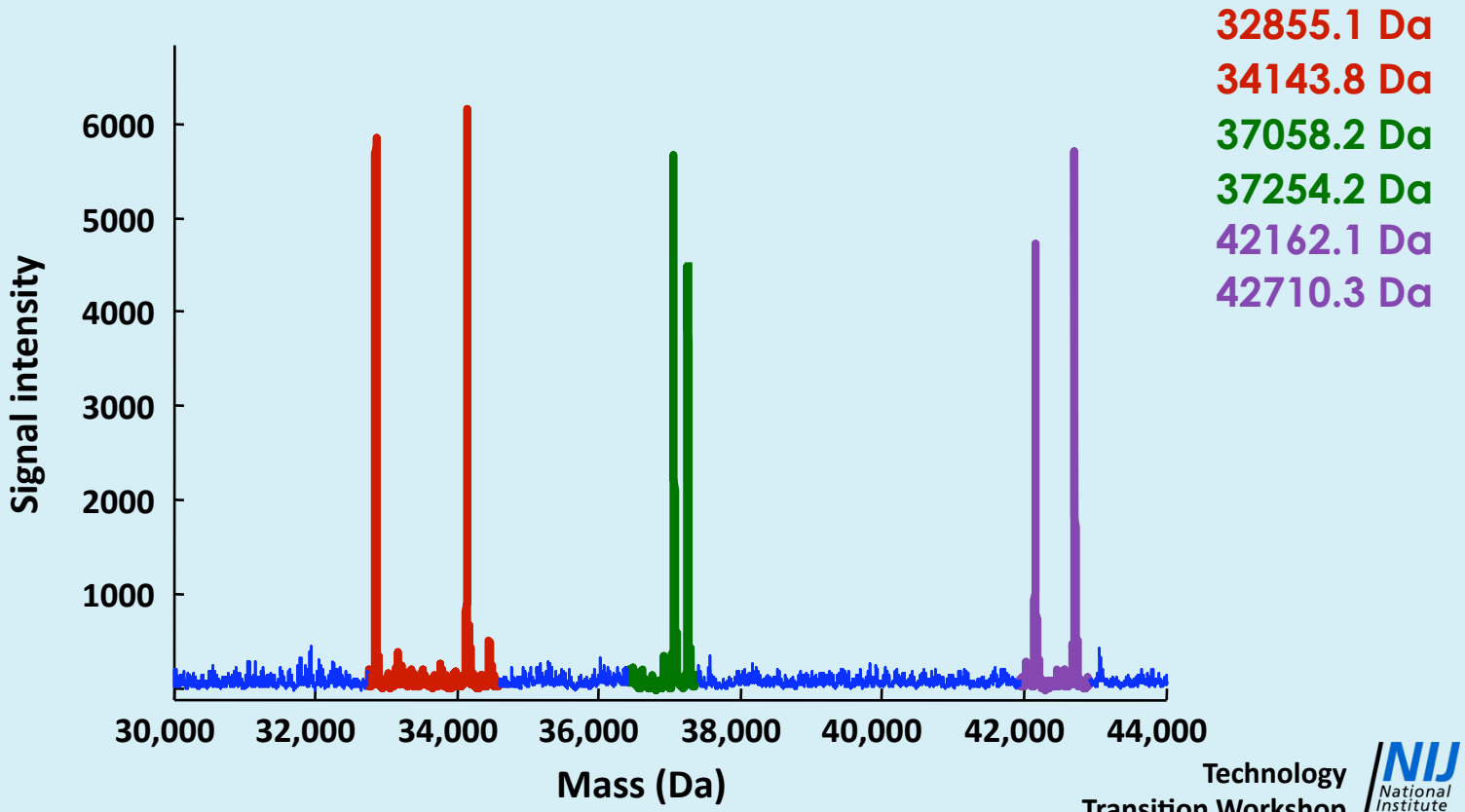
DS DNA Strand Association

- **Forward and reverse strands of a double stranded DNA can be associated by mass**



DS DNA Strand Association

- **Forward and reverse strands of a double stranded DNA can be associated by mass**



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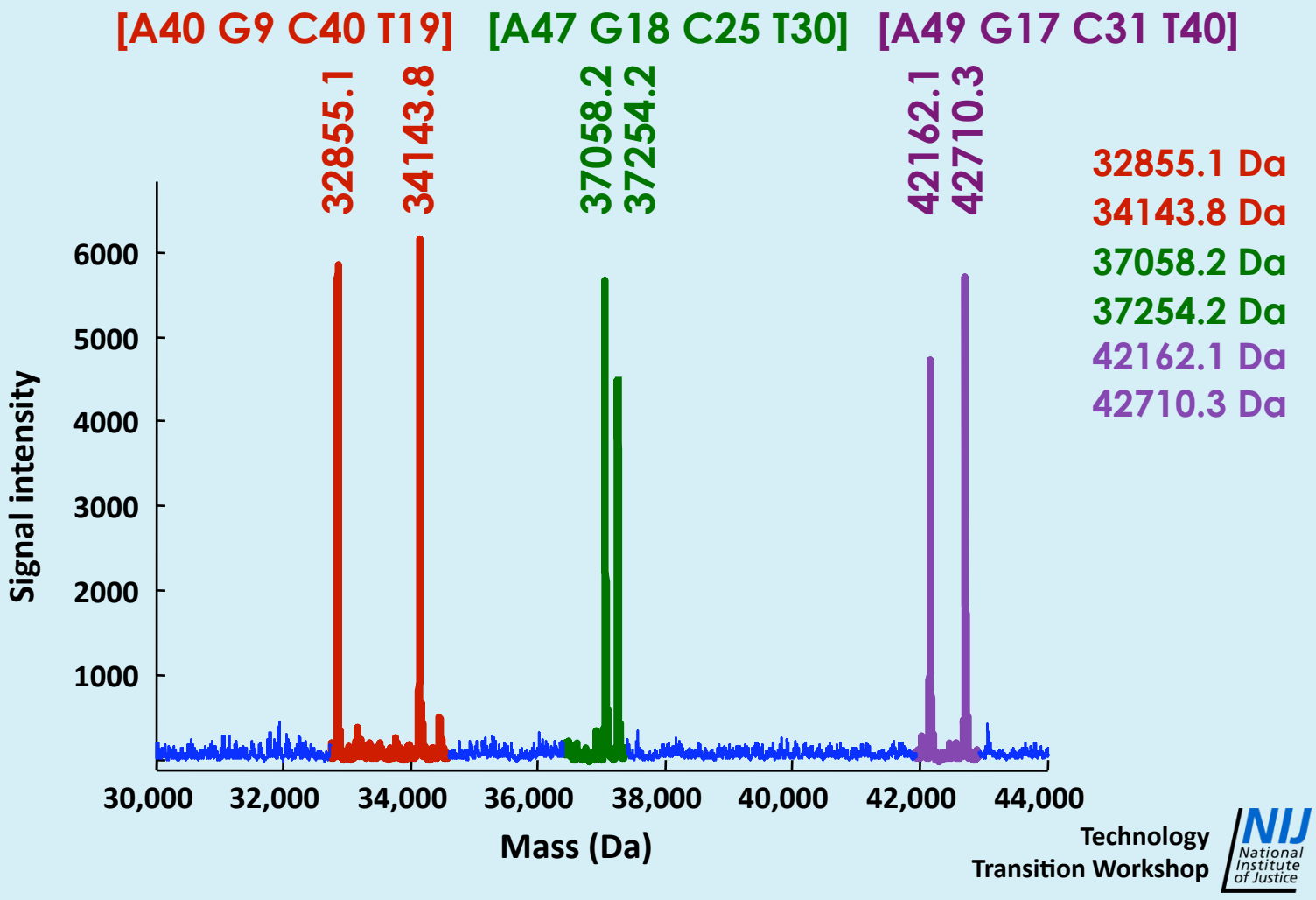
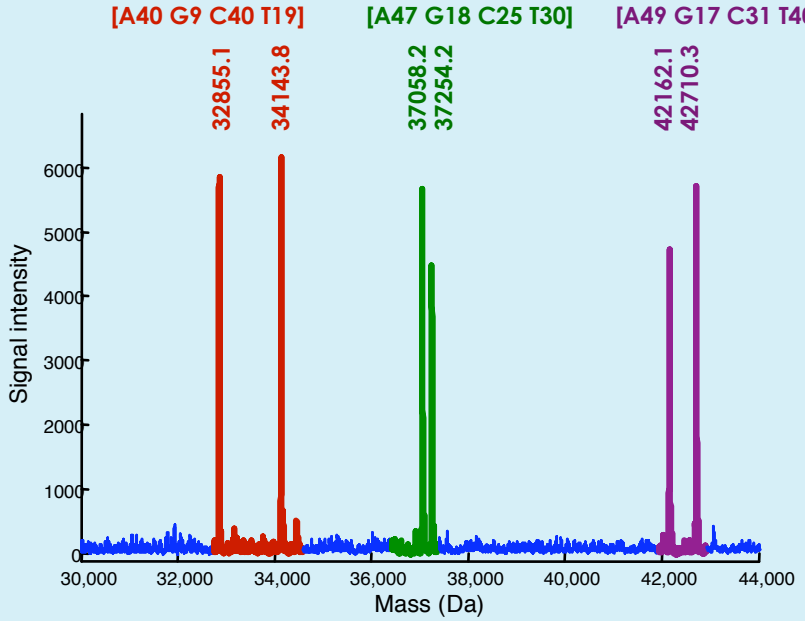
Base Composition Assignment



Base Composition Assignment



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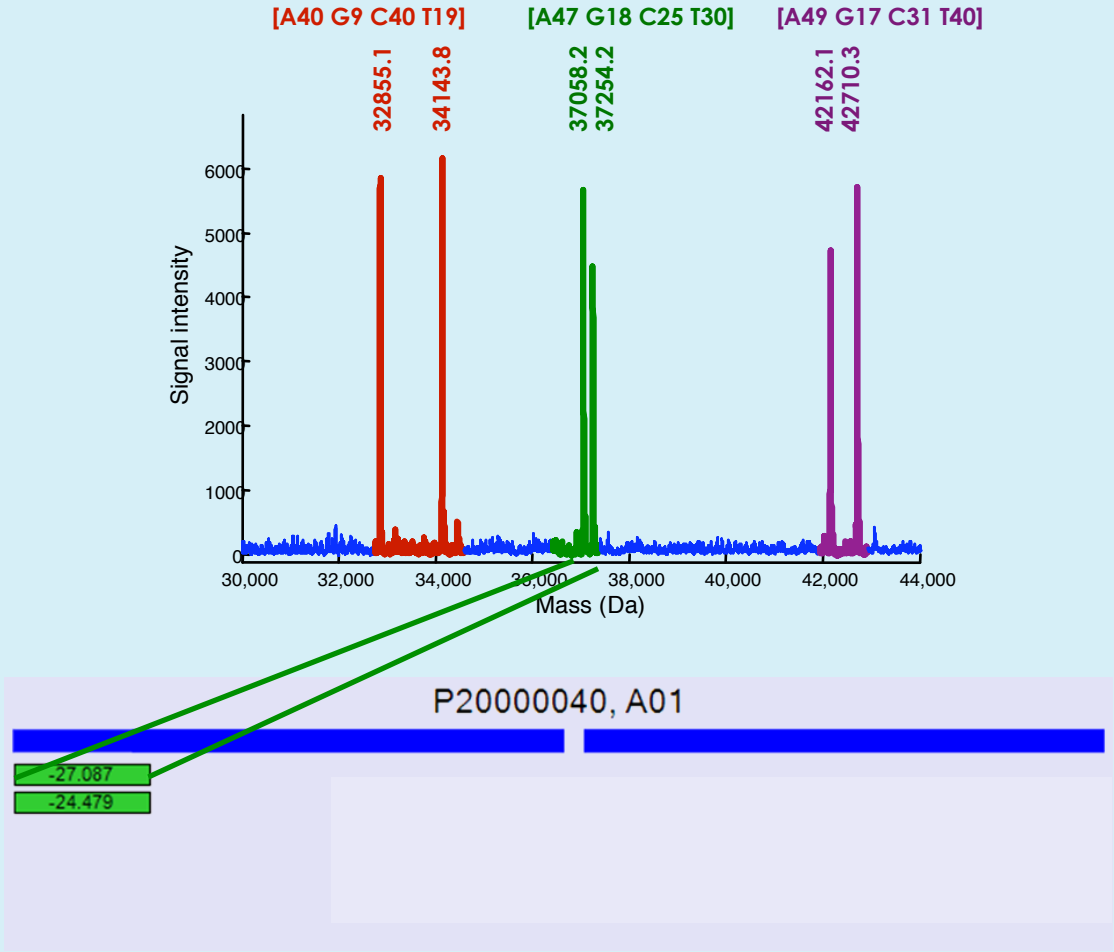
Final Product Assignment



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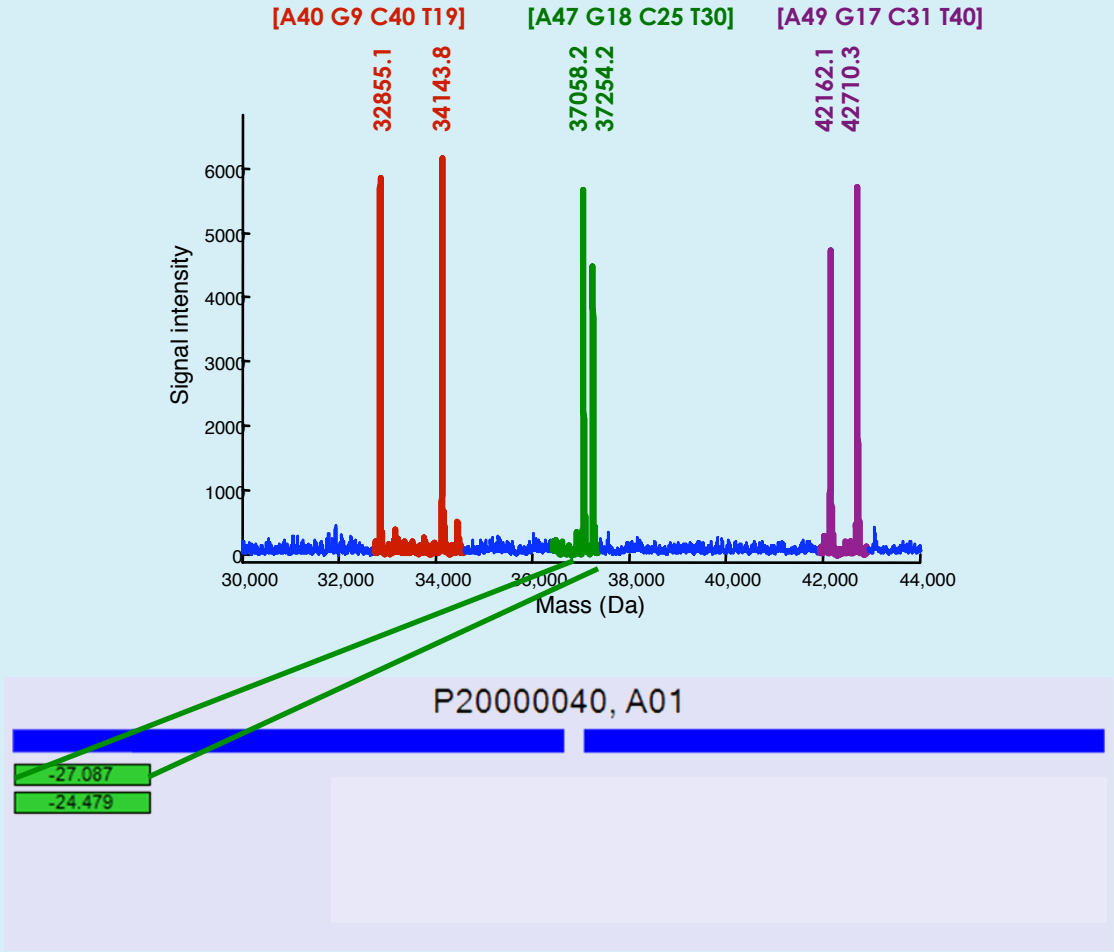
Final Product Assignment



15893..16012

[A47 G18 C25 T30]

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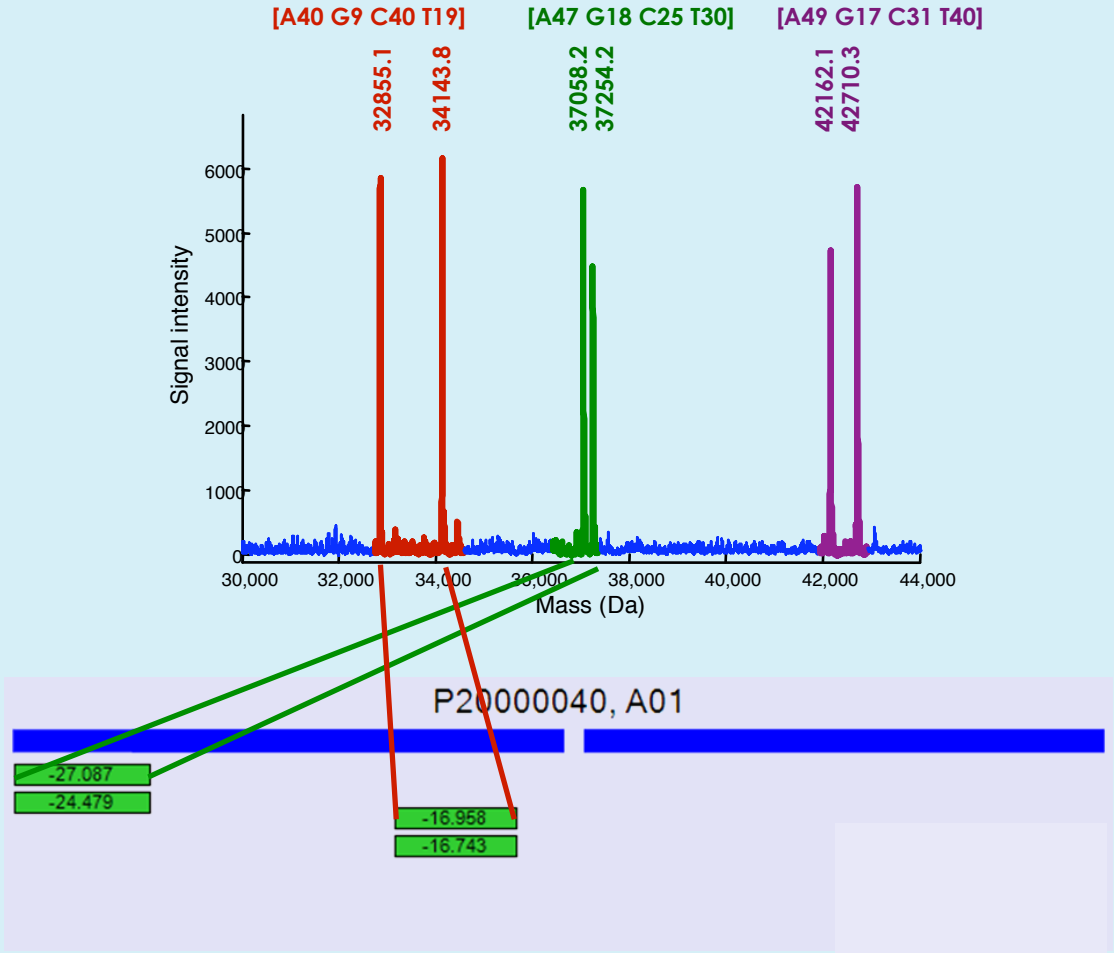
Final Product Assignment



15893..16012

[A47 G18 C25 T30]

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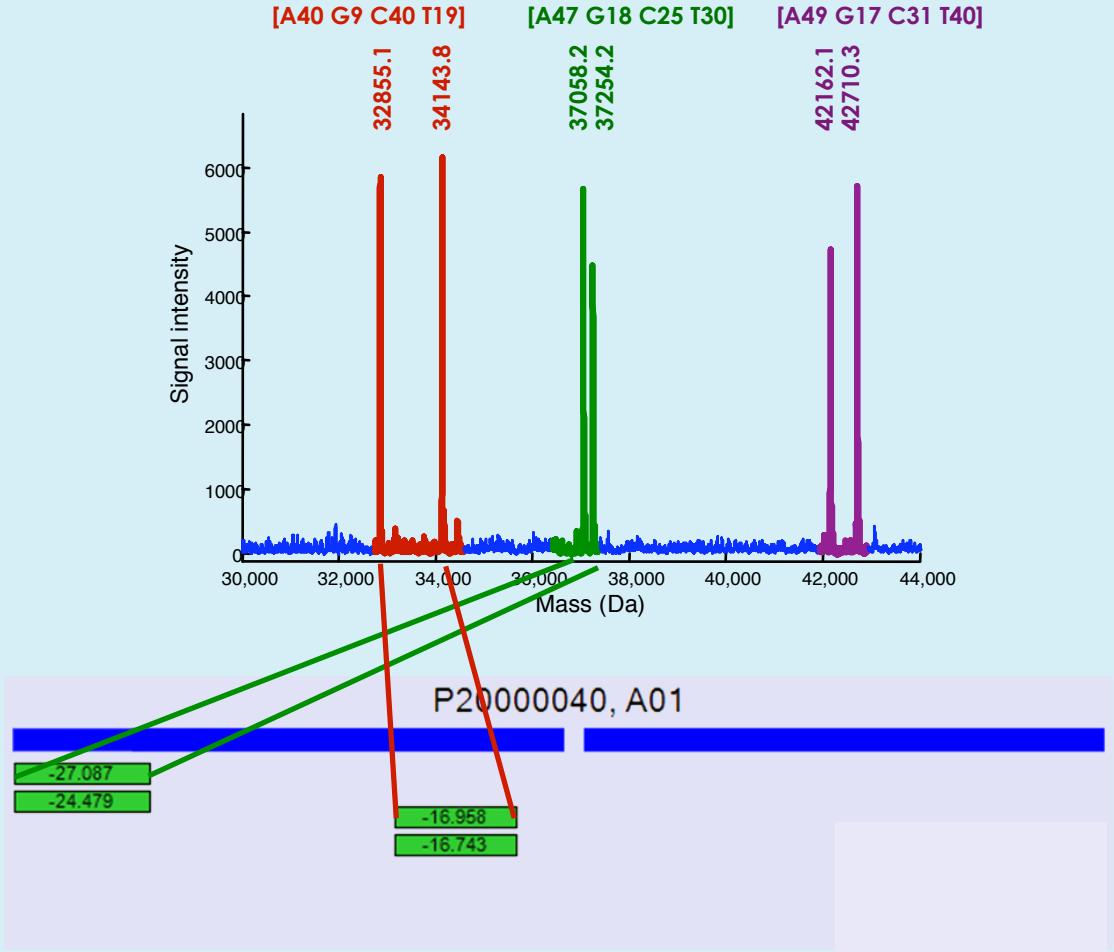
Final Product Assignment



15893..16012
[A47 G18 C25 T30]

16231..16338
[A40 G9 C40 T19]

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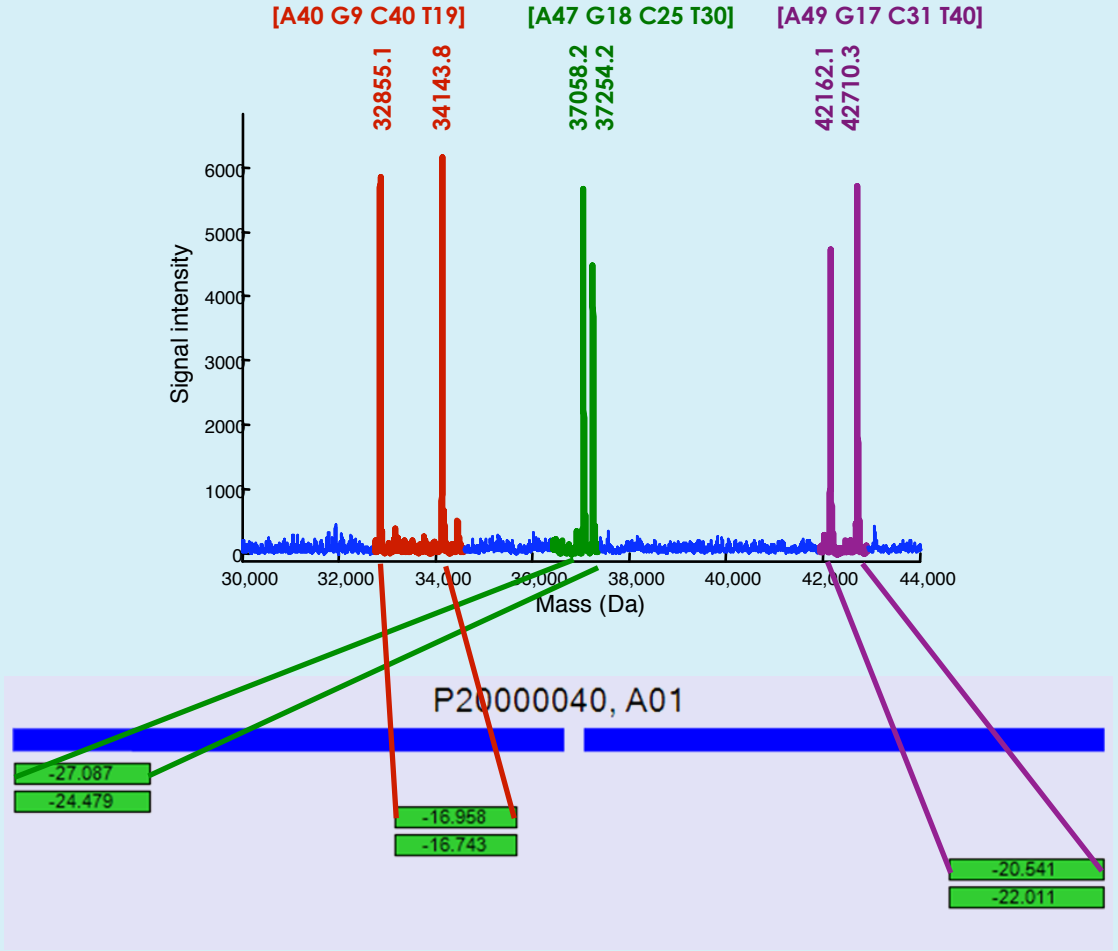
Final Product Assignment



15893..16012
[A47 G18 C25 T30]

16231..16338
[A40 G9 C40 T19]

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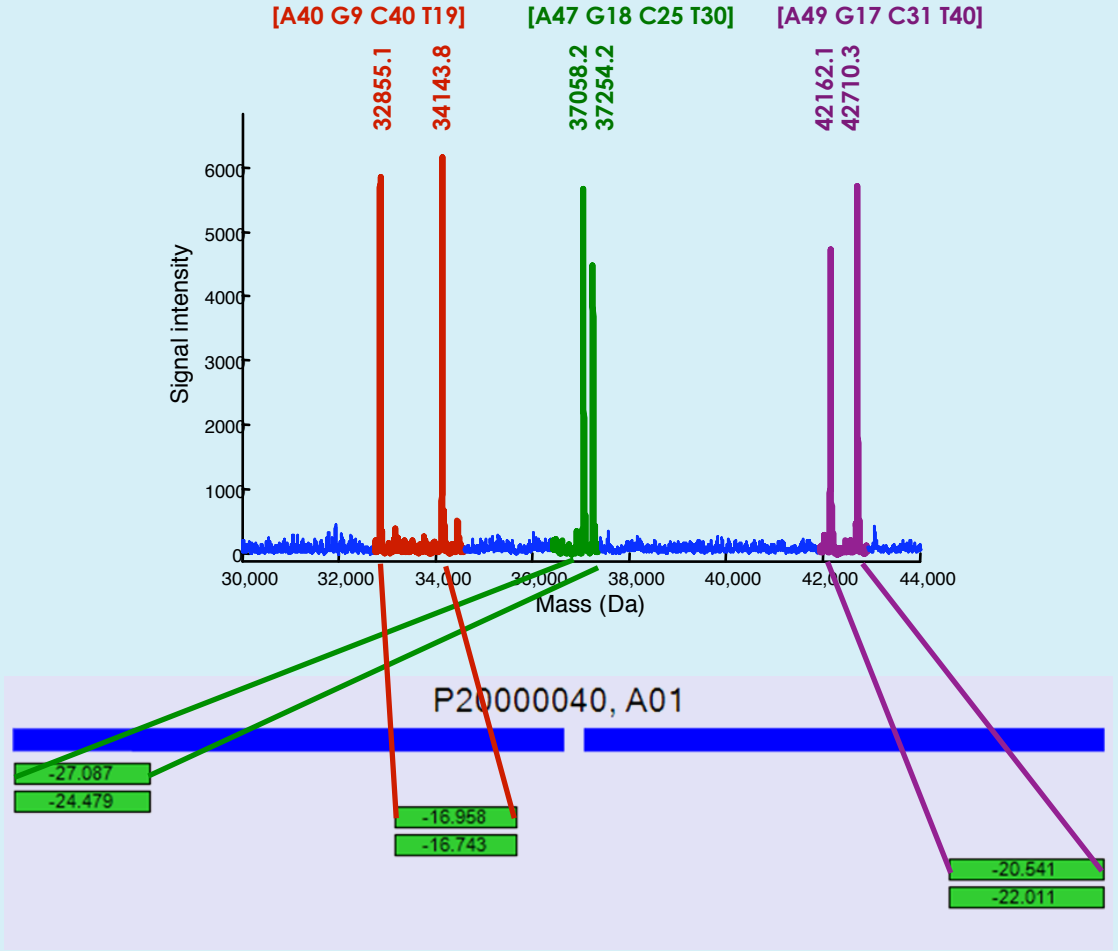
Final Product Assignment



15893..16012
[A47 G18 C25 T30]

16231..16338
[A40 G9 C40 T19]

154..290
[A49 G17 C31 T40]

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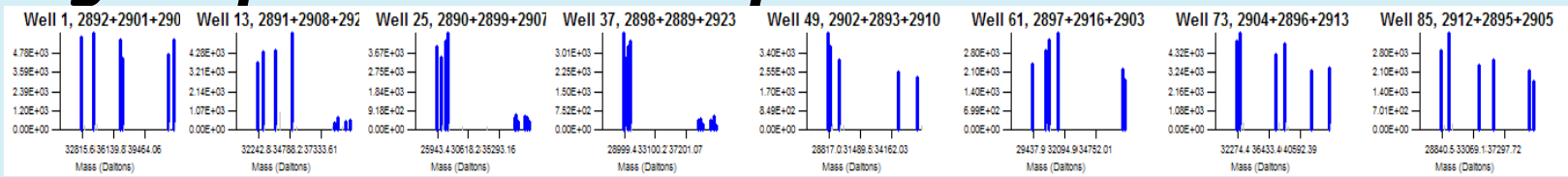
Eight Spectra Per Sample



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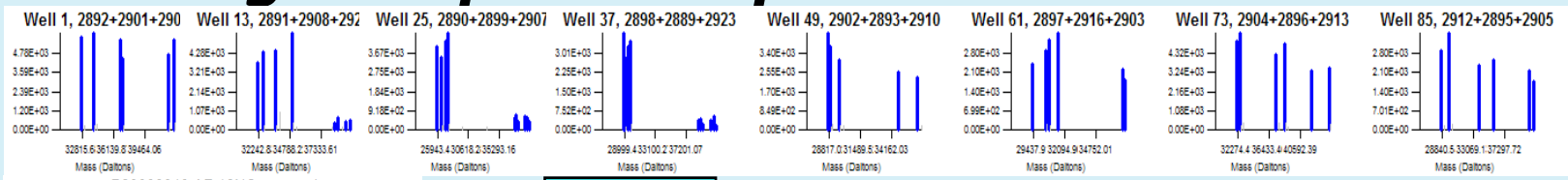
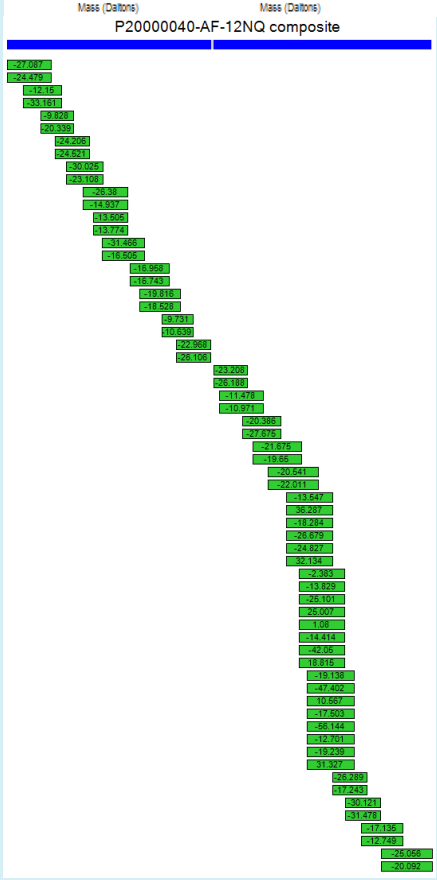
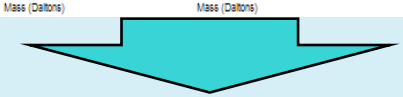
Coverage Map Development







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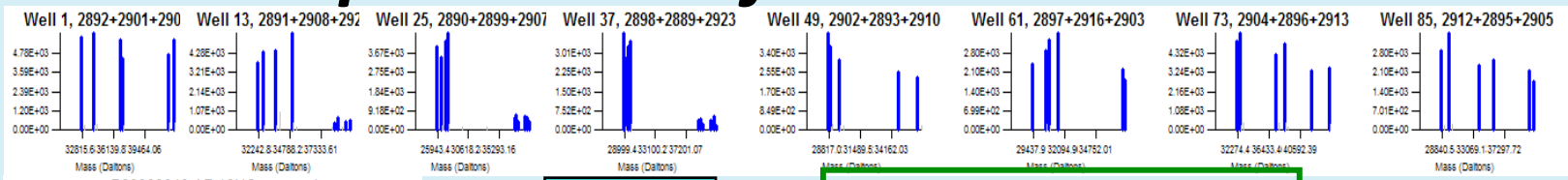
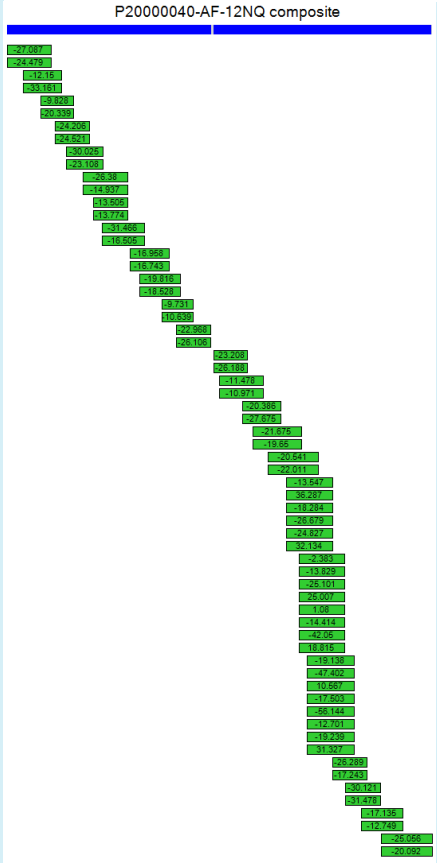
Base Composition Profile





Base composition profile

Base composition

rCRS coordinates

Primer pair

2901: 15893..16012: A47 G18 C25 T30
2925: 15937..16041: A35 G14 C24 T32
2899: 15985..16073: A26 G15 C21 T27
2898: 16025..16119: A26 G17 C26 T26
2897: 16055..16155: A31 G13 C30 T27
2896: 16102..16224: A45 G13 C42 T23
2895: 16130..16224: A36 G7 C33 T19
2893: 16154..16268: A44 G7 C46 T18
2892: 16231..16338: A40 G9 C40 T19
2891: 16256..16366: A37 G9 C41 T24
2890: 16318..16402: A20 G14 C30 T21
2889: 16357..16451: A21 G17 C36 T21
2902: 5..97: A19 G24 C24 T26
2903: 20..139: A24 G34 C29 T33
2904: 83..187: A23 G21 C29 T32
2905: 113..245: A39 G18 C28 T48
2906: 154..290: A49 G17 C31 T40
2908: 204..330: A42 G16 C35 T32
2908: 204..330: A42 G16 C36 T32
2908: 204..330: A42 G16 C37 T32
2908: 204..330: A42 G16 C38 T32
2907: 239..363: A43 G11 C46 T23
2907: 239..363: A43 G11 C47 T23
2907: 239..363: A43 G11 C48 T23
2907: 239..363: A43 G11 C49 T23
2923: 262..390: A47 G10 C50 T20
2923: 262..390: A47 G10 C51 T20
2923: 262..390: A47 G10 C52 T20
2923: 262..390: A47 G10 C53 T20
2910: 331..425: A33 G9 C27 T26
2916: 367..463: A27 G8 C32 T30
2912: 409..521: A32 G7 C48 T26
2913: 464..603: A44 G10 C63 T23

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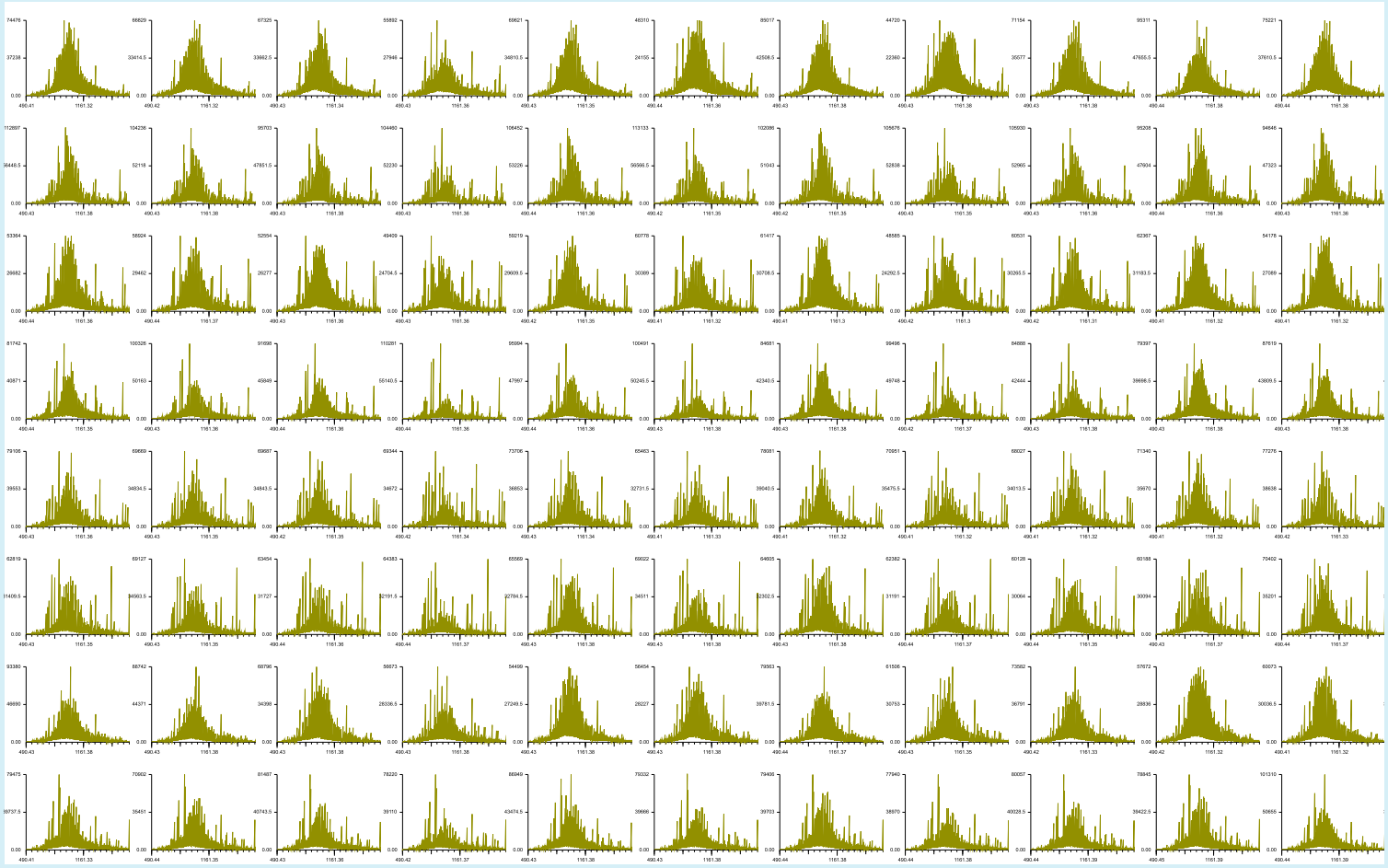
Multiple Assays Per Plate



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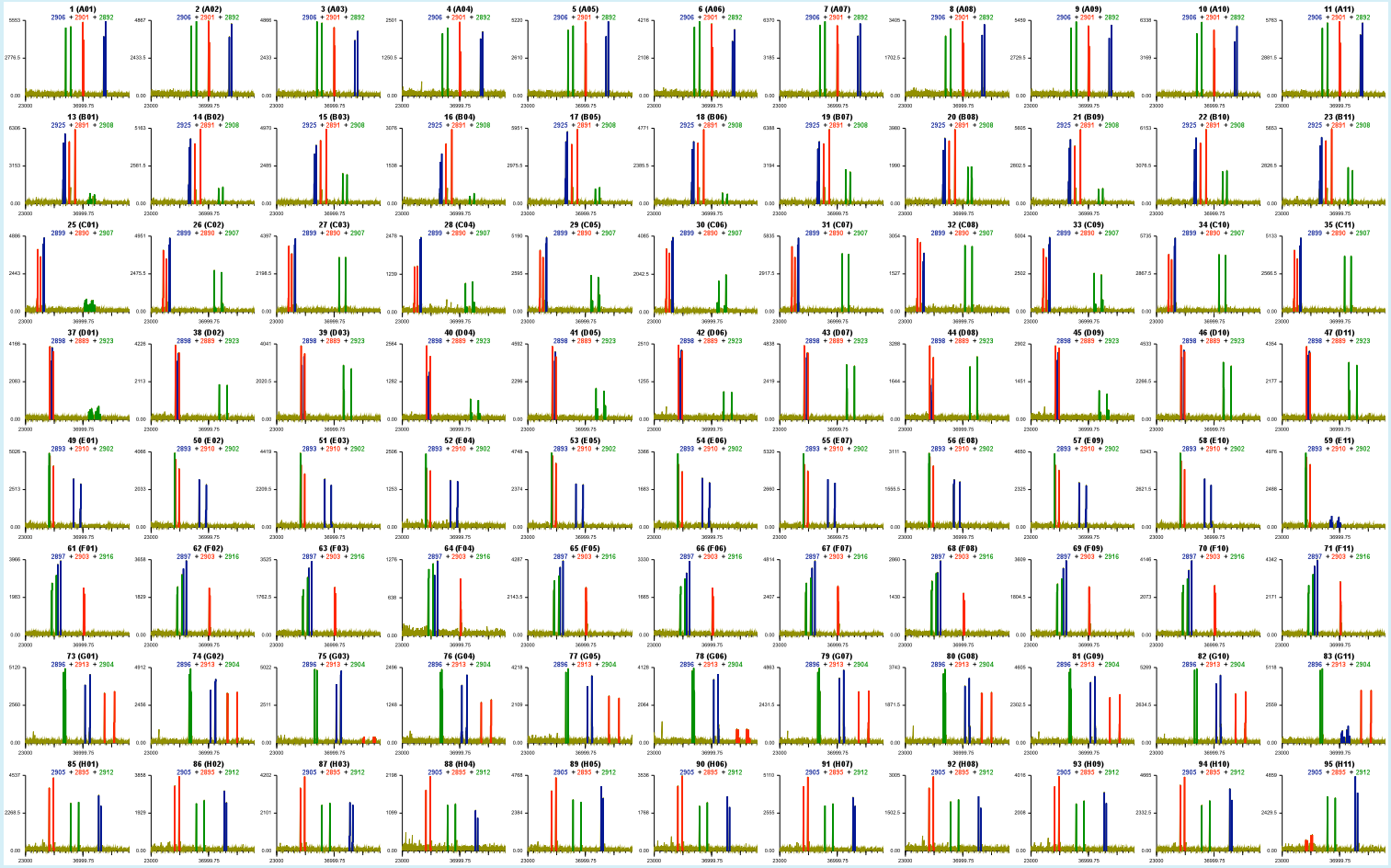
Multiple Mass Assignments



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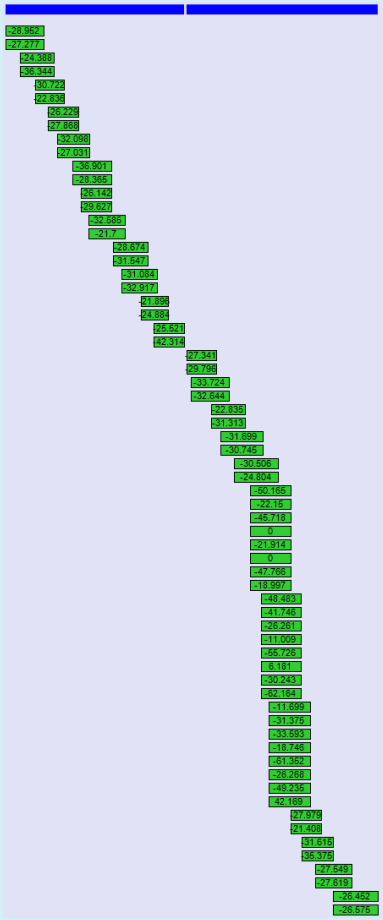
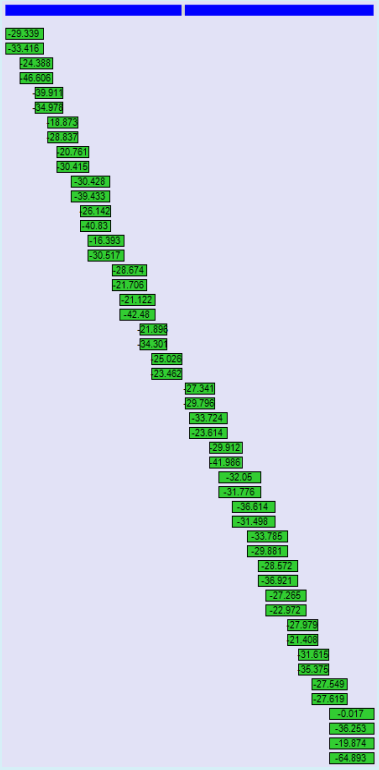
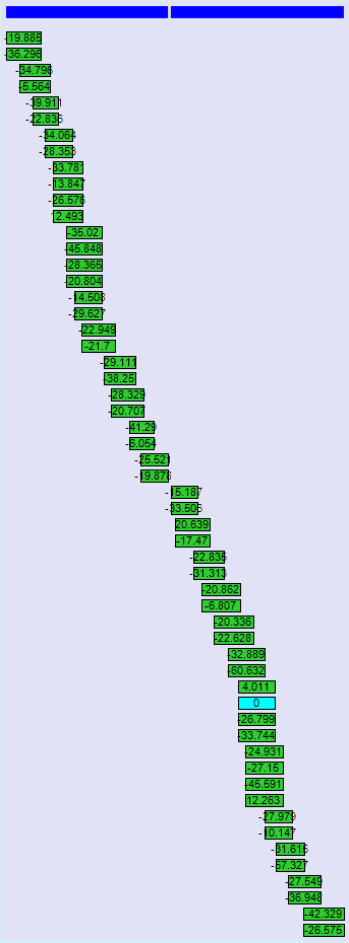
Multiple Samples Per Plate







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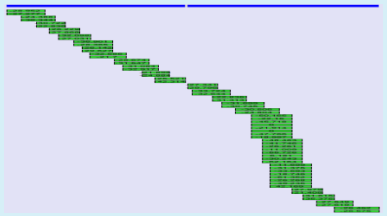
Multiple Profiles Per Plate



2901: 15893..16012: A47 G18 C25 T30
2925: 15937..16041: A35 G14 C24 T32
2899: 15985..16073: A26 G15 C21 T27
2898: 16025..16119: A26 G17 C26 T26
2897: 16055..16155: A31 G13 C30 T27
2896: 16102..16224: A45 G13 C42 T23
2895: 16130..16224: A36 G7 C33 T19
2893: 16154..16268: A44 G7 C46 T18
2892: 16231..16338: A40 G9 C40 T19
2891: 16256..16366: A37 G9 C41 T24
2890: 16318..16402: A20 G14 C30 T21
2889: 16357..16451: A21 G17 C36 T21
2902: 5..97: A19 G24 C24 T26
2903: 20..139: A24 G34 C29 T33
2904: 83..187: A23 G21 C29 T32
2905: 113..245: A39 G18 C28 T48
2906: 154..290: A49 G17 C31 T40
2908: 204..330: A42 G16 C35 T32
2908: 204..330: A42 G16 C36 T32
2908: 204..330: A42 G16 C37 T32
2908: 204..330: A42 G16 C38 T32
2907: 239..363: A43 G11 C46 T23
2907: 239..363: A43 G11 C47 T23
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2907: 239..363: A43 G11 C49 T23
2923: 262..390: A47 G10 C50 T20
2923: 262..390: A47 G10 C51 T20
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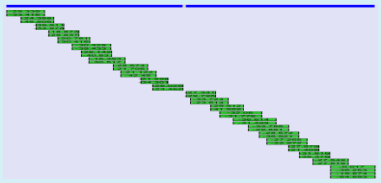

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2899: 15985..16073: A26 G15 C21 T27
2898: 16025..16119: A26 G17 C28 T24
2897: 16055..16155: A32 G12 C30 T27
2896: 16102..16224: A46 G12 C41 T24
2895: 16130..16224: A36 G7 C33 T19
2893: 16154..16268: A44 G7 C45 T19
2892: 16231..16338: A40 G9 C40 T19
2891: 16256..16366: A37 G9 C41 T24
2890: 16318..16402: A20 G14 C30 T21
2889: 16357..16451: A22 G16 C36 T21
2902: 5..97: A19 G24 C24 T26
2903: 20..139: A24 G34 C29 T33
2904: 83..187: A23 G21 C30 T31
2905: 113..245: A39 G18 C31 T45
2906: 154..290: A48 G18 C35 T36
2908: 204..330: A42 G16 C40 T30
2907: 239..363: A43 G11 C49 T23
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2910: 331..425: A33 G9 C27 T26
2916: 367..463: A27 G8 C32 T30
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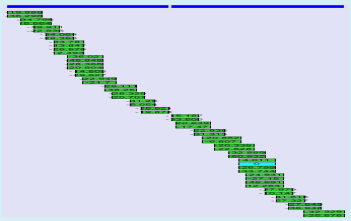

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2896: 16102..16224: A46 G12 C42 T23
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2895: 16130..16224: A36 G7 C33 T19
2893: 16154..16268: A44 G7 C46 T18
2892: 16231..16338: A39 G10 C40 T19
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2910: 331..425: A33 G9 C27 T26
2916: 367..463: A27 G8 C32 T30
2912: 409..521: A32 G7 C48 T26
2913: 464..603: A44 G10 C63 T23

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Tiling Compared to Sequencing

- 1266 unique tiling region sequences were selected from GenBank genomes
  - Each sequence differed from all others by at least one base
  - C-stretch length differences ignored
- Sequences converted to tiling base compositions
- Cross-compared for minimum differences using mtDNA search algorithm
  - Ignores C-stretch length differences
  - Corrects for primer pair overlaps
- 94.2% of unique tiling region sequences were uniquely discriminated by tiling assay

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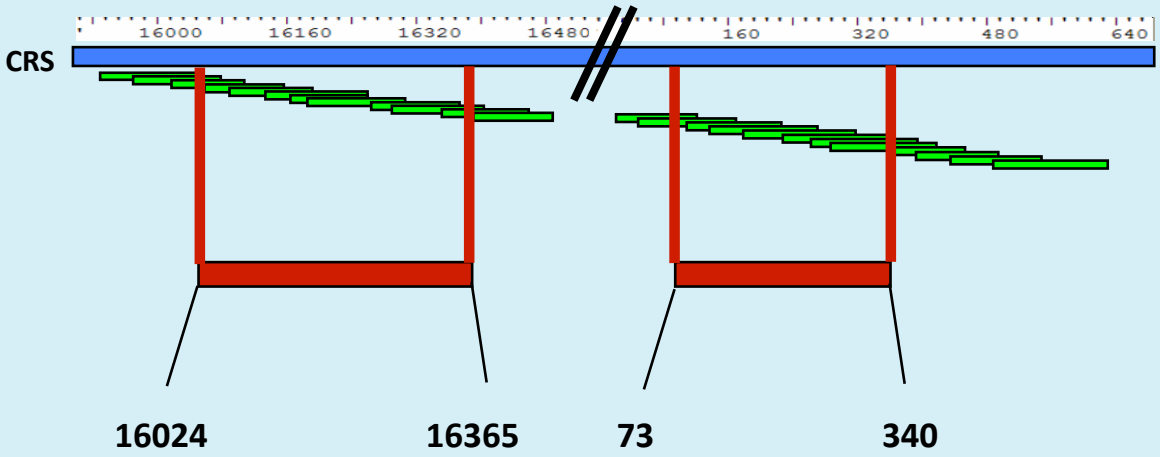
Tiling Compared to Sequencing



- **Tiling assay amplifies 15924-16428 and 31-576**
- **Minimum HVI +HVII sequences 16024-16365 and 73-340**

For the same set of 1266 unique sequences spanning mtDNA tiling coordinates:

- **94.2% can be differentiated with the tiling assay**
- **90.2% can be differentiated by sequencing HVI 16024-16365 and HV2 73-340**

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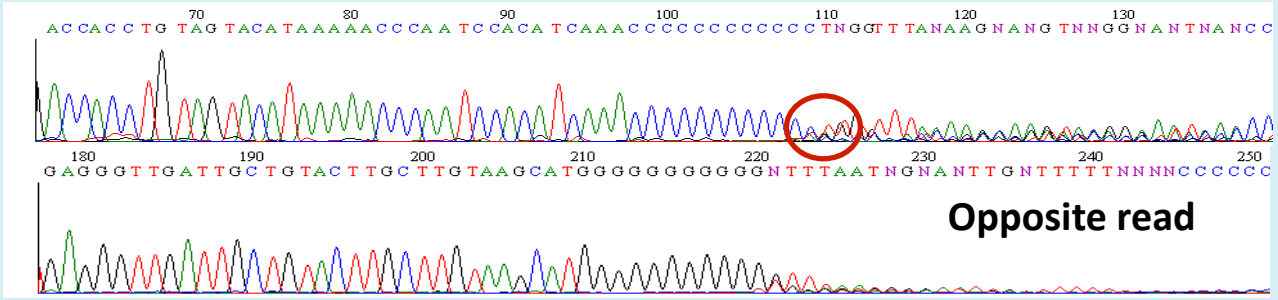
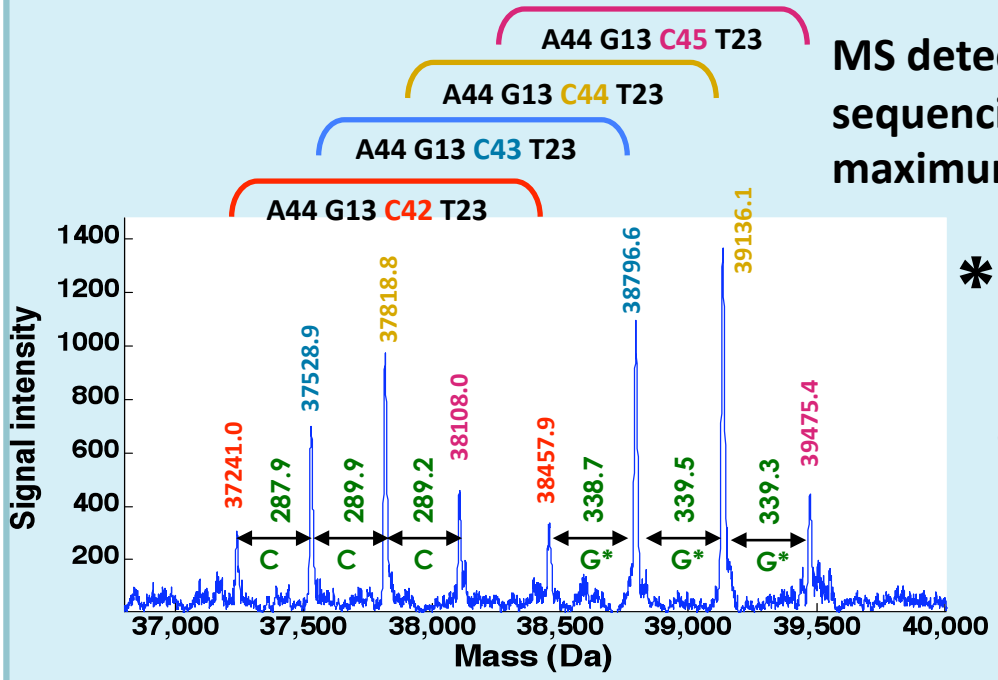
Length Heteroplasmy Detection

Example = USA.FBI.000009, region 16102..16224 *



Good sequence up to C stretch, then multiple overlapping reads



MS detects multiple products while sequencing requires a pure sample for maximum information content

***Happened to be same sample given as a blinded sample by Bruce Budowle in 2003.**
This was suggested by our assay in the FBI validation, then confirmed.

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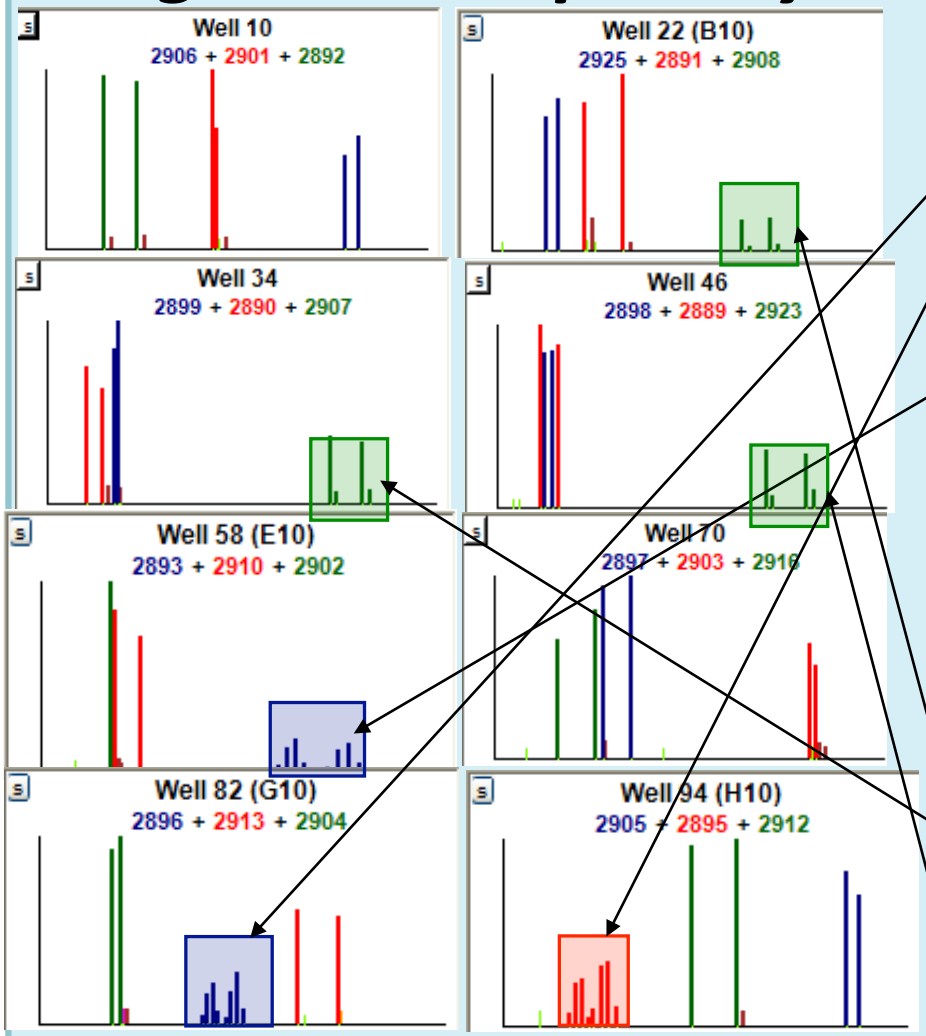
Length Heteroplasmy Example



2901: 15893..16012: A47 G18 C25 T30
2925: 15937..16041: A35 G14 C24 T32
2899: 15985..16073: A25 G16 C21 T27
2898: 16025..16119: A25 G18 C27 T25
2897: 16055..16155: A31 G12 C30 T28
2896: 16102..16224: A44 G12 C42 T24
2896: 16102..16224: A44 G12 C43 T24
2896: 16102..16224: A44 G12 C44 T24
2896: 16102..16224: A44 G12 C45 T24
2895: 16130..16224: A35 G7 C33 T19
2895: 16130..16224: A35 G7 C34 T19
2895: 16130..16224: A35 G7 C35 T19
2895: 16130..16224: A35 G7 C36 T19
2893: 16154..16268: A43 G7 C47 T17
2893: 16154..16268: A43 G7 C48 T17
2893: 16154..16268: A43 G7 C49 T17
2893: 16154..16268: A43 G7 C50 T17
2892: 16231..16338: A40 G9 C39 T20
2891: 16256..16366: A37 G9 C41 T24
2890: 16318..16402: A20 G14 C31 T20
2889: 16357..16451: A21 G17 C36 T21
2902: 5..97: A19 G24 C24 T26
2903: 20..139: A24 G34 C29 T33
2904: 83..187: A23 G21 C30 T31
2905: 113..245: A39 G18 C30 T46
2905: 113..245: A39 G18 C29 T47
2906: 154..290: A48 G18 C32 T39
2906: 154..290: A48 G18 C31 T40
2908: 204..330: A42 G16 C39 T32
2908: 204..330: A42 G16 C40 T32
2907: 239..363: A43 G11 C49 T24
2907: 239..363: A43 G11 C50 T24
2923: 262..390: A47 G10 C53 T21
2923: 262..390: A47 G10 C54 T21
2910: 331..425: A33 G9 C27 T26
2916: 367..463: A27 G8 C32 T30
2912: 409..521: A32 G7 C48 T26
2913: 464..603: A43 G11 C63 T23

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SNP Heteroplasmy Detection

From sequence profile

AF-4: 16024-16365

C 16176 N → C/T heteroplasmy

T 16362 C → C/T heteroplasmy

Observed profile

16102..16224: A45 G13 C41 T24

16102..16224: A45 G13 C40 T25

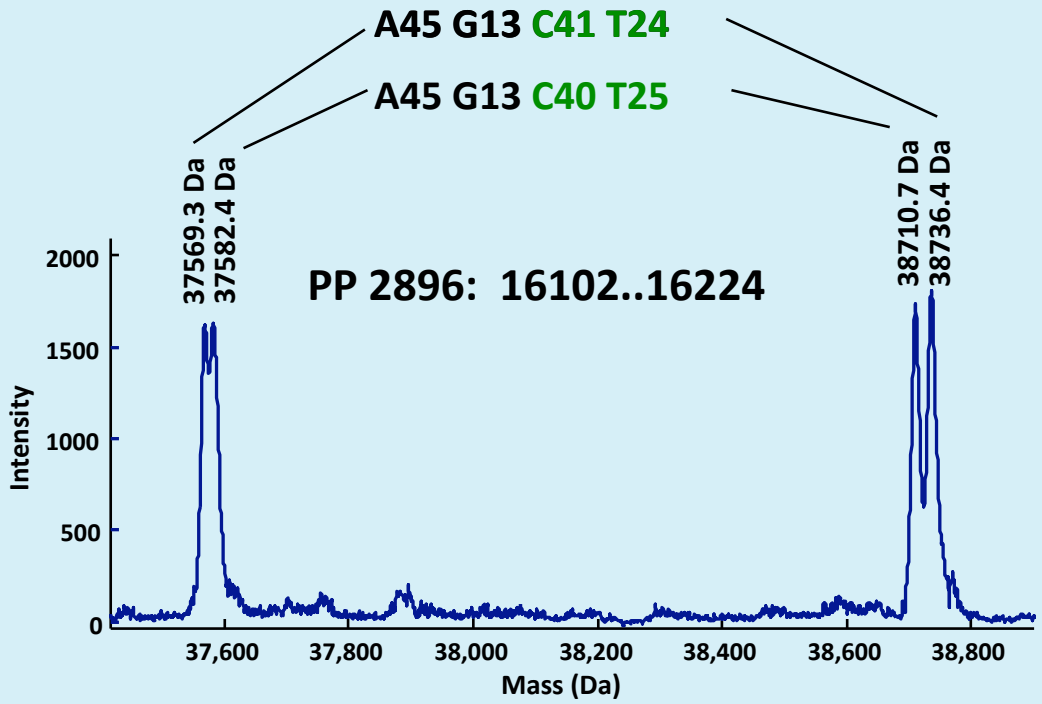
16130..16224: A36 G7 C33 T19

16130..16224: A36 G7 C32 T20

Calculated from truth key

16102..16224: A45 G13 C40 T24 N

16130..16224: A36 G7 C32 T19 N



From blinded sample AF-4

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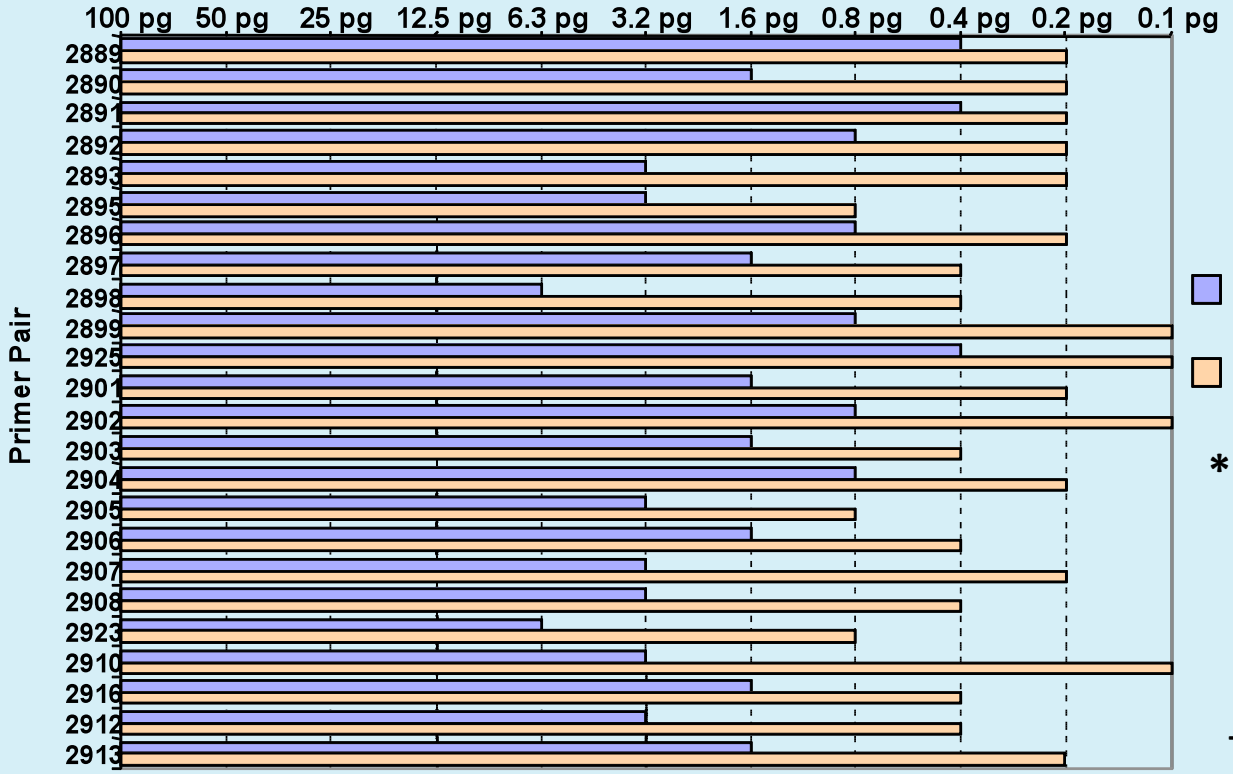
Sensitivity

- Analysis of five templates in dilution-to-extinction
- Sensitivity ranged from 0.1 pg to 6.3 pg template/primer pair*
- All templates had full profile at 6.3 pg or below per reaction
- Sensitivity criteria for standard QC plate set at 25 pg/reaction



* Based upon total nuclear DNA quantification with Quantifiler®

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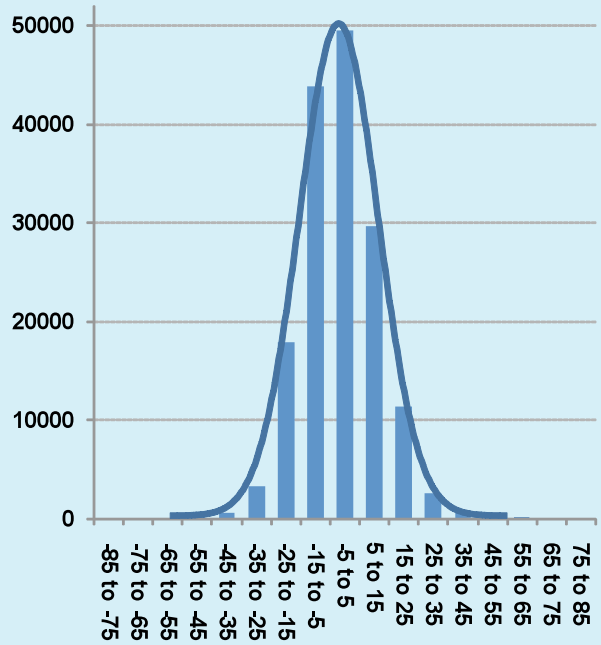
Reproducibility

- 3,331 trials of one positive control template run over the course of 27 months
- 500 pg template/reaction
- Automated data analysis
- 79,944 expected ds assignments
- 3,298 (99%) full, correct profiles

Distribution of mass measurement deviations for 159,688 DNA strand assignments

Ave. error magnitude was 10.12 ± 8.04 ppm



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Summary

- 1051 nucleotide positions covered by 24 primer pairs
- Accurate mass measurements and biochemical strategy allow mitochondrial base composition profiles to be developed
- Discrimination power is about 94% that of sequencing same region
- Discrimination power over tiling region can be greater than sequencing over minimum HV1 and HV2 ranges
- Base composition profiles can be compared to each other and to sequence profiles
- Databases can be searched and subjected to same type of statistics as a sequence database
- Mass spectrometry can resolve heteroplasmy (mixtures)

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SNP Assay Format

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Objective

- PCR/ESI-MS-based assay for human autosomal SNP analysis
- Exclude non-contributors to a DNA sample
- A random profile match should have very low probability
- Minimize population bias
  - Multiple markers with about 50% heterozygosity
  - Low F_{st} (distribution same in all populations)
  - Low detectable genetic linkage (low linkage disequilibrium)
  - Use product rule for probability estimates
  - Use global q or no q correction for population substructure
- 40 independent markers

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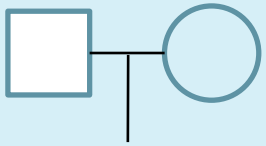
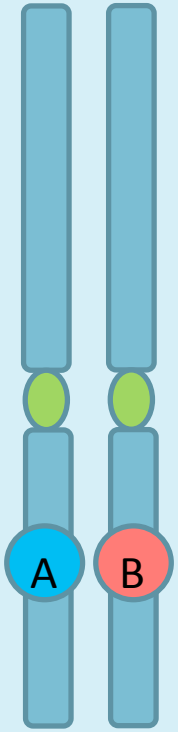
Ideal Bi-allelic Markers

One from father

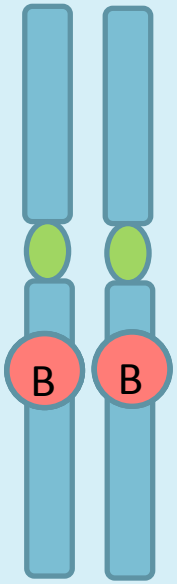
One from mother



Each autosomal chromosome comes as a pair



Bi-allelic markers have one of two states



Any marker for any individual can be both 'A' or both 'B' or one of each

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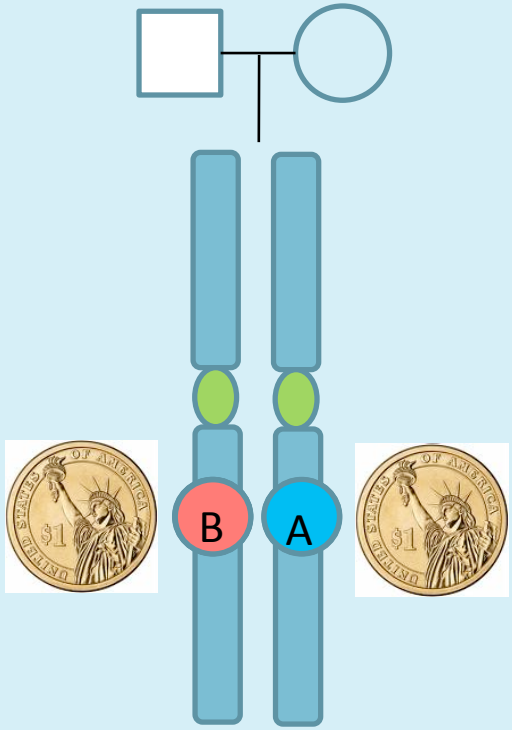
Ideal Bi-allelic Markers

There's a 50% chance of getting either allele on the father's chromosome



And a 50% chance of getting either allele on the mother's chromosome

Each marker is like a coin toss

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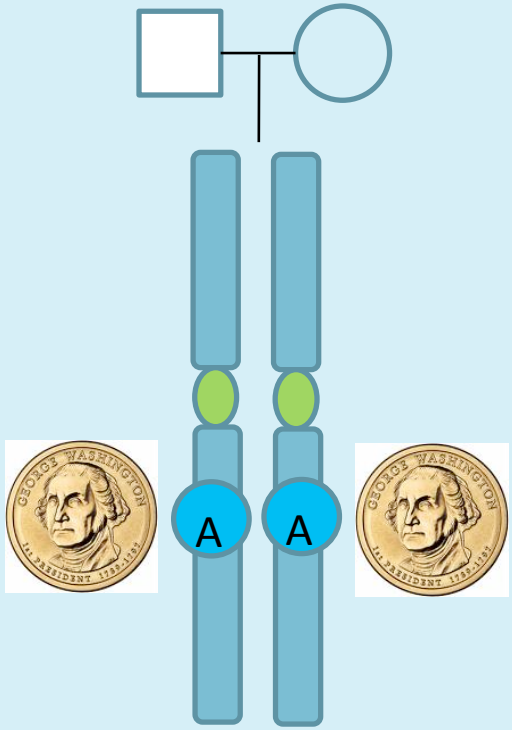
Ideal Bi-allelic Markers

There's a 50% chance of getting either allele on the father's chromosome



And a 50% chance of getting either allele on the mother's chromosome

Each marker is like a coin toss

That means 50% x 50% = 25% chance of getting two 'heads' (A allele)

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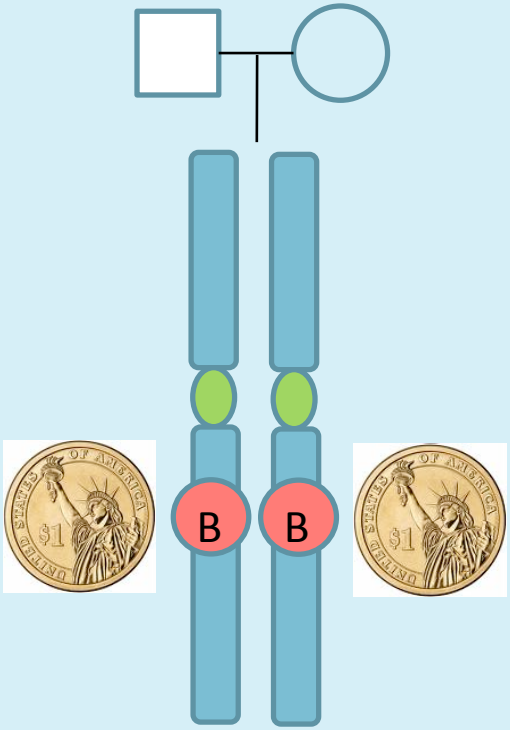
Ideal Bi-allelic Markers

There's a 50% chance of getting either allele on the father's chromosome



And a 50% chance of getting either allele on the mother's chromosome

Each marker is like a coin toss

And 50% x 50% = 25% chance of getting two 'tails' (B allele)

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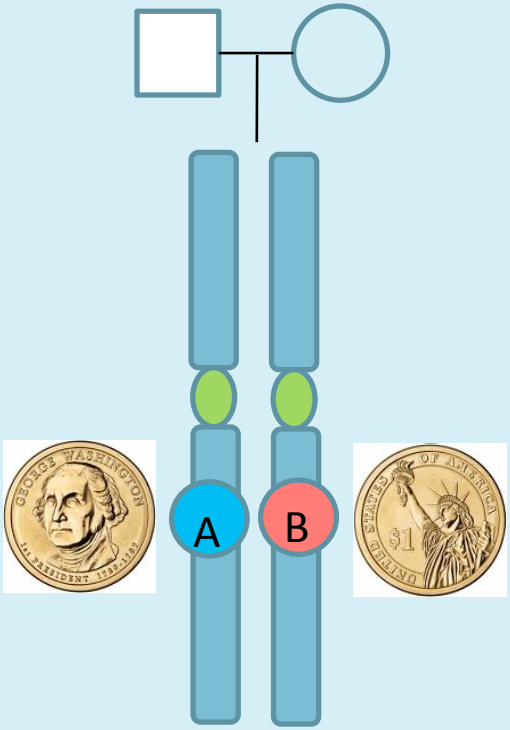
Ideal Bi-allelic Markers



Each marker is like a coin toss
And 50% x 50% = 25% chance of getting A + B

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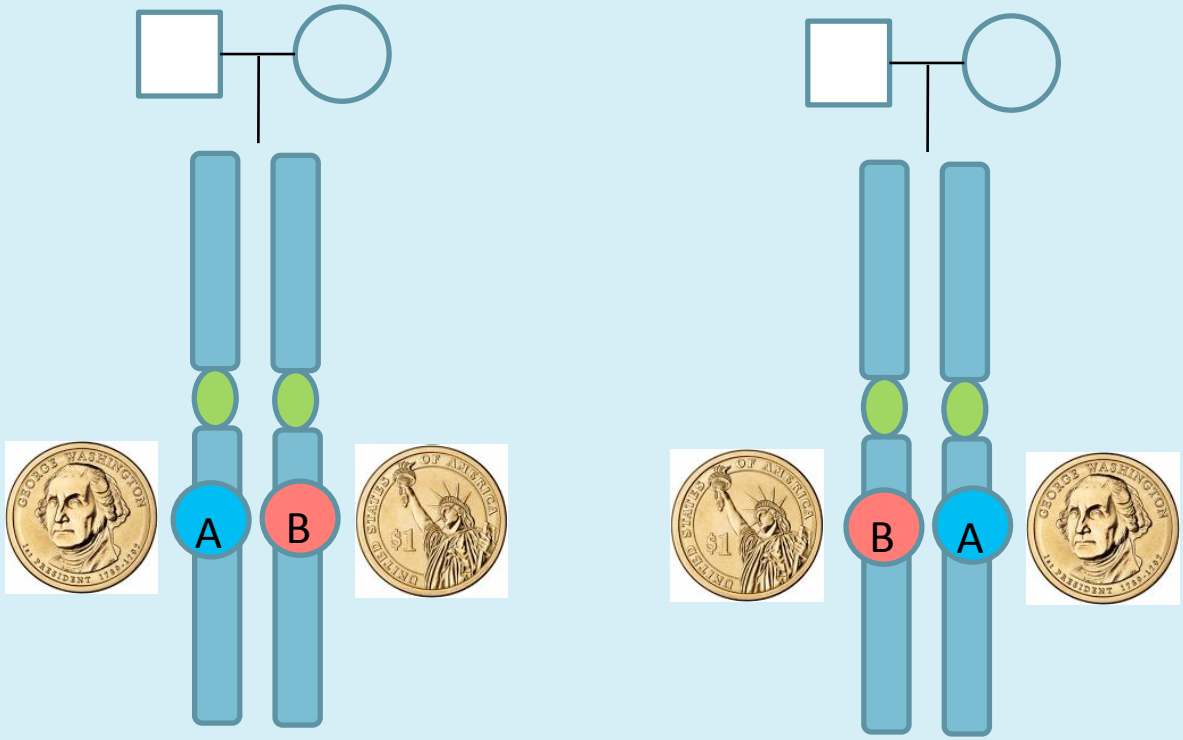
Ideal Bi-allelic Markers



Each marker is like a coin toss

And 50% x 50% = 25% chance of getting A + B

Plus 50% x 50% = 25% chance of getting B + A

= 50% chance of being heterozygous

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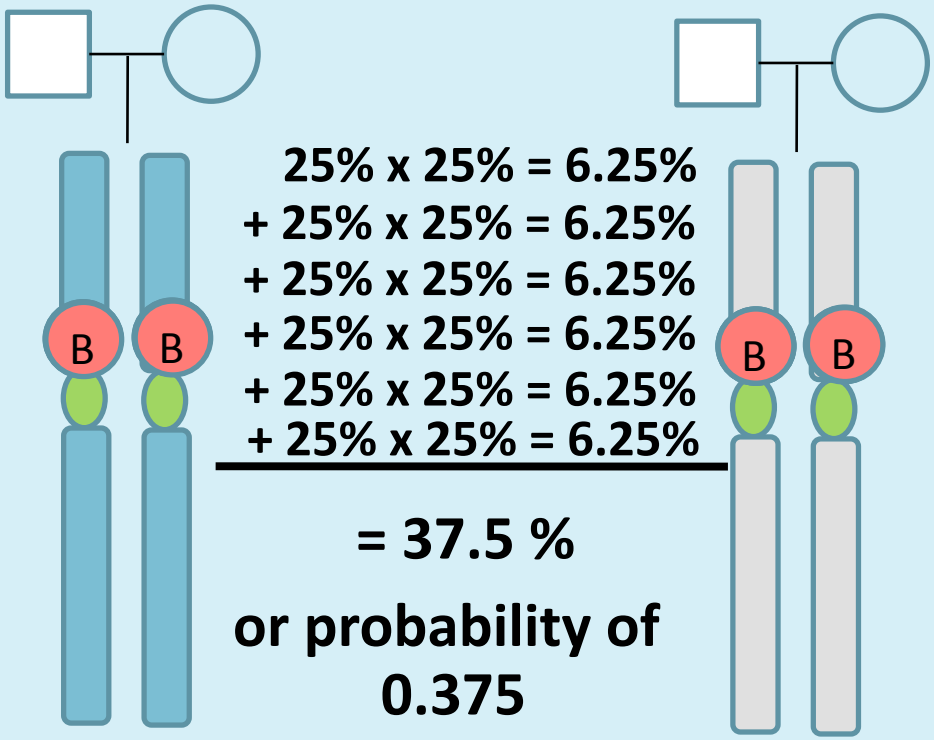
Ideal Bi-allelic Markers

For any two individuals,
the random odds they will match at any one locus

is



25% x 25% = 6.25%
+ 25% x 25% = 6.25%
+ 25% x 25% = 6.25%
+ 25% x 25% = 6.25%
+ 25% x 25% = 6.25%
+ 25% x 25% = 6.25%

= 37.5 %

or probability of 0.375

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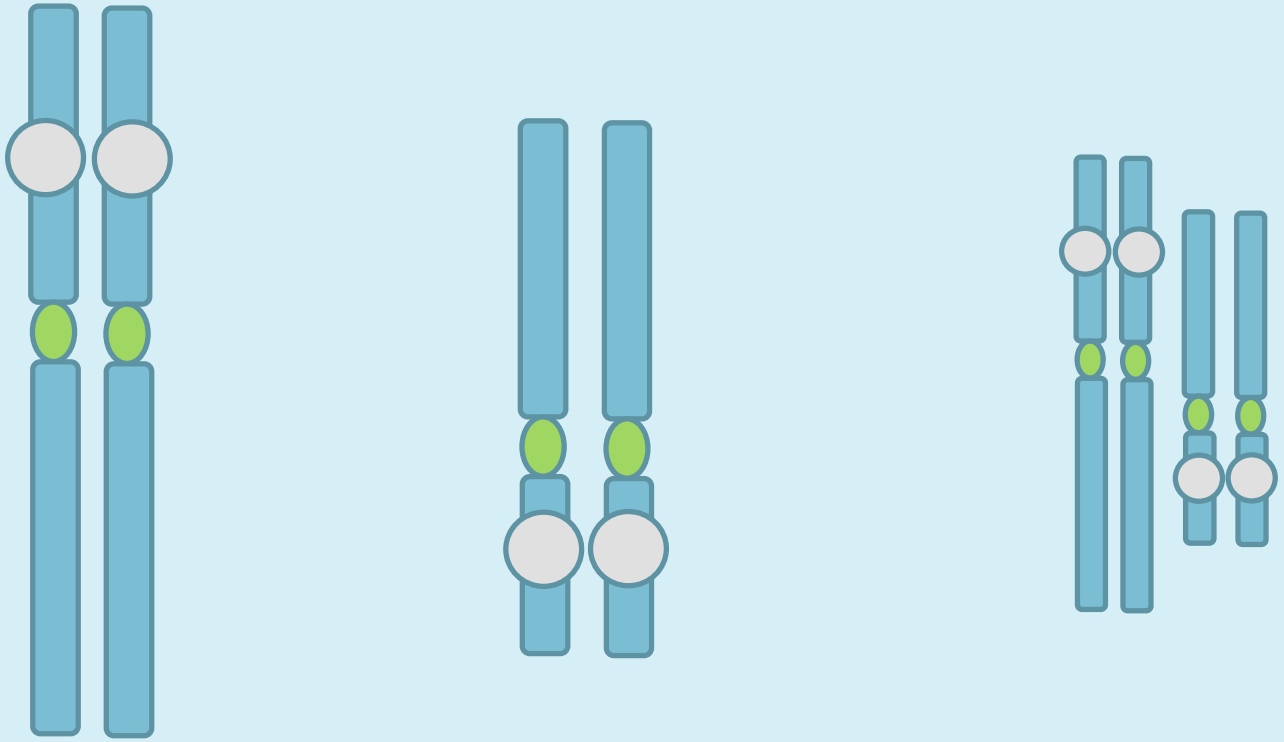
Ideal Bi-allelic Markers



Random match probability for one marker

0.375

X

Random match probability for another marker

0.375

=

Random match probability for two-marker profile

0.141

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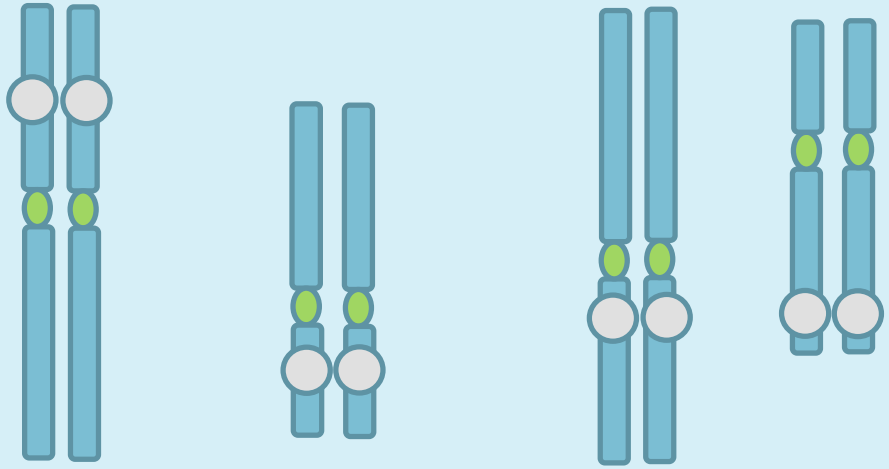
Ideal Bi-allelic Markers



Random match probability for four-marker profile

= 0.375 x 0.375 x 0.375 x 0.375

= 0.375^4

= 0.0198

Close to 99% with four perfectly distributed bi-allelic markers

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Ideal Bi-allelic Markers

- **With 40 unlinked and perfectly-distributed SNPs, the random match probability would ideally be 0.37540, or 9.15×10^{-18}**

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Kidd-40 SNP Panel

- Large collection of SNP positions with data for three major population groups
- Subpanel identified with low F_{st} and high heterozygosity
- Evaluated subpanel over seven populations
- 73 SNPs with $F_{st} < 0.02$ over seven populations
- 40 final SNPs with $F_{st} < 0.06$ and heterozygosity > 0.4 across 40 populations around the world
  - Reference: Pakstis, A., Speed, W., Kidd, J., Kidd, K. "Candidate SNPs for a Universal Individual Identification Panel." *Human Genetics* 121 (3 – 4) (May 2007): 305 – 317.

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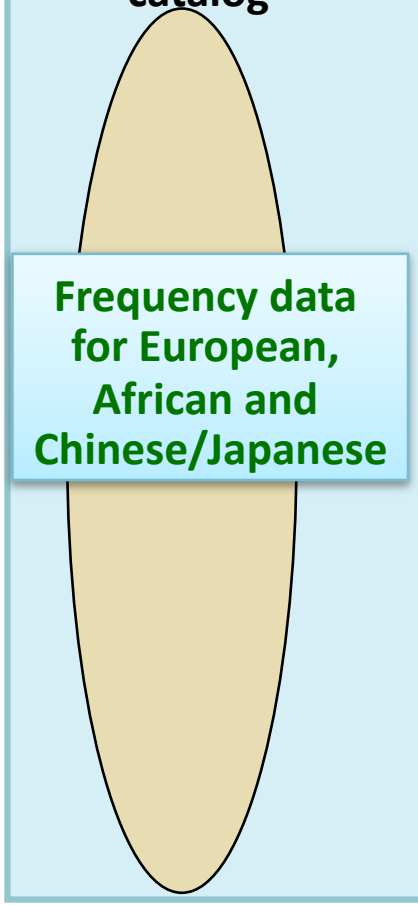
The Kidd Approach

90,483 SNPs from ABI catalog



Frequency data for European, African and Chinese/Japanese

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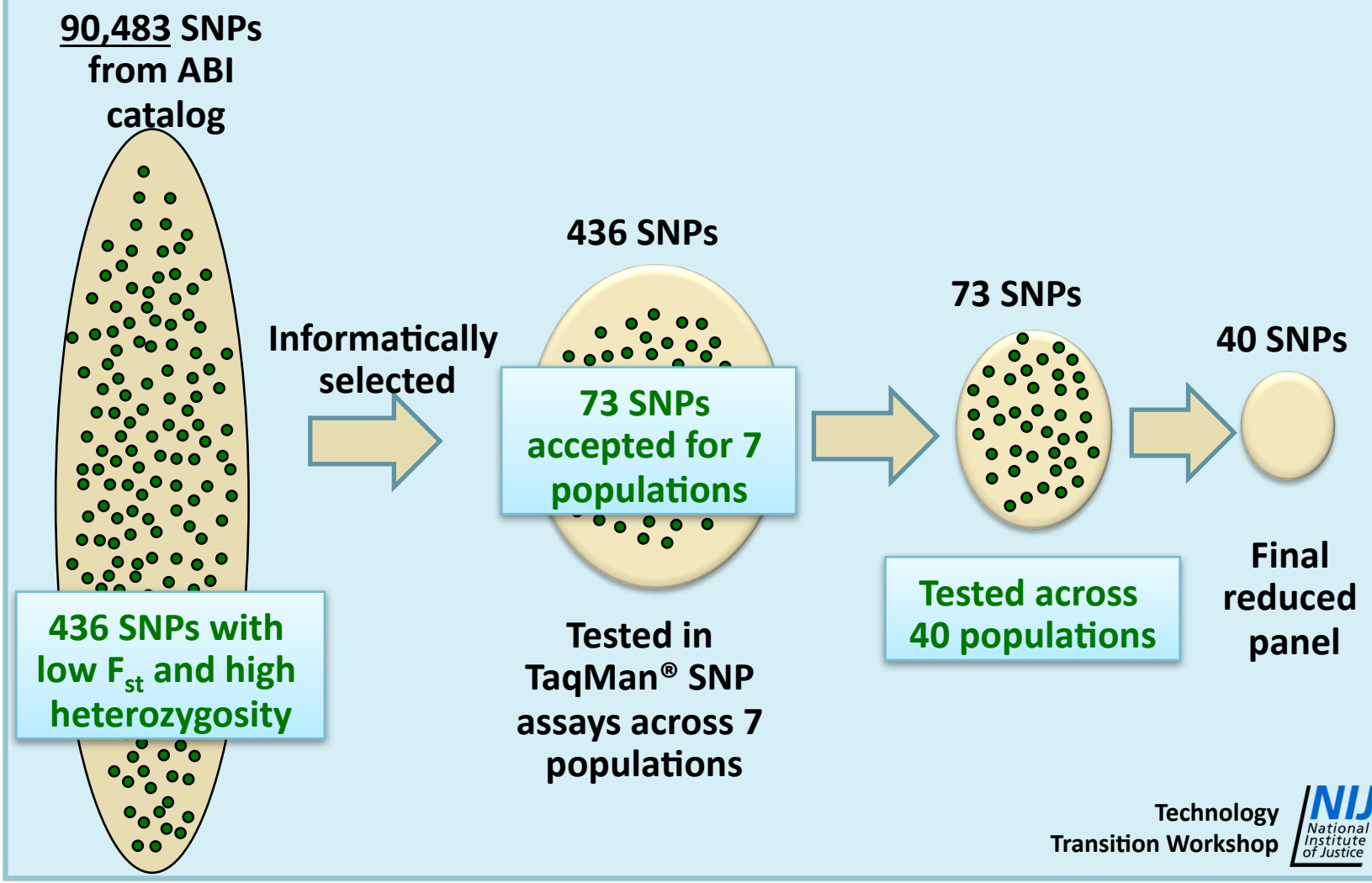
The Kidd Approach



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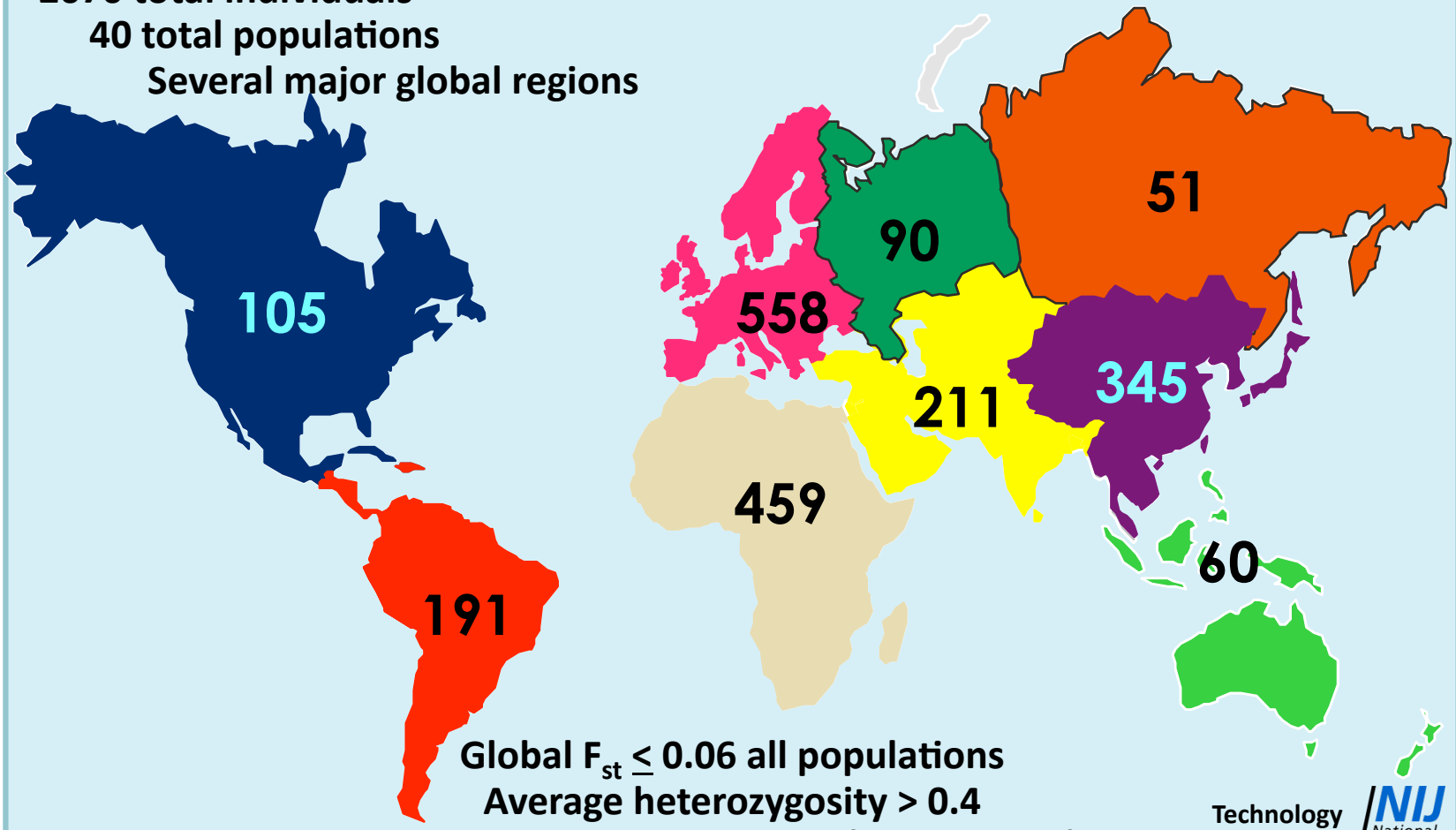
Kidd Population Coverage

2070 total individuals
40 total populations
Several major global regions



Global $F_{st} \leq 0.06$ all populations
Average heterozygosity > 0.4
Median LD = 0.010 (ave. = 0.029)

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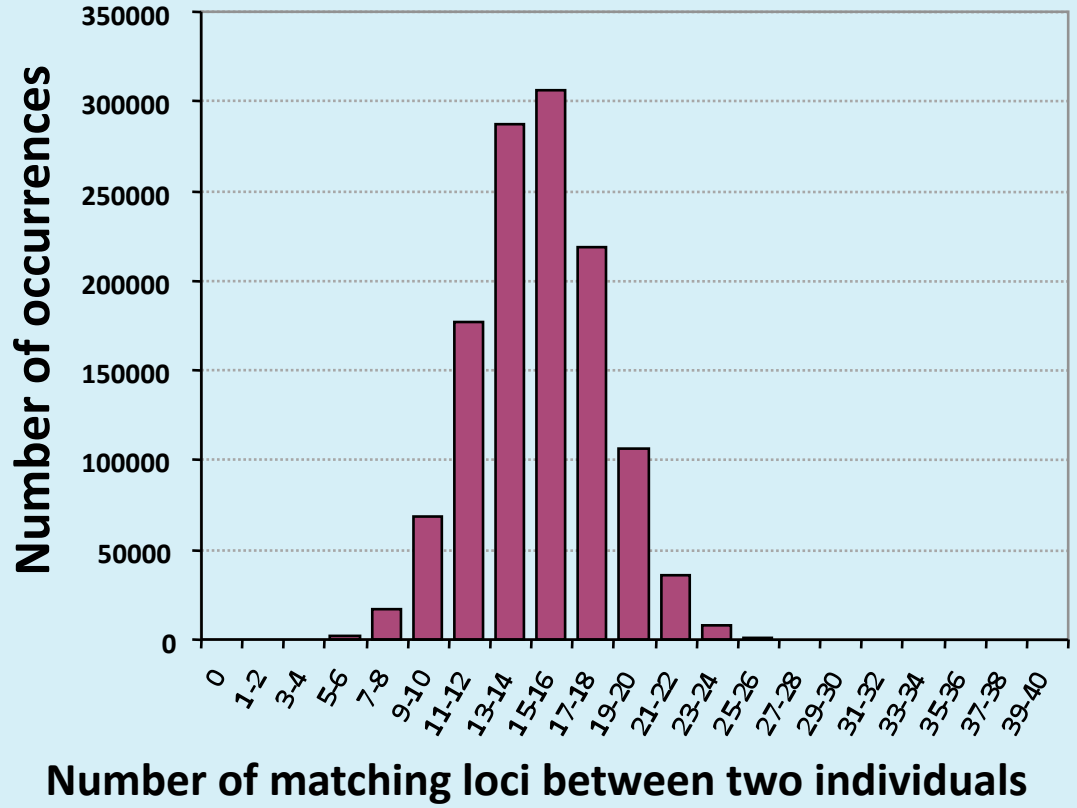
Resolving Power of Kidd SNP Panel

- **'Perfect' 40-SNP panel: random match with probability of 9.15×10^{-18}**
- **Kidd panel has ave. of about 1×10^{-15} match probability**
- **Within populations, ave. match probability ranged from 10^{-12} to 10^{-16}**



- **1,568 full profiles**
- **40 populations**
- **All pairwise profile-to-profile comparisons**
- **1,228,528 pairwise comparisons**
- **Most people will differ at 15 – 16 loci**

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Why Use Mass Spectrometry?

- **Unified platform for major DNA forensics applications**
  - **Mitochondrial DNA profiling**
  - **STR analysis**
  - **Autosomal SNP analysis**
  - **SNPs, STRs and/or mtDNA can be analyzed automatically on one instrument in the same run**
- **High degree of accuracy**
  - **Potential rare variant will be resolved rather than missed (could a C←→T position ever present an 'A' or a 'G'?)**

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Initial Development: Eight 5-plex Reactions

	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 plate and thermocycle

Sample 1, Sample 2, Sample 3, Sample 4, Sample 5, Sample 6, Sample 7, Sample 8, Sample 9, Sample 10, Positive, Negative

	1	2	3	4	5	6	7	8	9	10	11	12
A	RS1058083 + RS13182883 + RS1821380 + RS214955 + RS7704770											
B	RS10488710 + RS1478829 + RS7205345 + RS985492 + RS987640											
C	RS1410059 + RS1554472 + RS2073383 + RS279844 + RS6444724											
D	RS13134862 + RS1523537 + RS560681 + RS6811238 + RS9951171											
E	RS2272998 + RS321198 + RS3780962 + RS445251 + RS6591147											
F	RS1019029 + RS12997453 + RS1358856 + RS2503107 + RS740598											
G	RS1109037 + RS2567608 + RS315791 + RS447818 + RS7229946											
H	RS10092491 + RS13218440 + RS1336071 + RS338882 + RS7520386											

Each sample is distributed across one column of an assay plate

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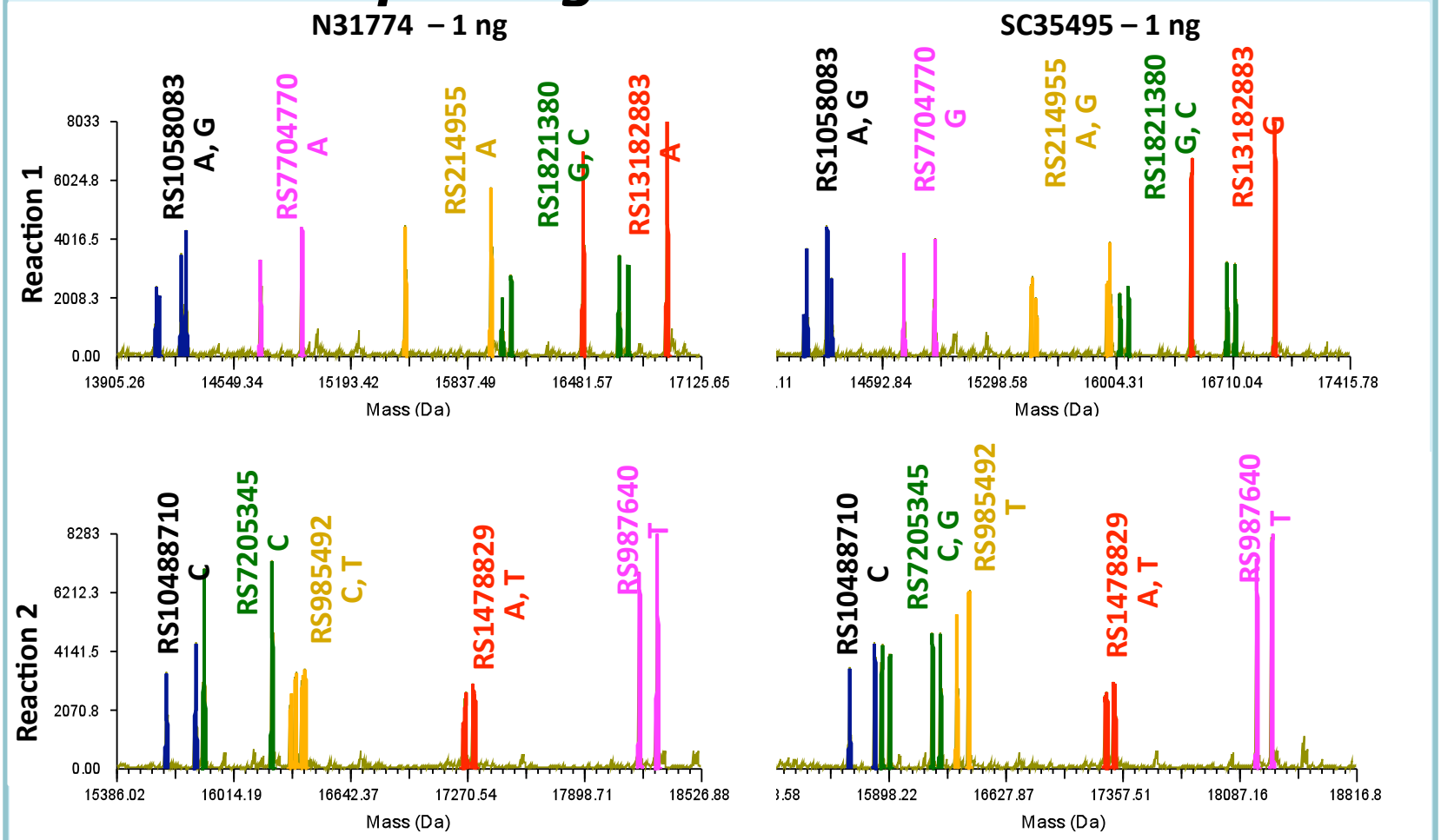
Initial Multiplexing Results



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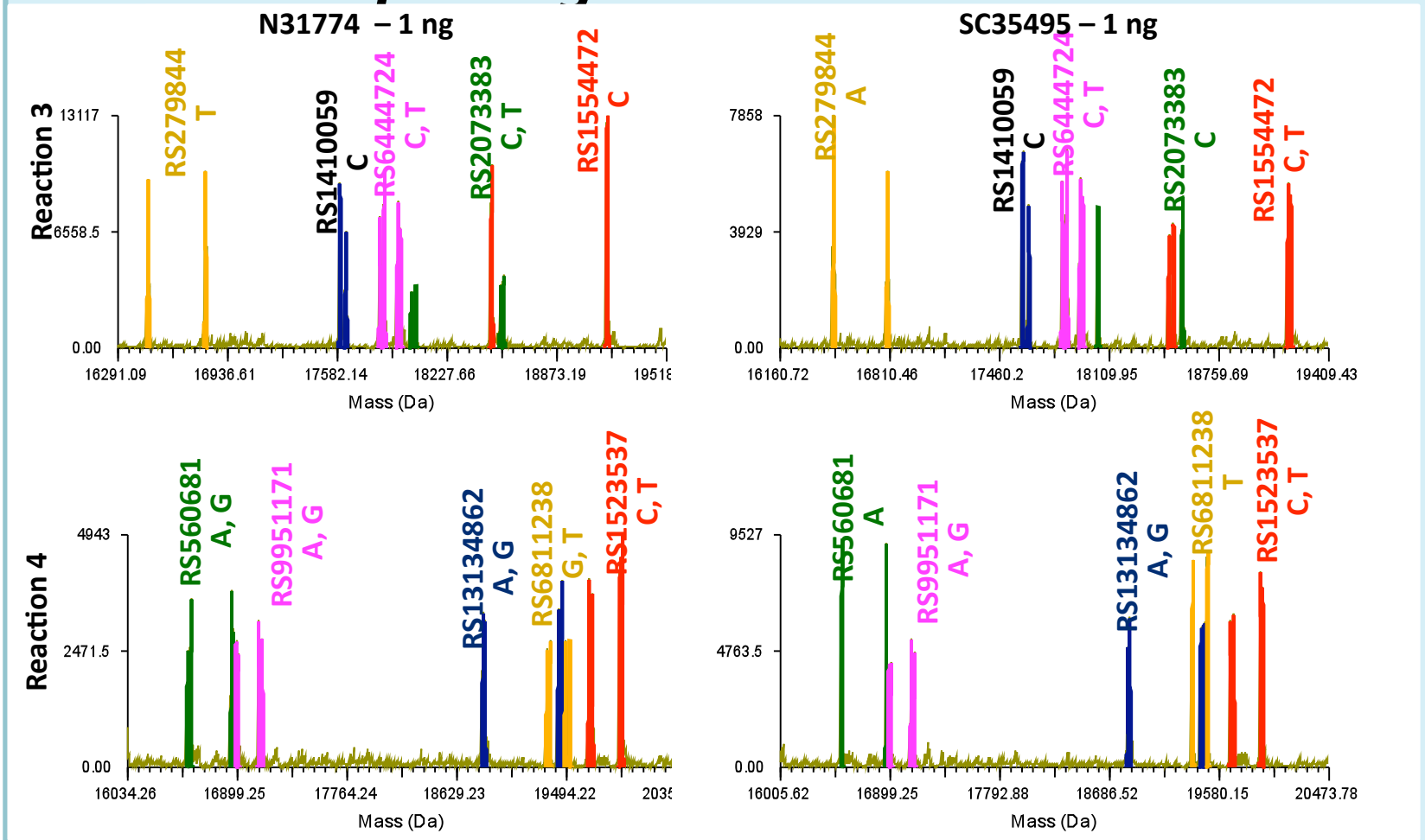
Initial Multiplexing Results



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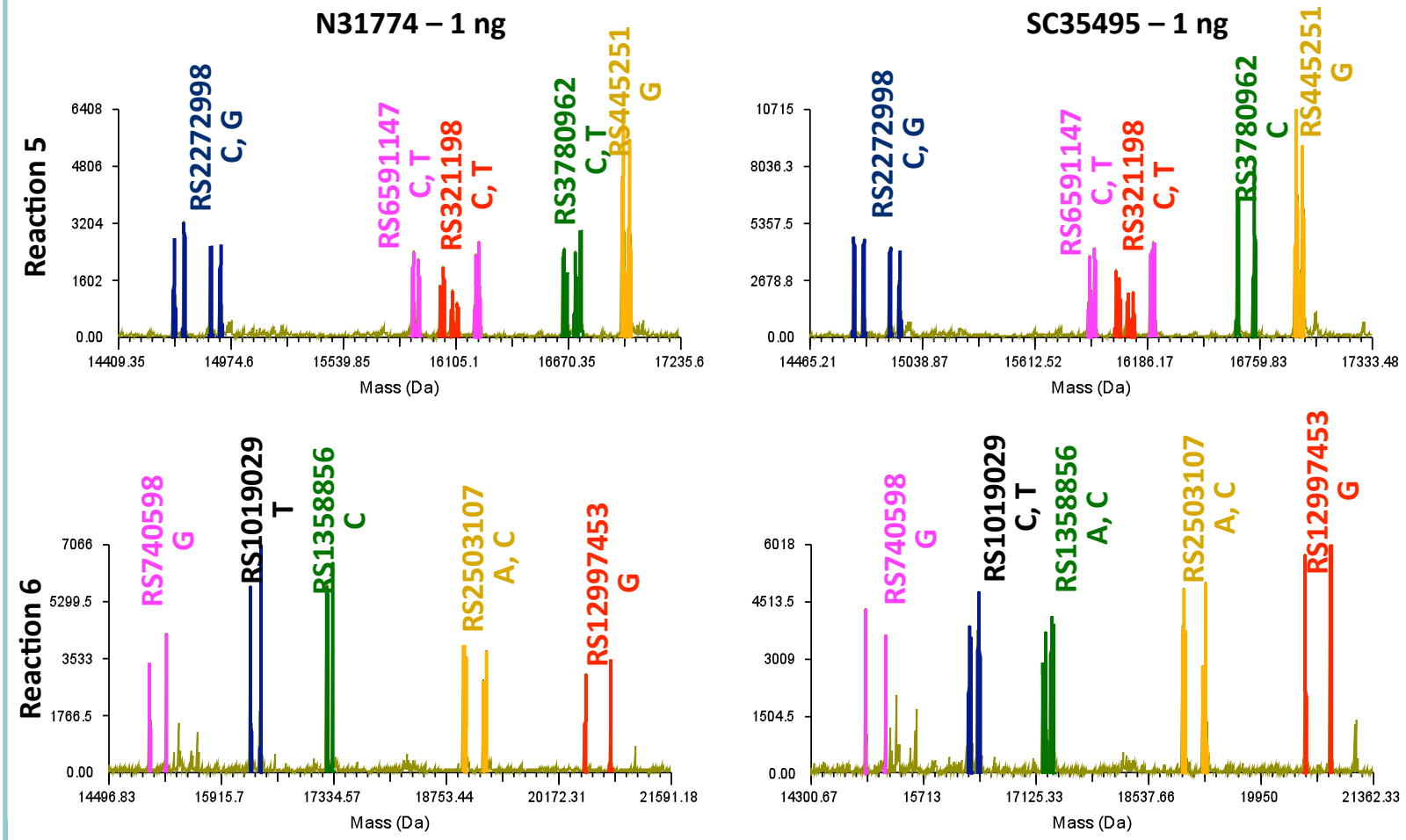
Initial Multiplexing Results



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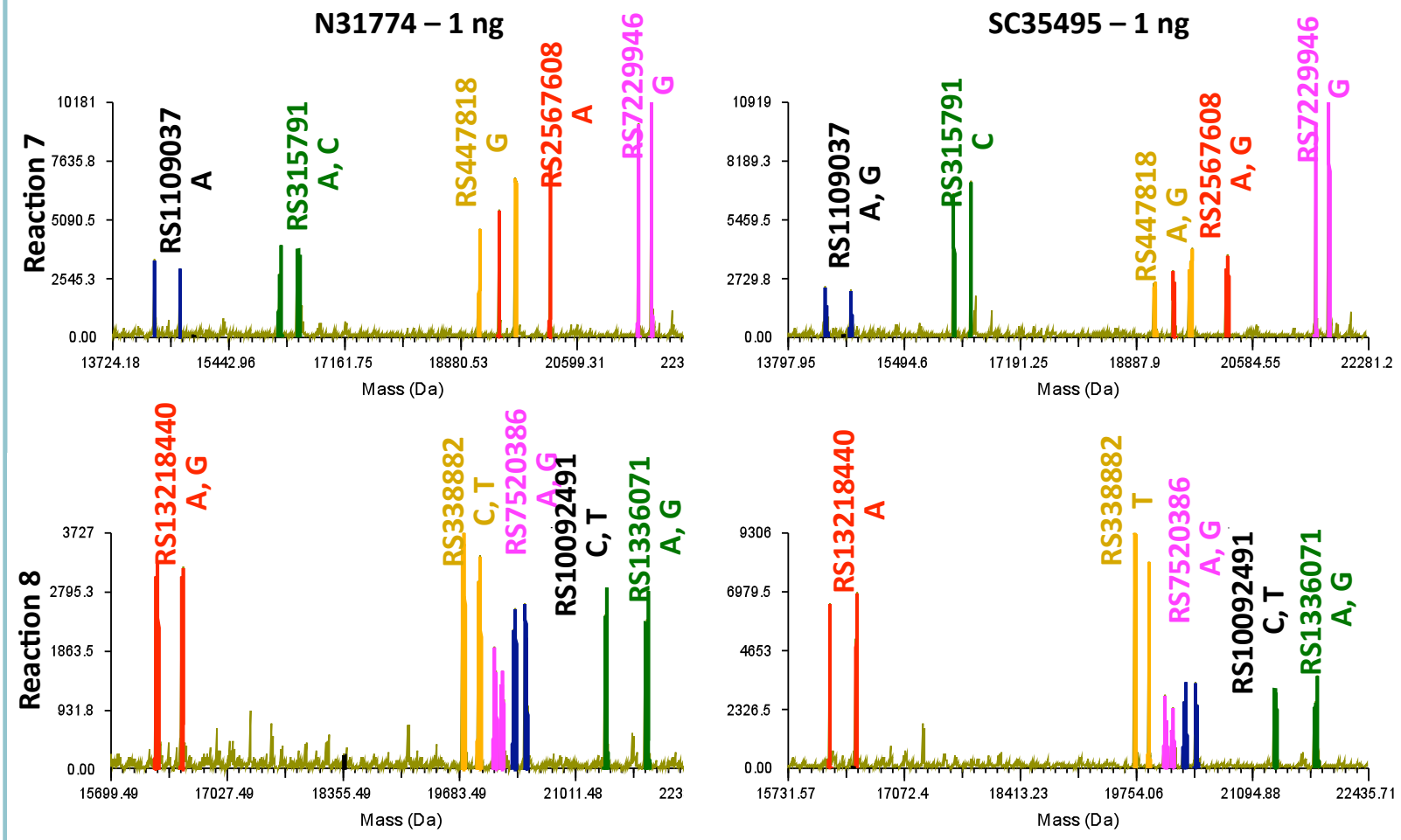
Initial Multiplexing Results



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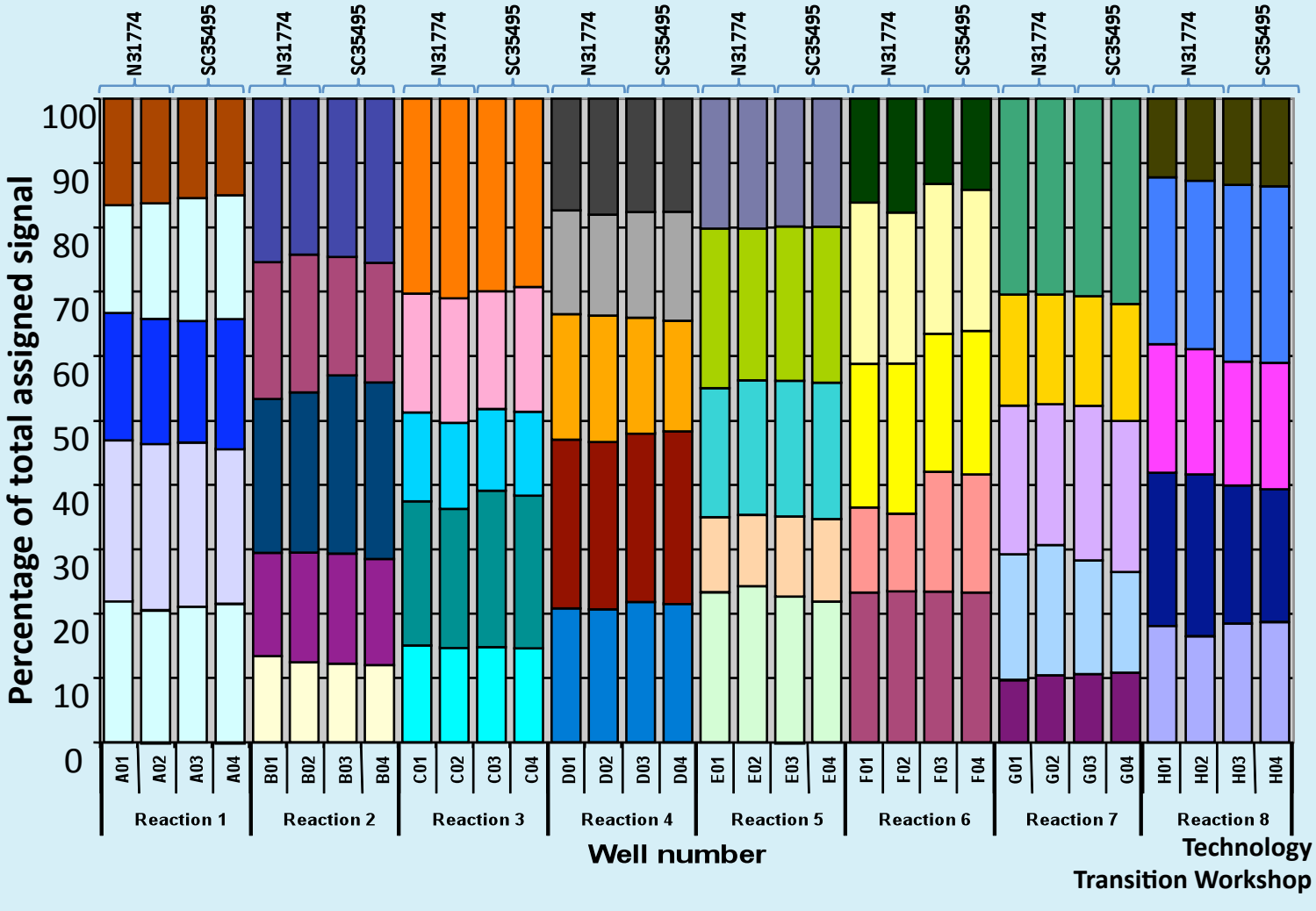
Initial Intra-locus Strand Balance



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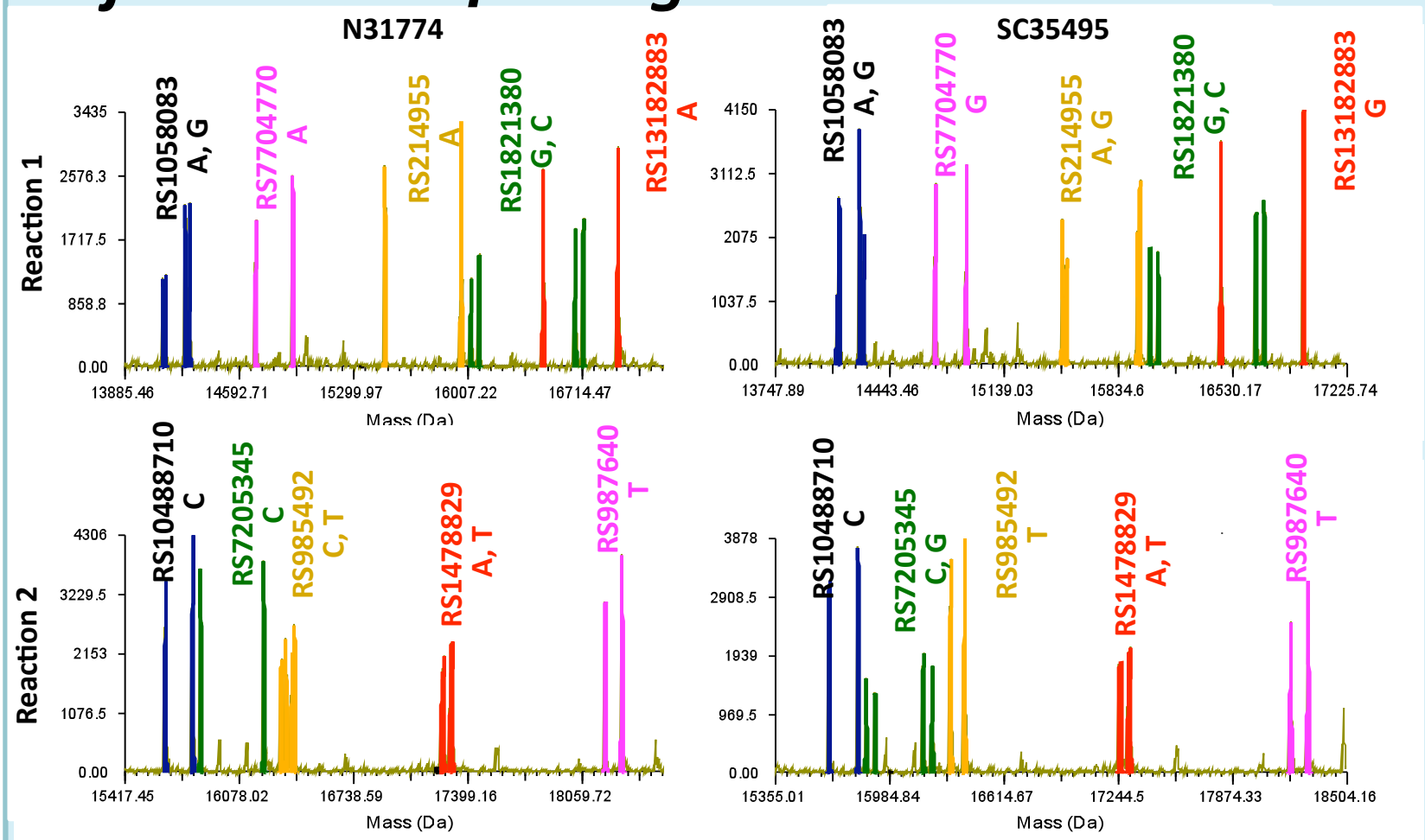
Adjusted Multiplexing Results



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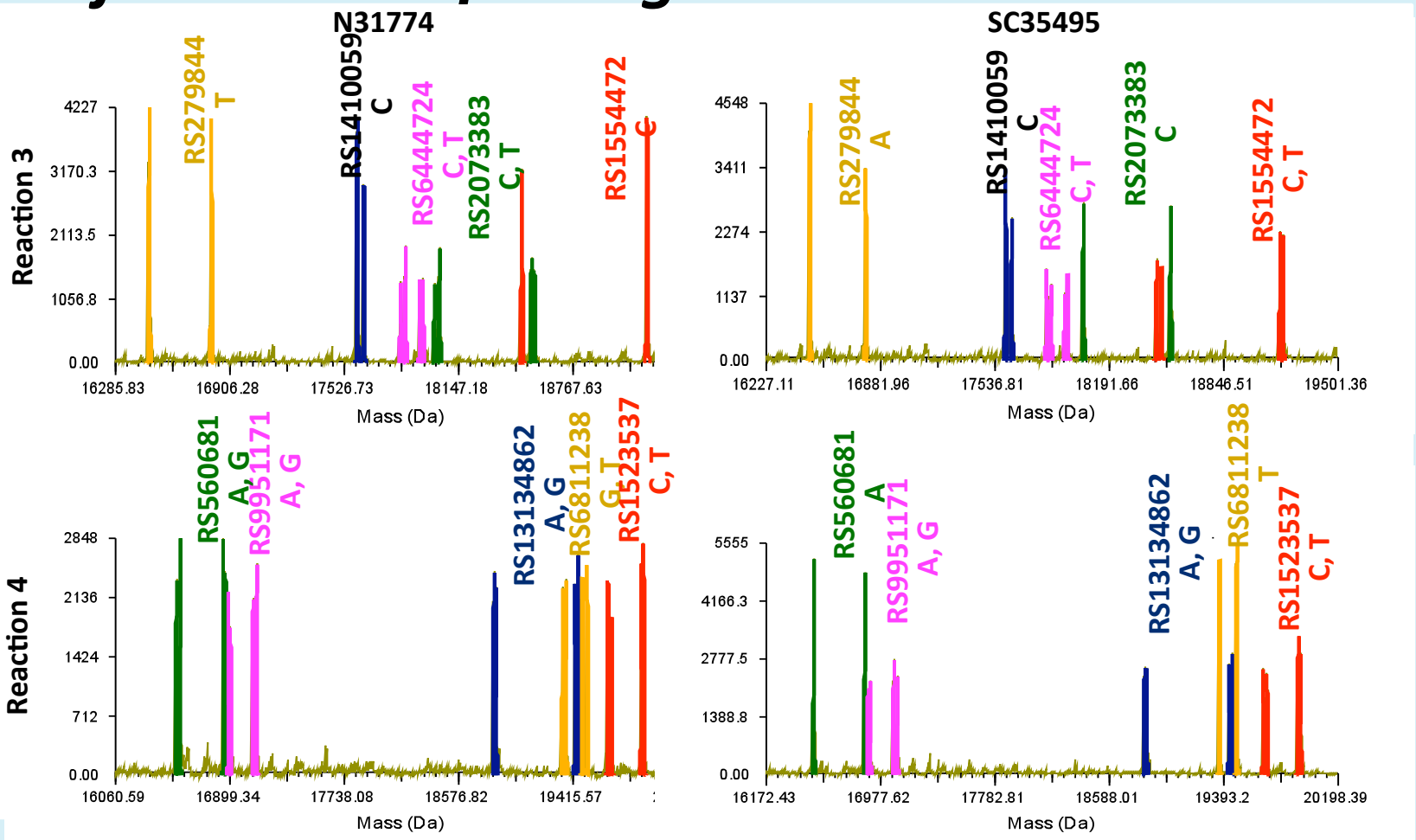
Adjusted Multiplexing Results



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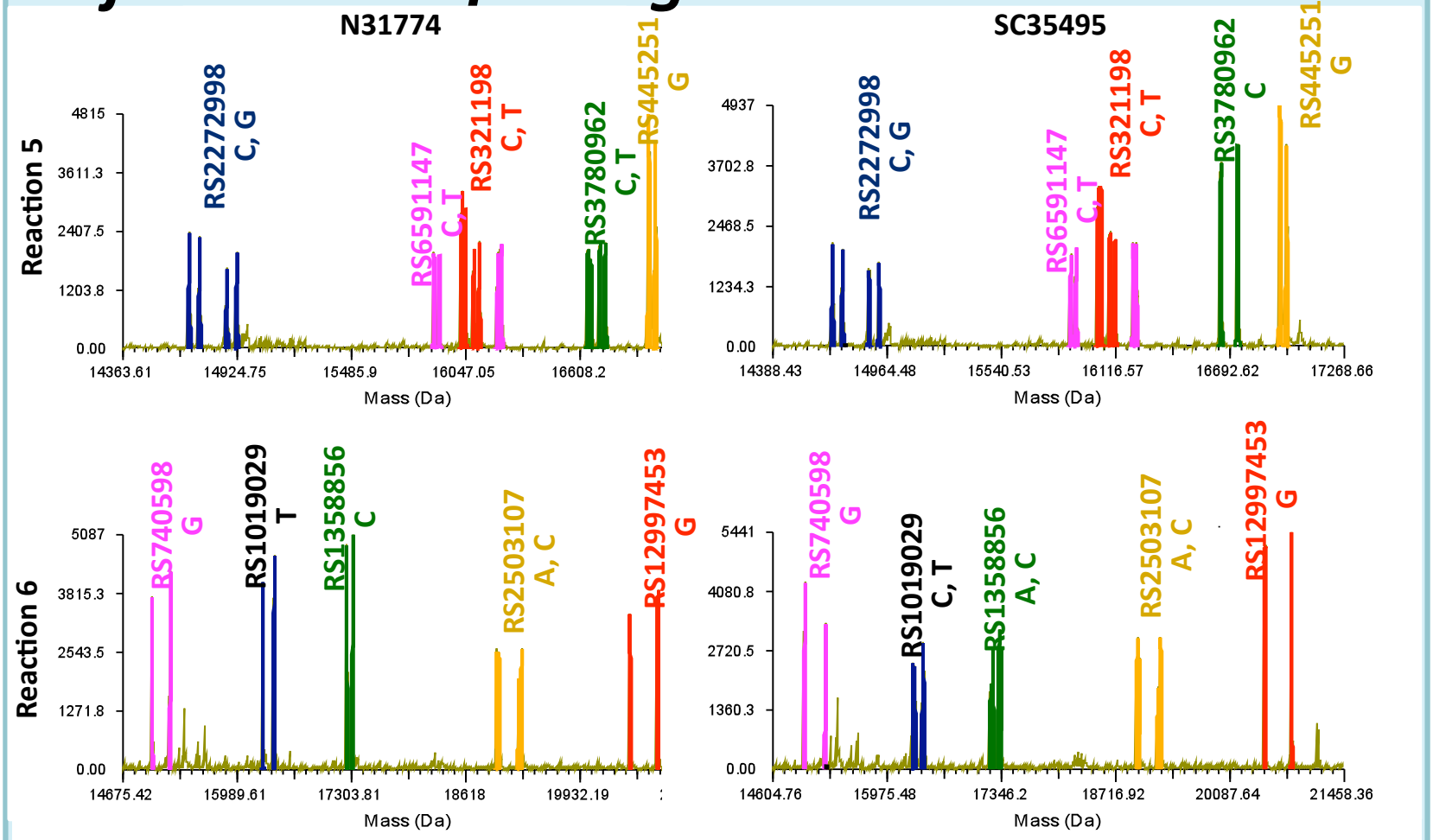
Adjusted Multiplexing Results



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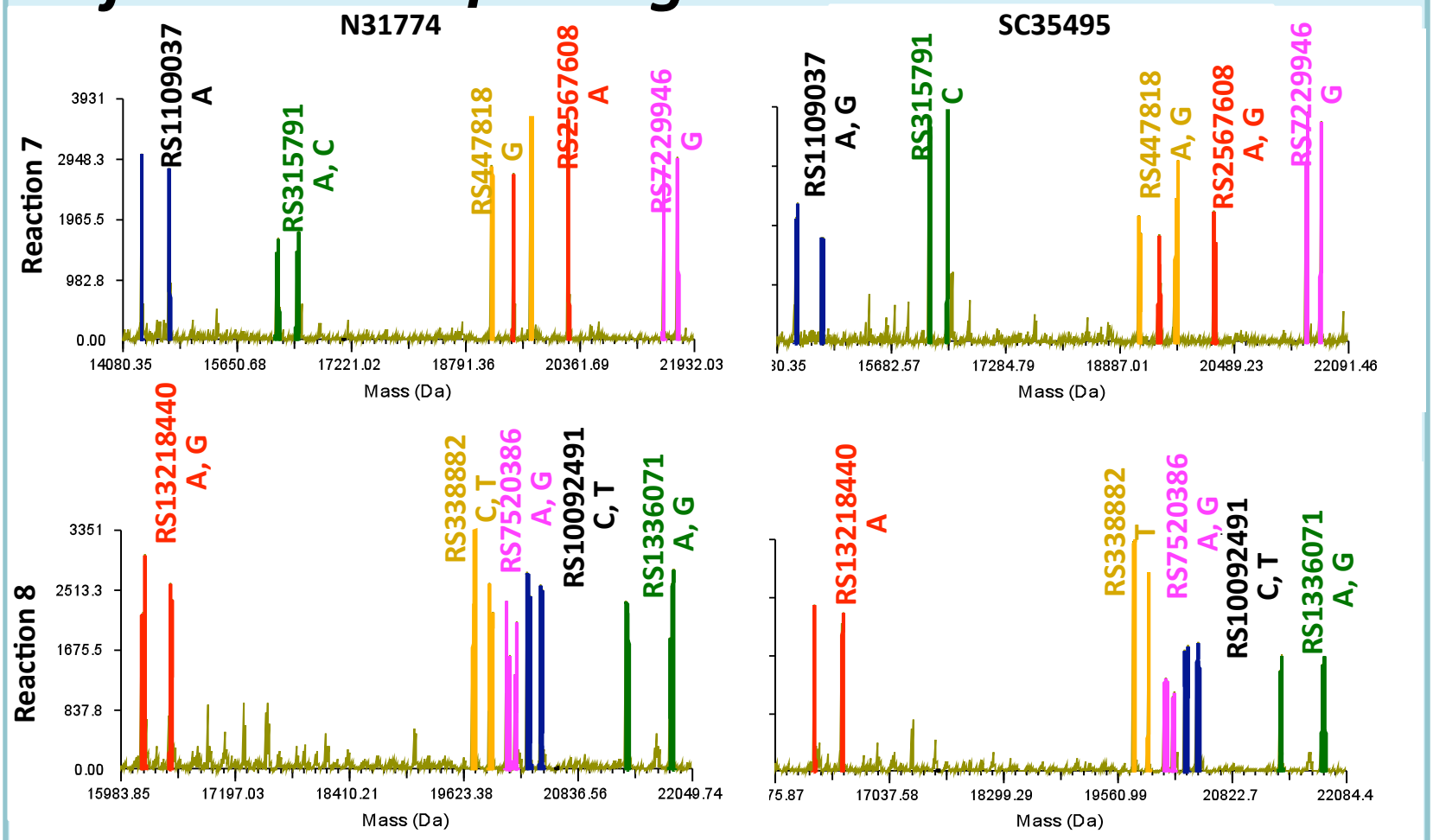
Adjusted Multiplexing Results



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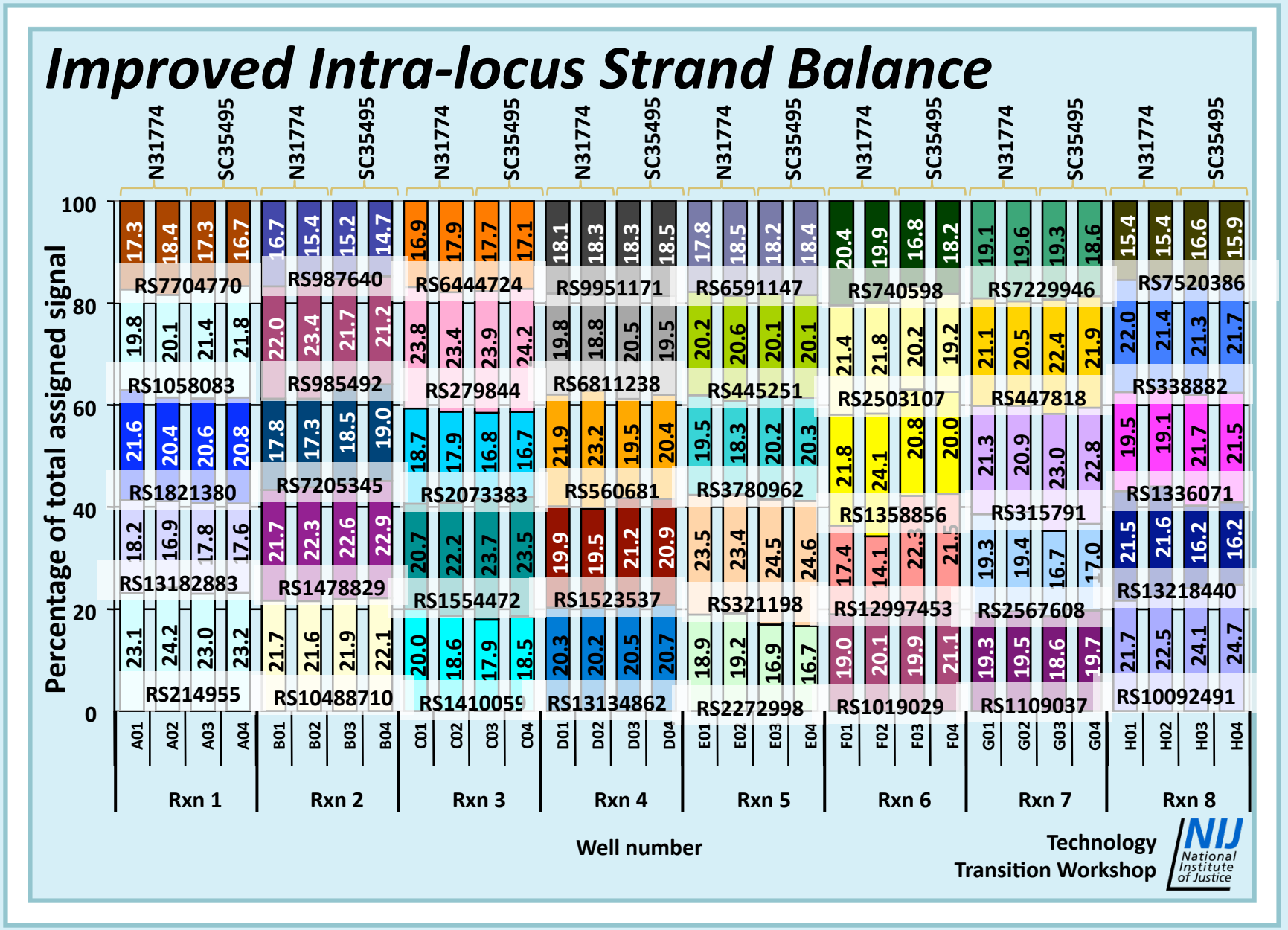
Improved Intra-locus Strand Balance
N31774
SC35495
Percentage of total assigned signal
100
80
60
40
20
0
RS7704770
RS1058083
RS1821380
RS13182883
RS214955
RS987640
RS985492
RS7205345
RS1478829
RS10488710
RS6444724
RS279844
RS2073383
RS1554472
RS1410059
RS9951171
RS6811238
RS560681
RS1523537
RS13134862
RS6591147
RS445251
RS3780962
RS321198
RS2272998
RS740598
RS2503107
RS1358856
RS12997453
RS1019029
RS7229946
RS447818
RS315791
RS2567608
RS1109037
RS7520386
RS338882
RS1336071
RS13218440
RS10092491
Rxn 1
Rxn 2
Rxn 3
Rxn 4
Rxn 5
Rxn 6
Rxn 7
Rxn 8
Well number
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Sensitivity

Full profile obtained at 63 pg/reaction

Reaction quality degraded at 125 pg and below

Locus	2000	1000	500	250	125	63	31	16	8
RS10092491	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	no data
RS1019029	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, ---	C, T
RS10488710	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---
RS1058083	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G
RS1109037	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, ---	A, ---
RS12997453	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---
RS13134862	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, ---	no data
RS13182883	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	A, G	G, ---
RS13218440	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---
RS1336071	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, ---	A, ---
RS1358856	A, C	A, C	A, C	A, C	A, C	A, C	A, C	no data	C, ---
RS1410059	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---
RS1478829	A, T	A, T	A, T	A, T	A, T	A, T	A, ---	T, ---	A, T
RS1523537	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	no data
RS1554472	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	no data
RS1821380	C, G	C, G	C, G	C, G	C, G	C, G	C, G	G, ---	C, G
RS2073383	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---
RS214955	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, ---
RS2272998	C, G	C, G	C, G	C, G	C, G	C, G	C, G	C, G	C, ---
RS2503107	A, C	A, C	A, C	A, C	A, C	A, C	A, C	A, C	C, ---
RS2567608	A, G	A, G	A, G	A, G	A, G	A, G	A, ---	A, G	no data
RS279844	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---
RS315791	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---
RS321198	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	no data
RS338882	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---
RS3780962	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	no data	C, ---
RS445251	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---
RS447818	A, G	A, G	A, G	A, G	A, G	A, G	G, ---	G, ---	A, G
RS560681	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---
RS6444724	C, T	C, T	C, T	C, T	C, T	C, T	C, ---	C, T	no data
RS6591147	C, T	C, T	C, T	C, T	C, T	C, T	C, ---	C, T	C, T
RS6811238	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---
RS7205345	C, G	C, G	C, G	C, G	C, G	C, G	C, G	C, G	C, G
RS7229946	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---
RS740598	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	no data
RS7520386	A, G	A, G	A, G	A, G	A, G	A, G	A, ---	A, G	A, G
RS7704770	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---
RS985492	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---
RS987640	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---
RS9951171	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, ---	A, G

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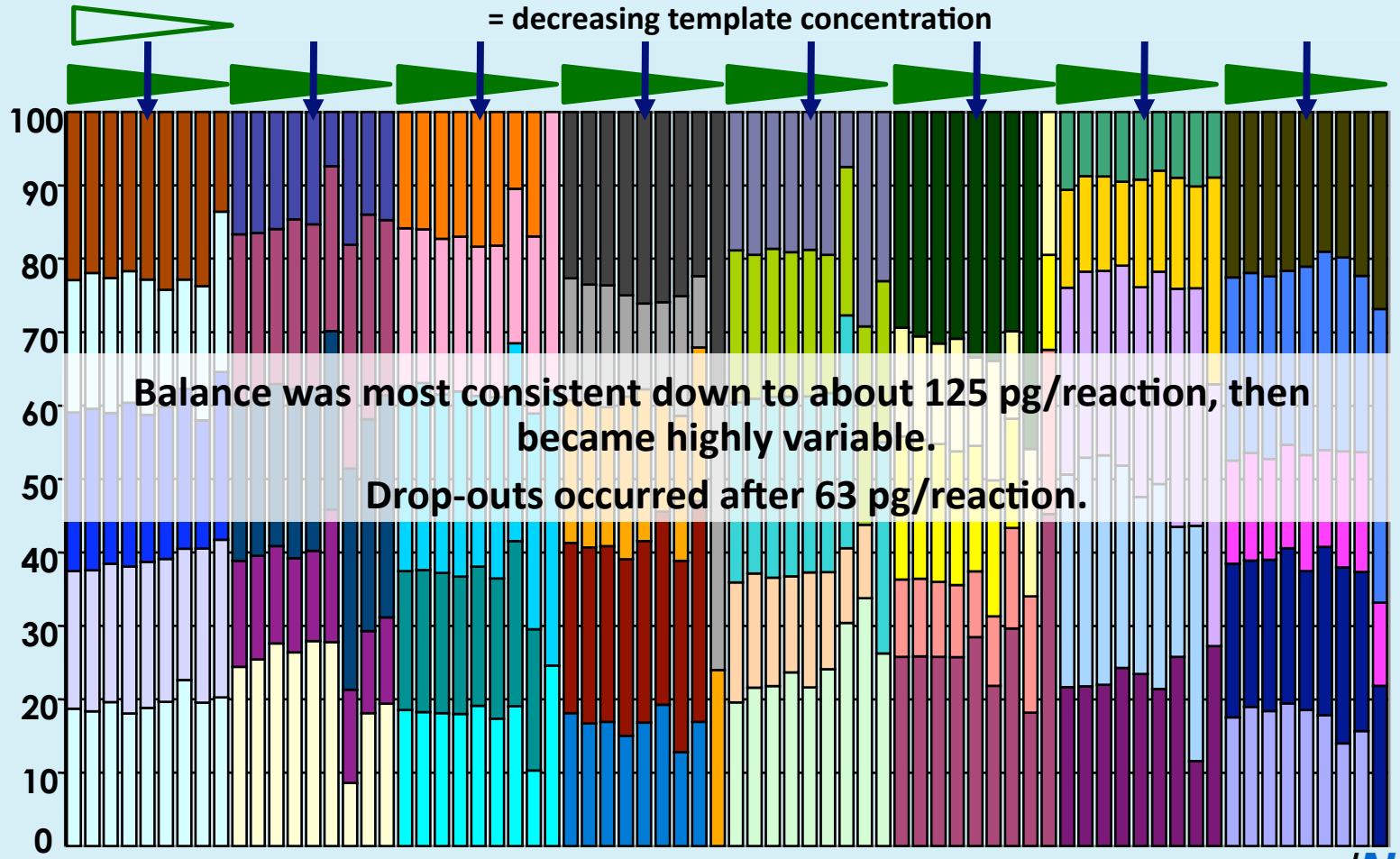
Inter-Locus 5-plex Balance in Dilutions



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Species Specificity

- **Human blood-derived DNA sample was tested in duplicate in the presence of 10-fold excess of exogenous DNA**
- **1 ng of human DNA per reaction**
- **10 ng exogenous DNA**
  - **Dog (male American Eskimo – buccal swab)**
  - **Cat (male long-hair, buccal swab)**
  - **Candida albicans (yeast)**
  - **Aspergillus oryzae (environmental filamentous fungus)**
  - **Escherichia coli (gram negative bacterium)**
  - **Staphylococcus aureus (gram positive bacterium)**
- **All tests with exogenous DNA gave a full profile**

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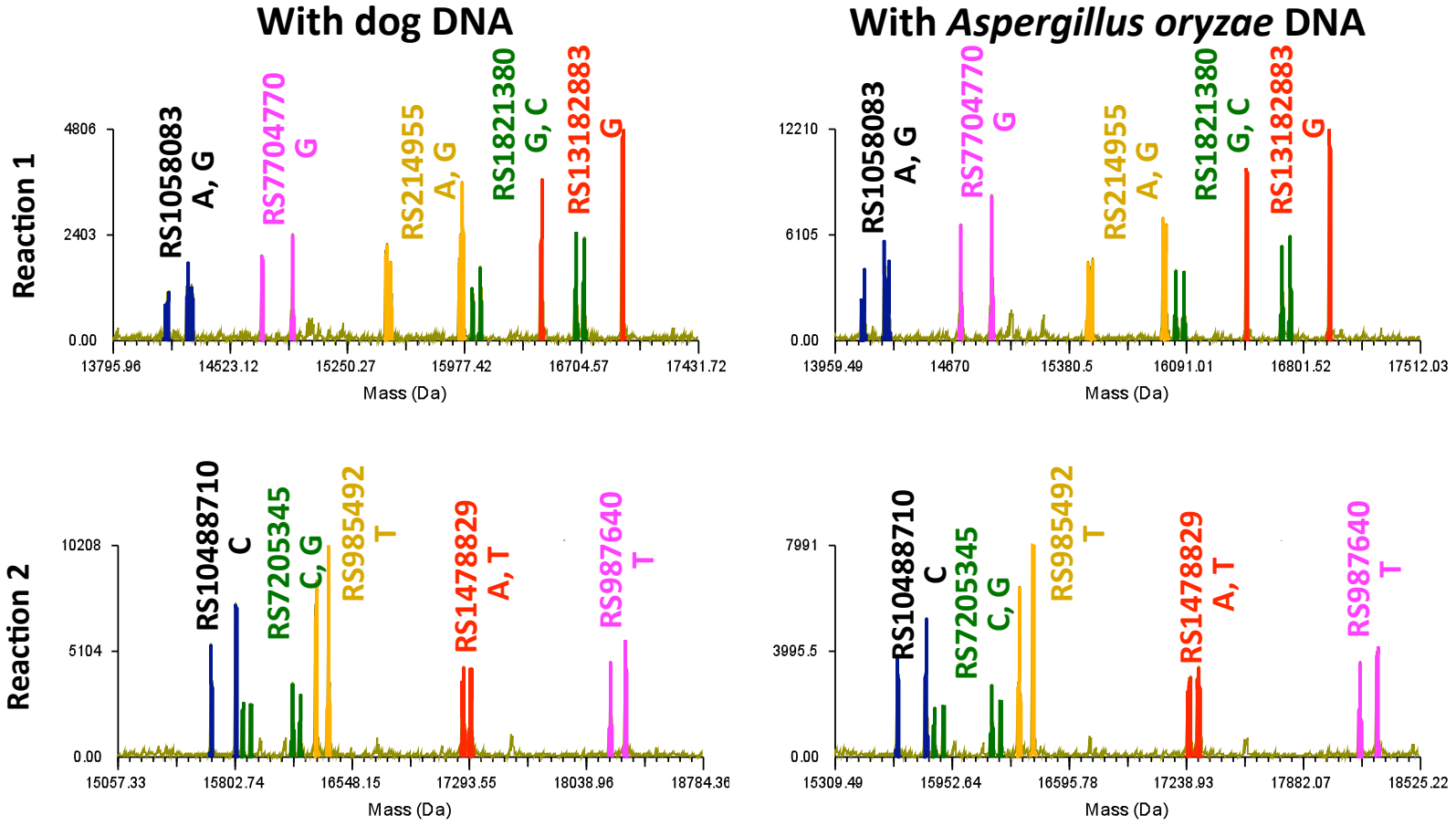
Species Specificity



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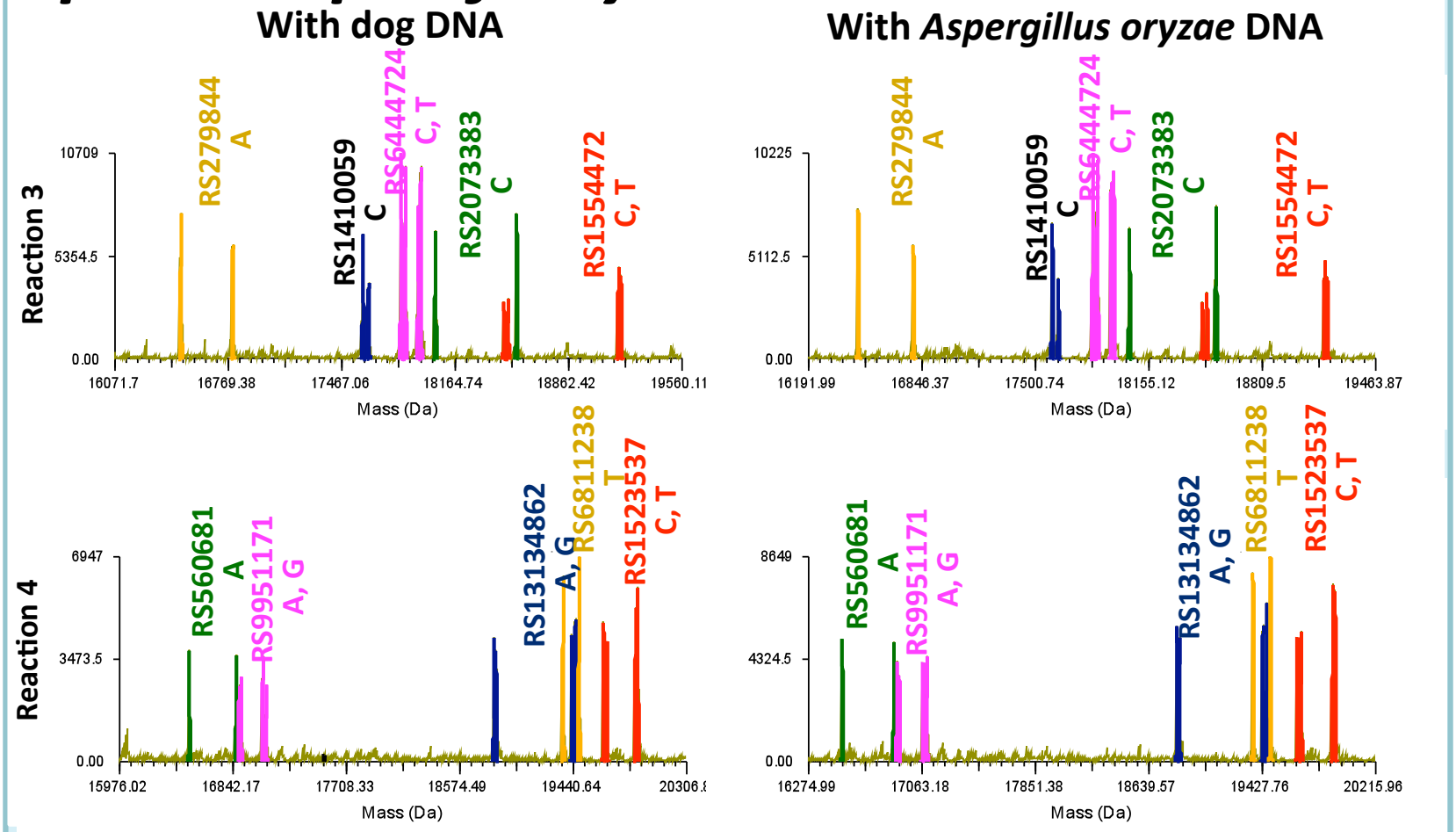
Species Specificity



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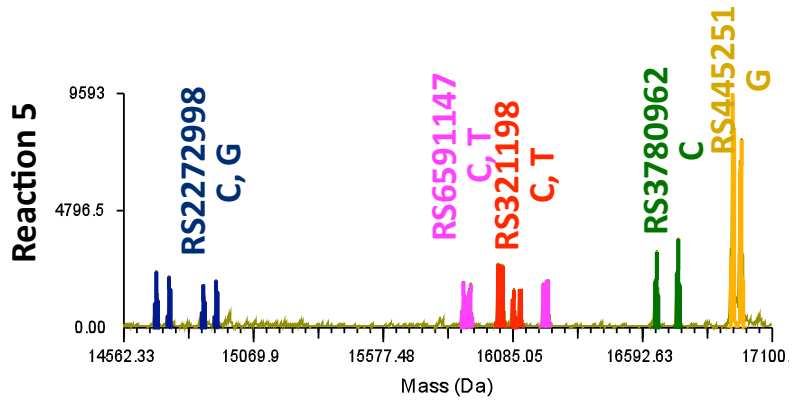
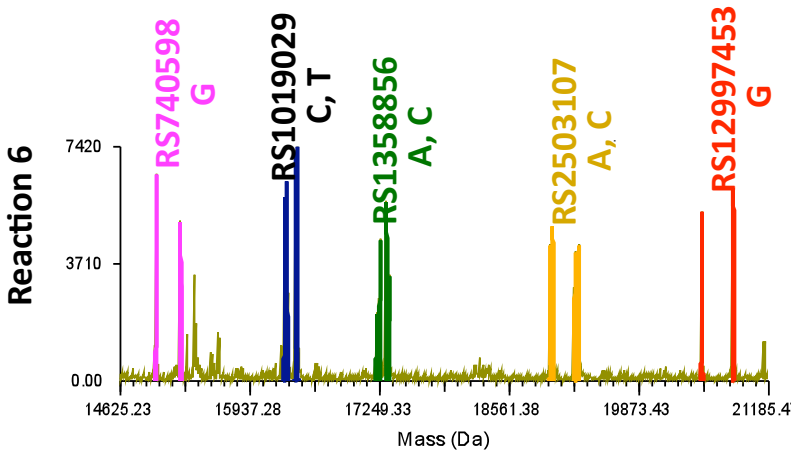
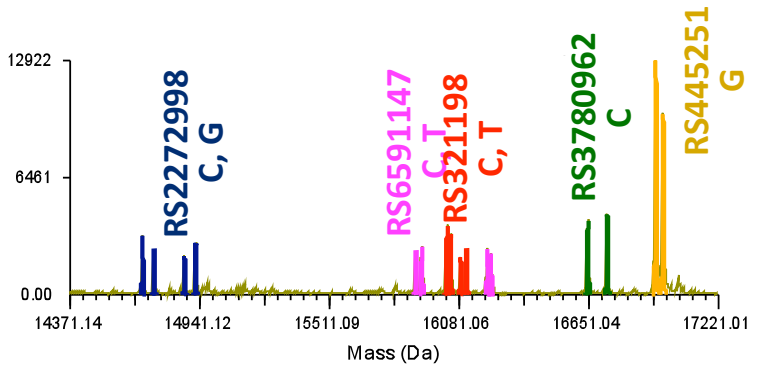
Species Specificity

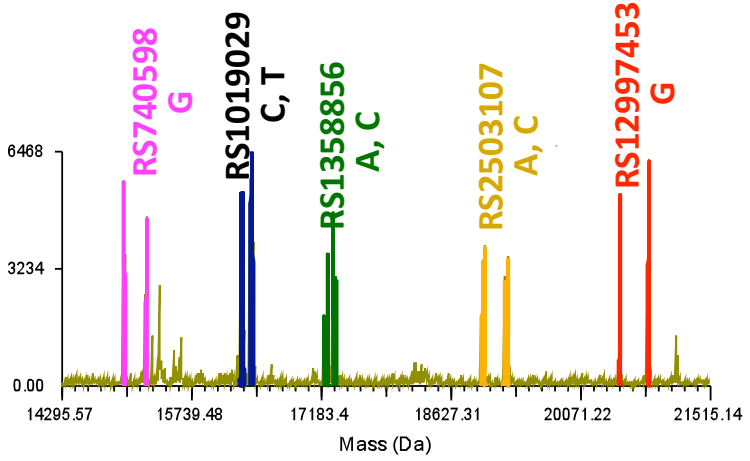
With dog DNA





With *Aspergillus oryzae* DNA





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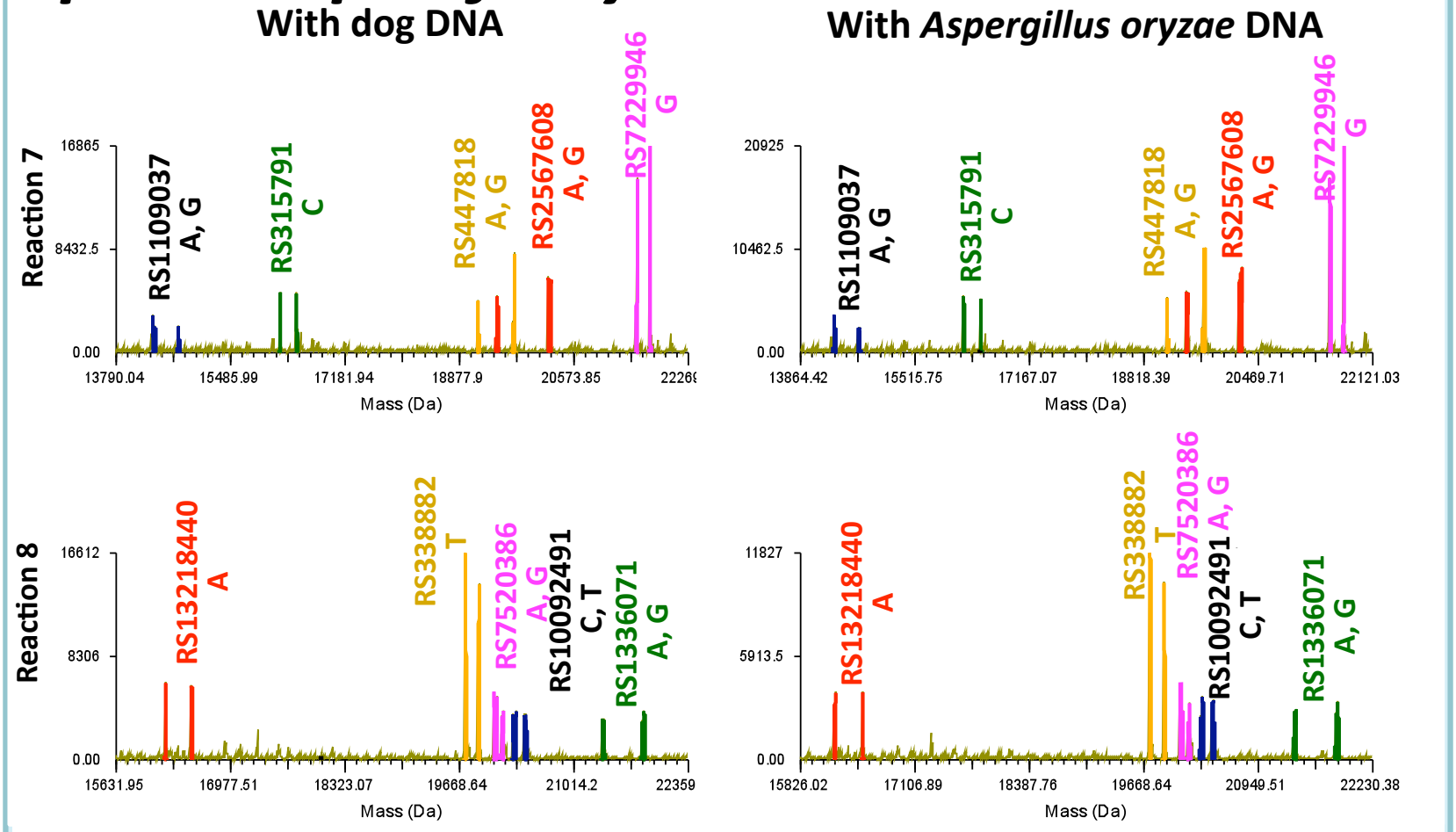
Species Specificity



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Species Specificity

10-fold excess of bacterial DNA

Pos

Pos

E. coli

E. coli

E. Coli + human

E. Coli + human

S. aureus

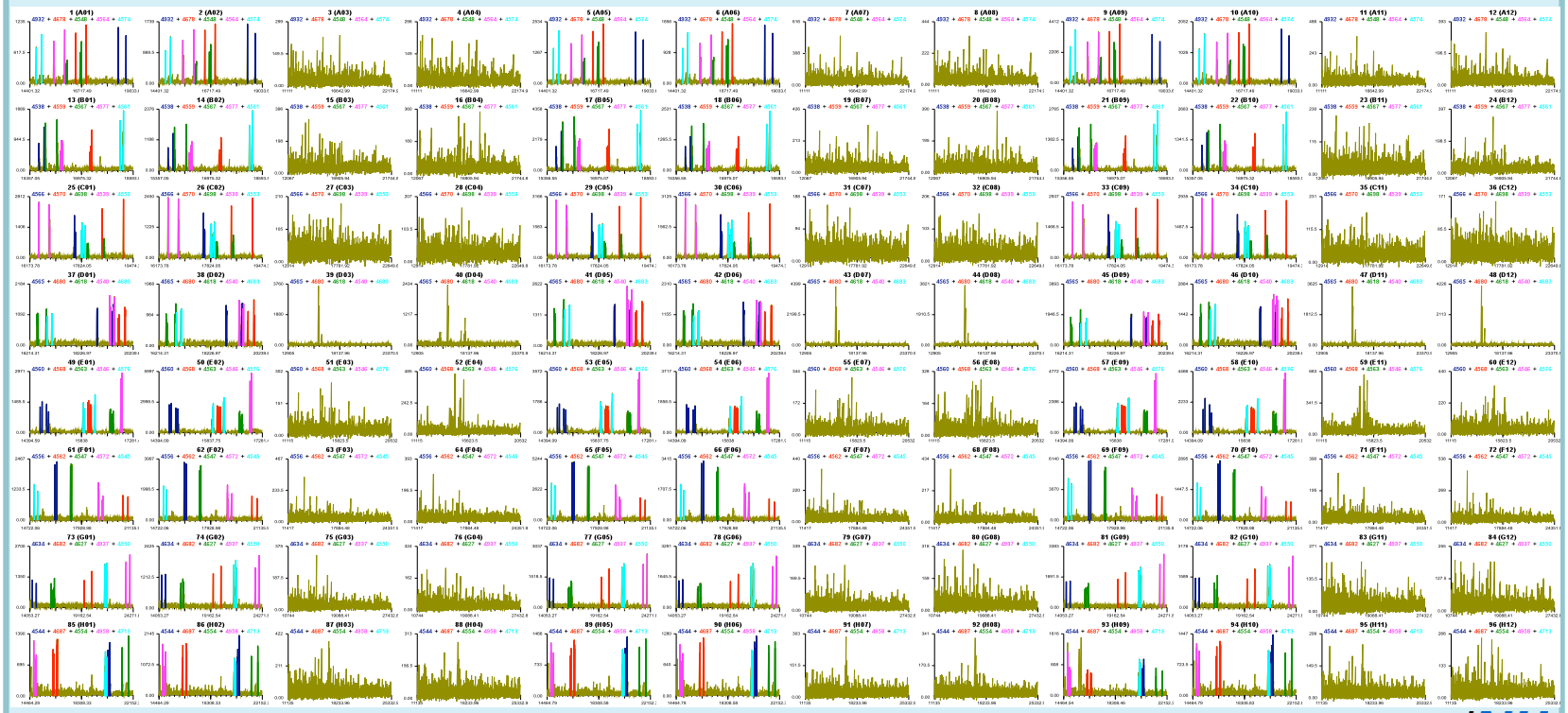
S. aureus

S. aureus + human

S. aureus + human

neg

neg



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Species Specificity

Full profiles were obtained for all replicates in the presence of 10-fold excess of exogenous DNA from six different sources

Locus	Dog	Cat	*C. alb*	*A. ory*	*E. coli*	*S. aur*	Dog	Cat	*C. alb*	*A. ory*	*E. coli*	*S. aur*
RS10092491	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T
RS1019029	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T
RS10488710	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---
RS1058083	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G
RS1109037	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G
RS12997453	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---
RS13134862	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G
RS13182883	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---
RS13218440	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---
RS1336071	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G
RS1358856	A, C	A, C	A, C	A, C	A, C	A, C	A, C	A, C	A, C	A, C	A, C	A, C
RS1410059	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---
RS1478829	A, T	A, T	A, T	A, T	A, T	A, T	A, T	A, T	A, T	A, T	A, T	A, T
RS1523537	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T
RS1554472	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T
RS1821380	C, G	C, G	C, G	C, G	C, G	C, G	C, G	C, G	C, G	C, G	C, G	C, G
RS2073383	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---
RS214955	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G
RS2272998	C, G	C, G	C, G	C, G	C, G	C, G	C, G	C, G	C, G	C, G	C, G	C, G
RS2503107	A, C	A, C	A, C	A, C	A, C	A, C	A, C	A, C	A, C	A, C	A, C	A, C
RS2567608	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G
RS279844	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---
RS315791	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---
RS321198	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T
RS338882	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---
RS3780962	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---	C, ---
RS445251	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---
RS447818	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G
RS560681	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---
RS6444724	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T
RS6591147	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T	C, T
RS6811238	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---
RS7205345	C, G	C, G	C, G	C, G	C, G	C, G	C, G	C, G	C, G	C, G	C, G	C, G
RS7229946	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---
RS740598	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---
RS7520386	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G
RS7704770	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---
RS985492	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---
RS987640	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---
RS9951171	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G

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Small Test Panel: 96 Individuals

- 10 buccal swab DNAs
- 38 blood samples obtained commercially
  - 20 samples tested in parallel with 40 individual TaqMan® assays
- 50 blinded DNA samples from UNTHSC (John Planz)
  - Tested at UNTHSC with AB GenPlex™ Kidd-40 panel
- Buccal swabs run at a set dilution factor (corresponded to between 300 pg and 1.8 ng per reaction)
- Blood samples were run at 500 pg/reaction
- Samples were run in duplicate
- Each sample gave a consistent profile between duplicates
- 100% concordance between TaqMan® and Ibis™ assays
- Concordance between Ibis™ and GenPlex™ for all loci except one
  - rs2073383 was 100% concordant between Ibis™ and TaqMan®
  - rs2073383 disconcordant between GenPlex™ and TaqMan®

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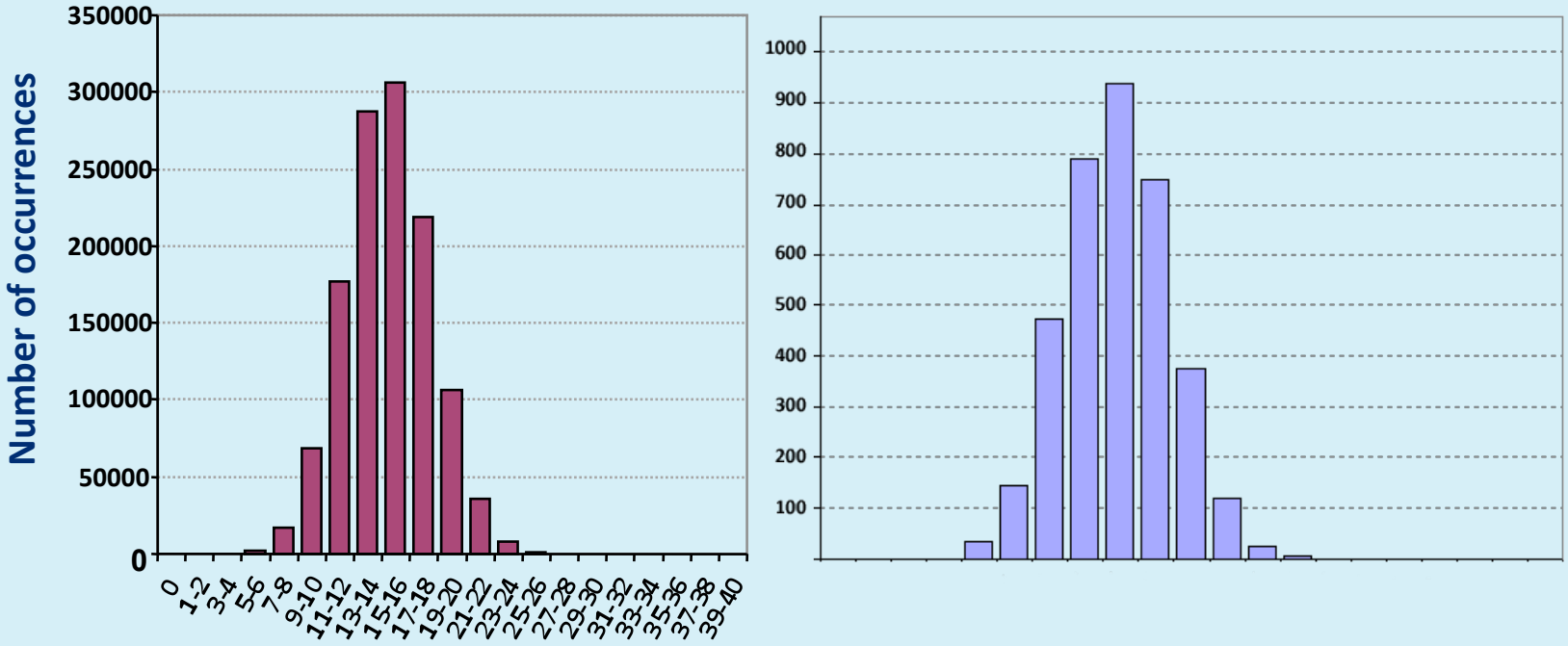
Comparison of 86 Samples Run with Ibis™ Kit to Distribution from Kidd Paper

Pair-wise comparisons between 86 individuals – 3655 comparisons



Number of matching loci between 2 individuals

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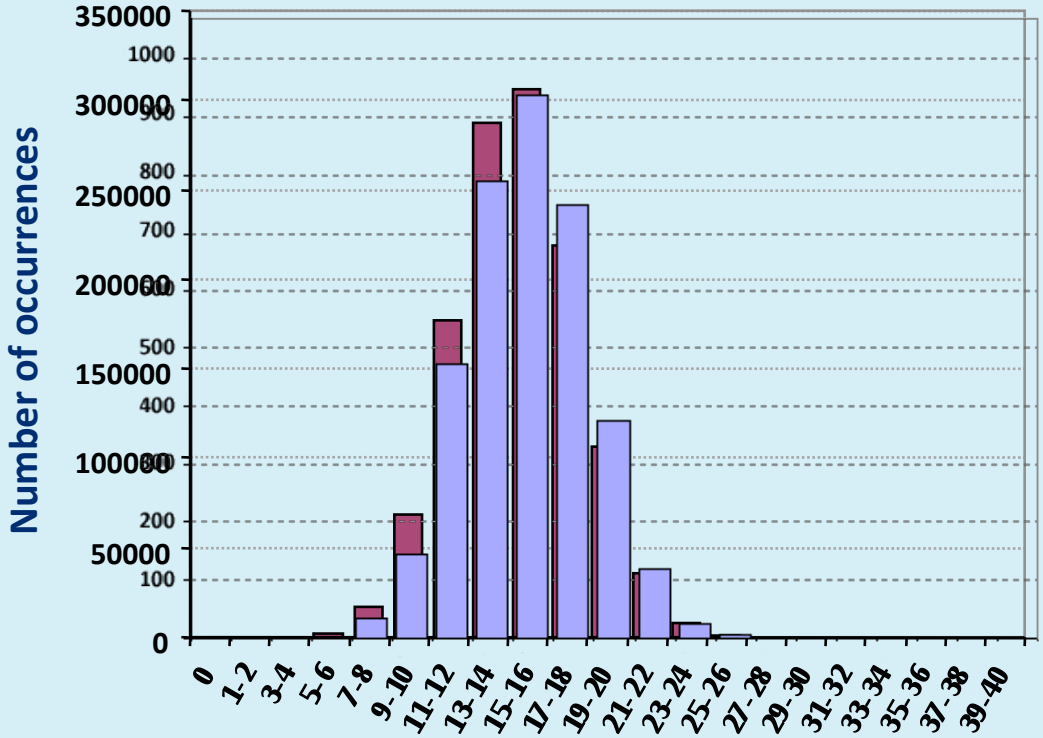
Comparison of 86 Samples Run with Ibis™ Kit to Distribution from Kidd Paper

Pair-wise comparisons between 86 individuals – 3655 comparisons



Number of matching loci between 2 individuals

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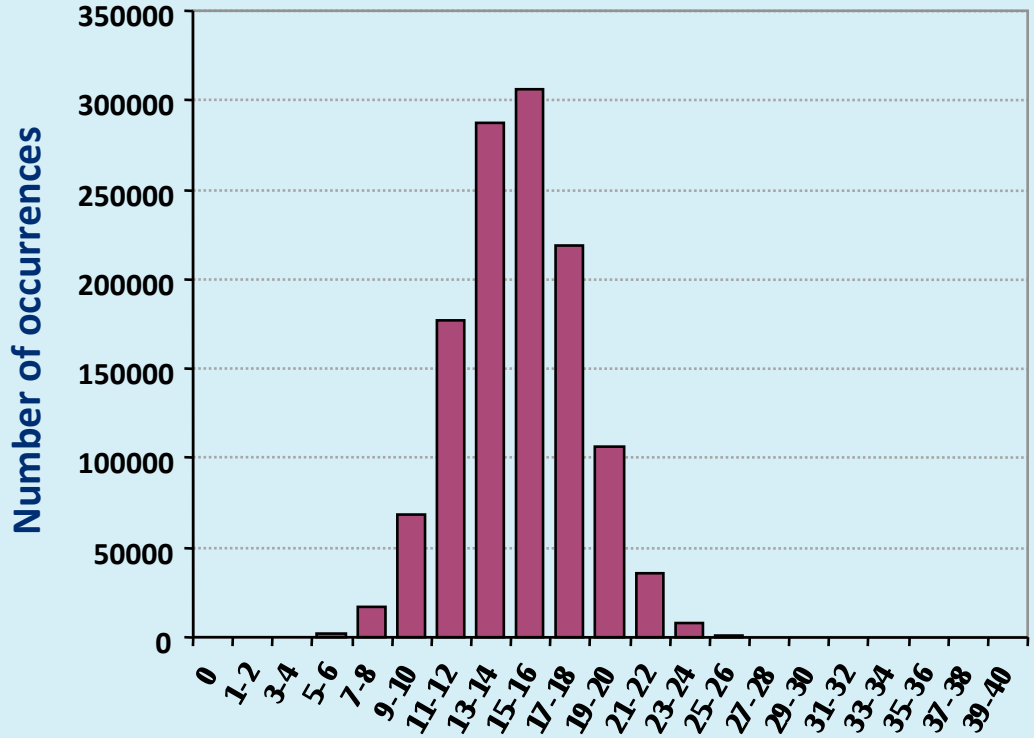
Pair-wise comparisons between 86 individuals – 3655 comparisons



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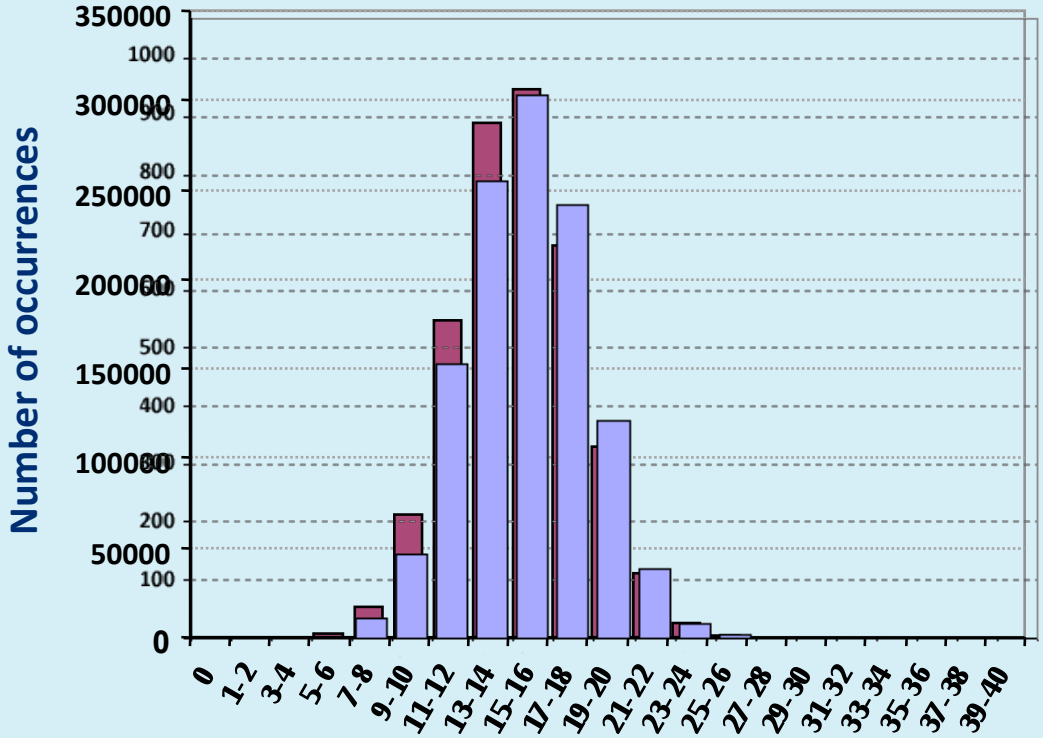
Comparison of 86 Samples Run with Ibis™ Kit to Distribution from Kidd Paper

Pair-wise comparisons between 86 individuals – 3655 comparisons



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Summary

- **Basic 40-Kidd-SNP assay defined and moving into validation**
- **Limit of sensitivity down to 63 – 125 pg/reaction**
- **Addition of mammal, fungal or bacterial DNA does not appear to interfere with the assay**
- **100% concordance with AB TaqMan® assays for all loci**
- **Small panel of samples showed average locus differences comparable to Kidd results**
- **Integrated software developed and in process**

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Questions?

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***Note**:*
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