



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

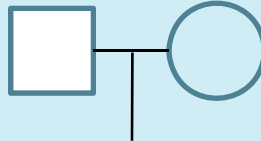
Overview of the Ibis™ SNP Assay

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**

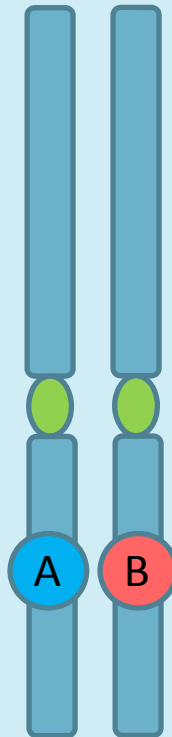
Ideal Bi-allelic Markers

One from
father

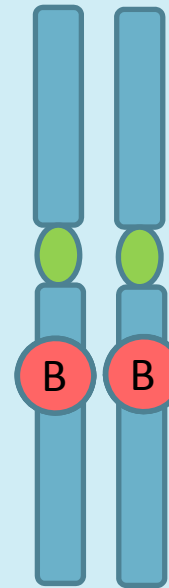


One from
mother

Each autosomal
chromosome comes
as a pair



Bi-allelic markers
have one of two
states



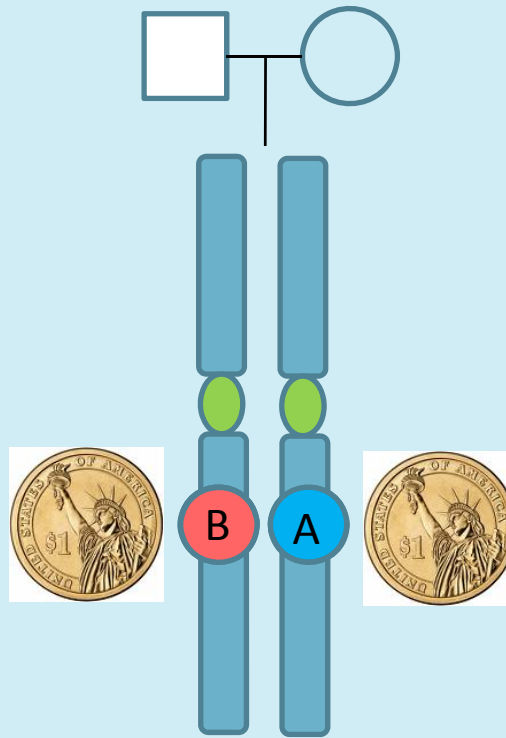
Any marker
for any individual
can be both 'A'
or both 'B'
or one of each

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Ideal Bi-allelic Markers

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

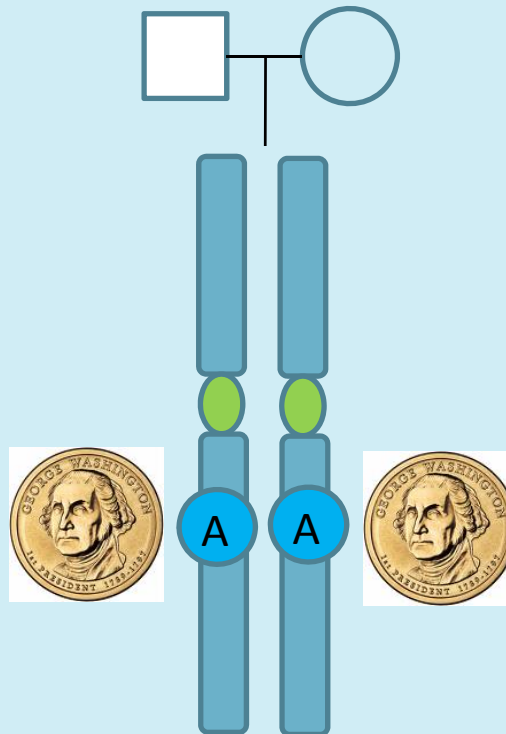


And a 50% chance of getting either allele on the mother's chromosome

Each marker is like a coin toss

Ideal Bi-allelic Markers

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



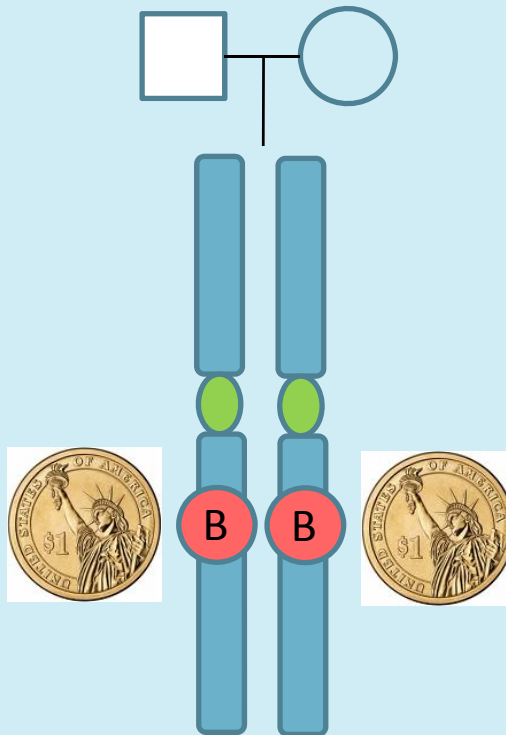
And a 50% chance of getting either allele on the mother's chromosome

Each marker is like a coin toss

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

Ideal Bi-allelic Markers

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

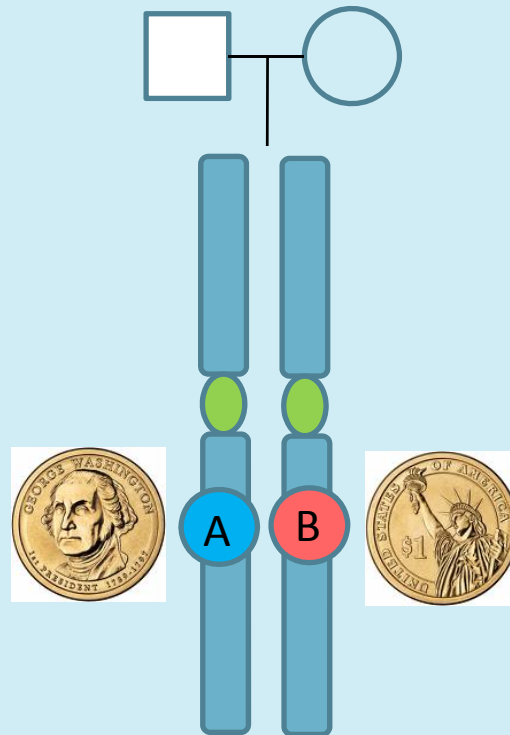


**And a 50% chance of
getting either allele
on the mother's
chromosome**

**Each marker is like a
coin toss**

And $50\% \times 50\% = 25\%$ chance of getting two 'tails' (B allele)

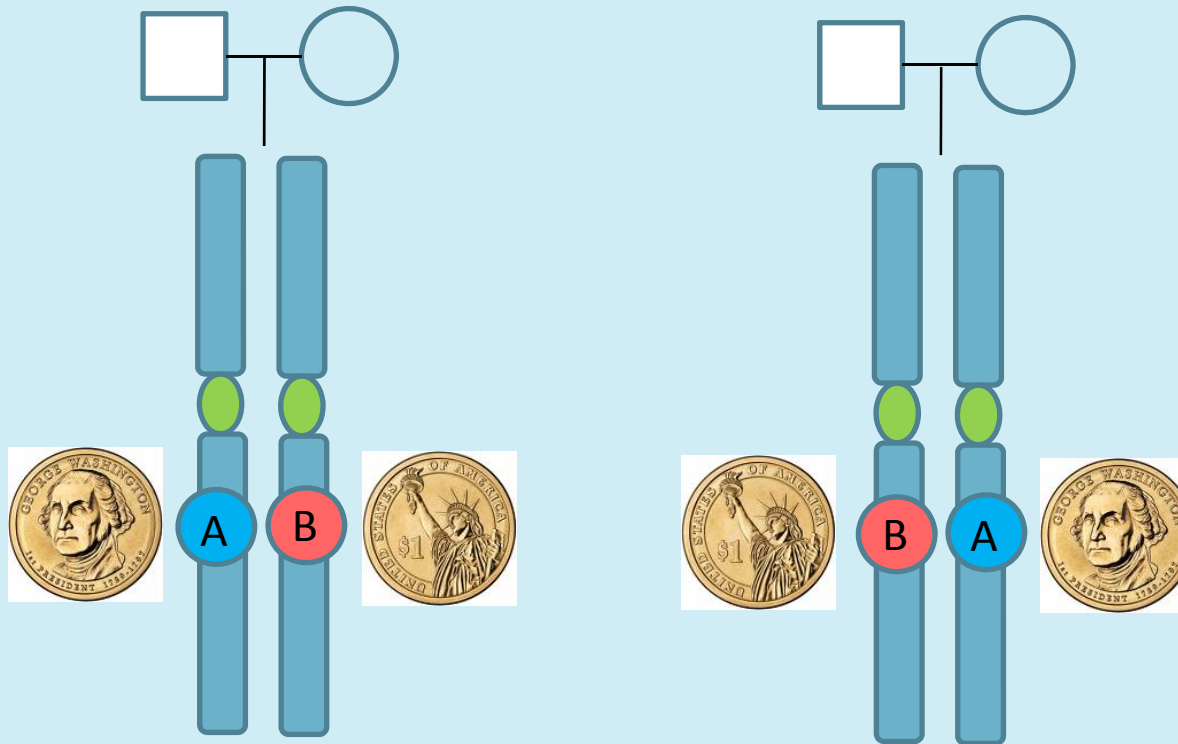
Ideal Bi-allelic Markers



Each marker is like a
coin toss

And $50\% \times 50\% = 25\%$ chance of getting A + B

Ideal Bi-allelic Markers



Each marker is like a
coin toss

And $50\% \times 50\% = 25\%$ chance of getting A + B

Plus $50\% \times 50\% = 25\%$ chance of getting B + A

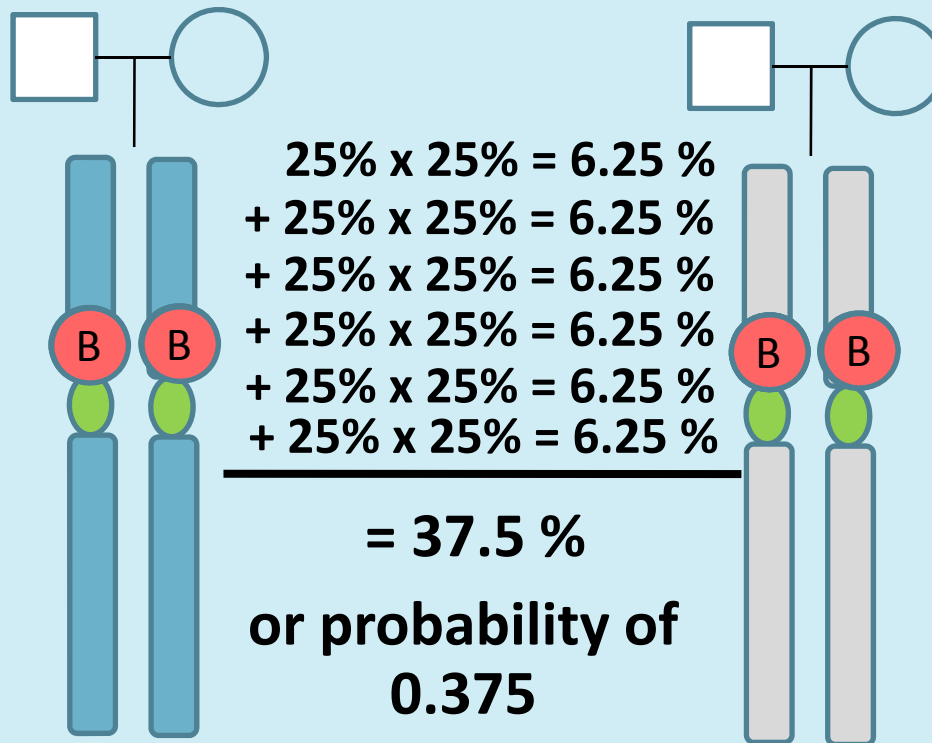
= 50% chance of being heterozygous

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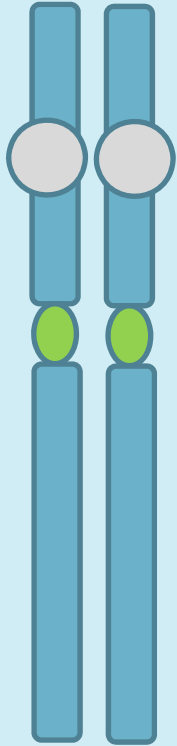


Ideal Bi-allelic Markers

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

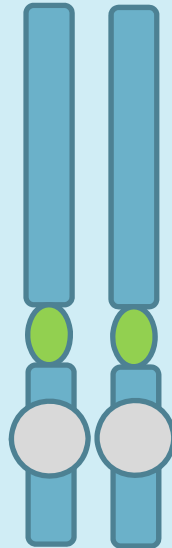


Ideal Bi-allelic Markers



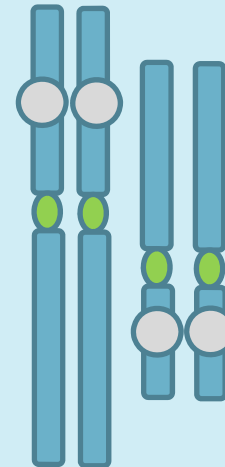
Random match
probability for
one marker
0.375

X



Random match
probability for
another marker
0.375

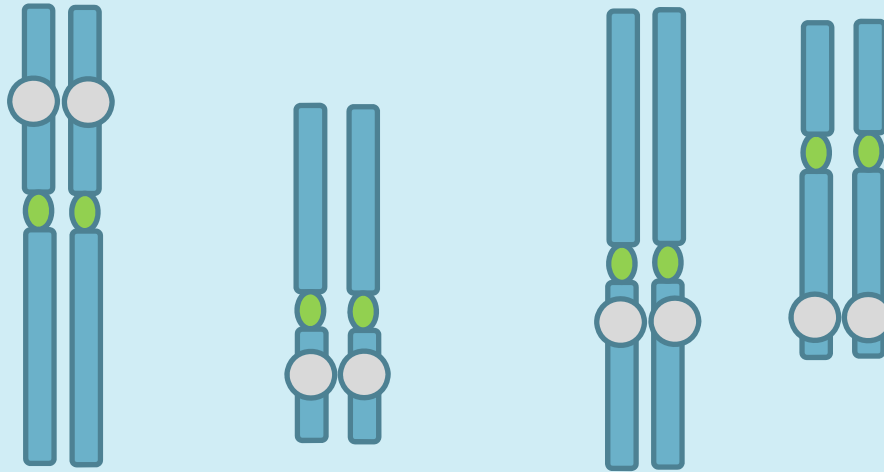
=



Random match probability
for two-marker profile
0.141

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Ideal Bi-allelic Markers



Random match probability for four-marker profile

$$= 0.375 \times 0.375 \times 0.375 \times 0.375$$

$$= 0.375^4$$

$$= 0.0198$$

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

Ideal Bi-allelic Markers

With 40 unlinked and perfectly-distributed SNPs, the random match probability would ideally be 0.37540, or **9.15 x 10⁻¹⁸**.

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.

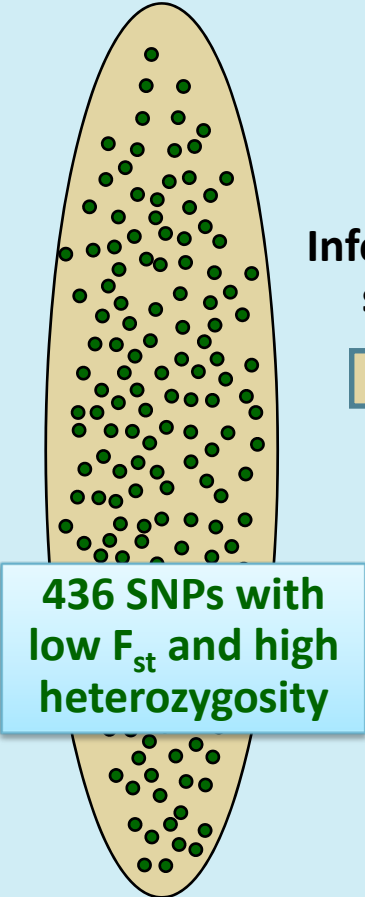
The Kidd Approach

90,483 SNPs
from ABI
catalog

Frequency data
for European,
African and
Chinese/Japanese

The Kidd Approach

90,483 SNPs
from ABI
catalog



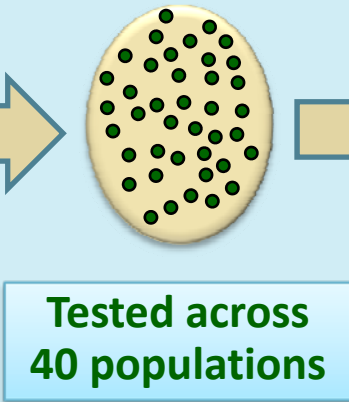
Informatically
selected

436 SNPs

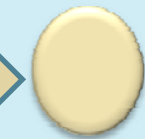


Tested in
TaqMan® SNP
assays across 7
populations

73 SNPs



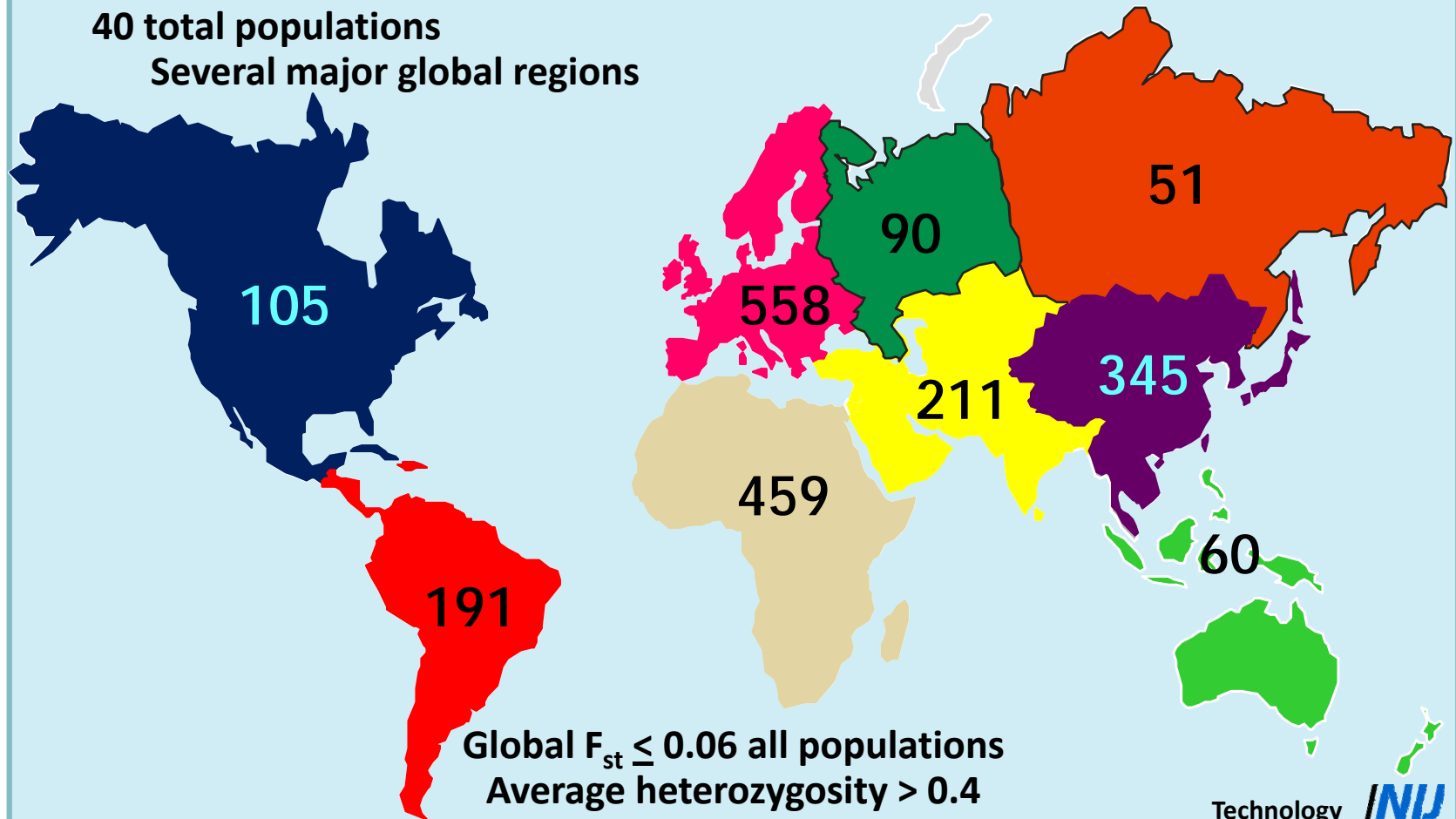
40 SNPs



Final
reduced
panel

Kidd Population Coverage

2070 total individuals
40 total populations
Several major global regions



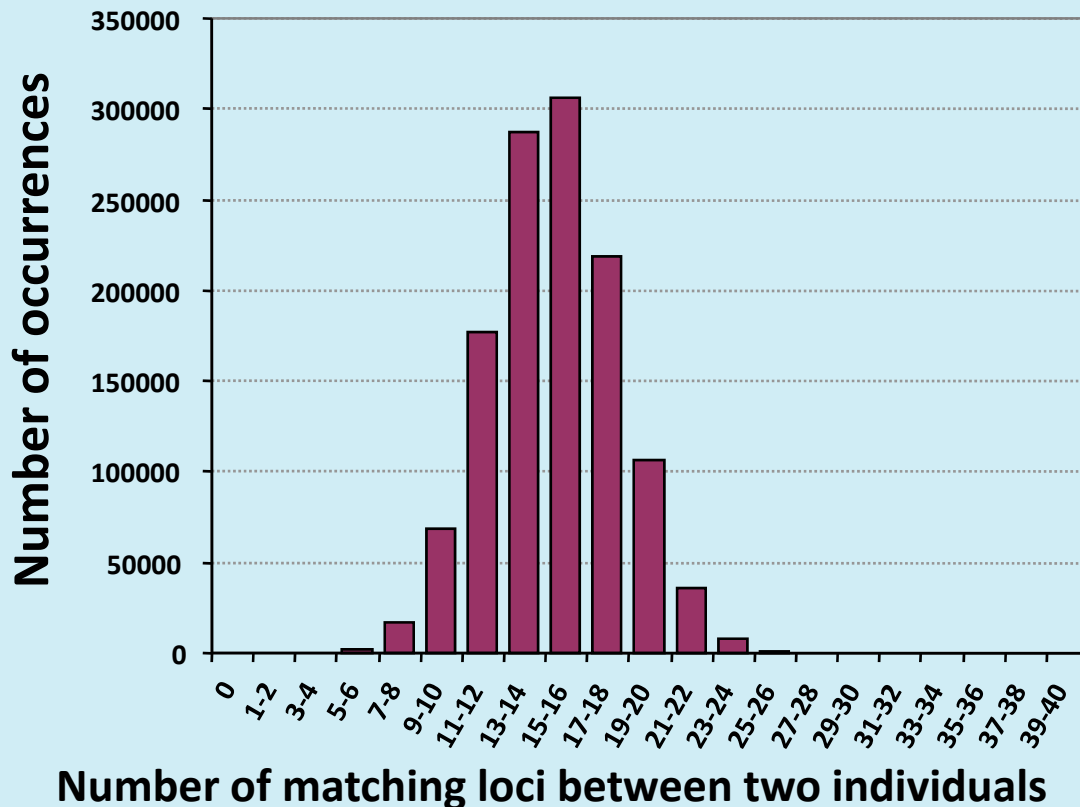
Global $F_{st} \leq 0.06$ all populations
Average heterozygosity > 0.4
Median LD = 0.010 (ave = 0.029)

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Resolving Power of Kidd SNP Panel

- ‘Perfect’ 40-SNP panel: random match with probability of 9.15×10^{-18}
- Kidd-40 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
- 2,457,056 pairwise comparisons
- Most people will differ at 15 – 16 loci

Why Use Mass Spectrometry?

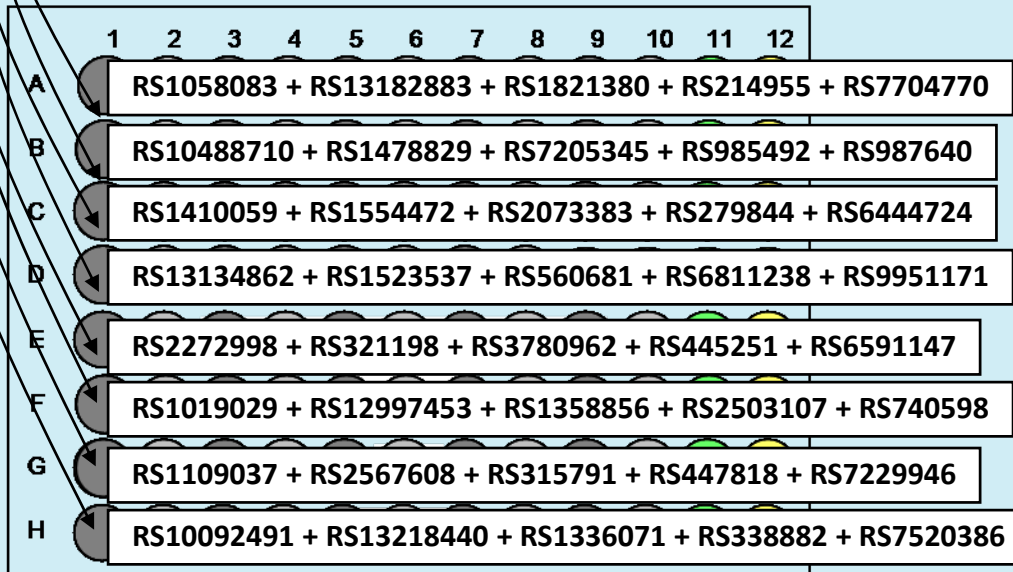
- **Unified platform for major DNA forensics applications**
 - **Mitochondrial DNA profiling**
 - **STR analysis**
 - **Autosomal SNP analysis**
 - **SNPs, STRs and / or mtDNA could be analyzed automatically on one instrument in the same run**
- **High degree of accuracy**
 - **Potential rare variant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

Initial Development: Eight 5-plex Reactions

	1	2	3	4	5	6	7	8	9	10	11	12
A		WT57318	WT51362	WA29594	JT51471	OT05897	PT84223	PT84232	GT37778	GT37900	TT51422	ZT80786
B	UT57300	WT51342	WT51373	WA29612	JT51499	OT05898	PT84224	PT84234	GT37812	GT37913	TT51435	ZT80815
C	UT57307	WT51343	WT51378	ZT81387	OT05888	OT05899	PT84225	PT84236	GT37828	JT52076	TT51483	ZT80826
D	UT57312	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

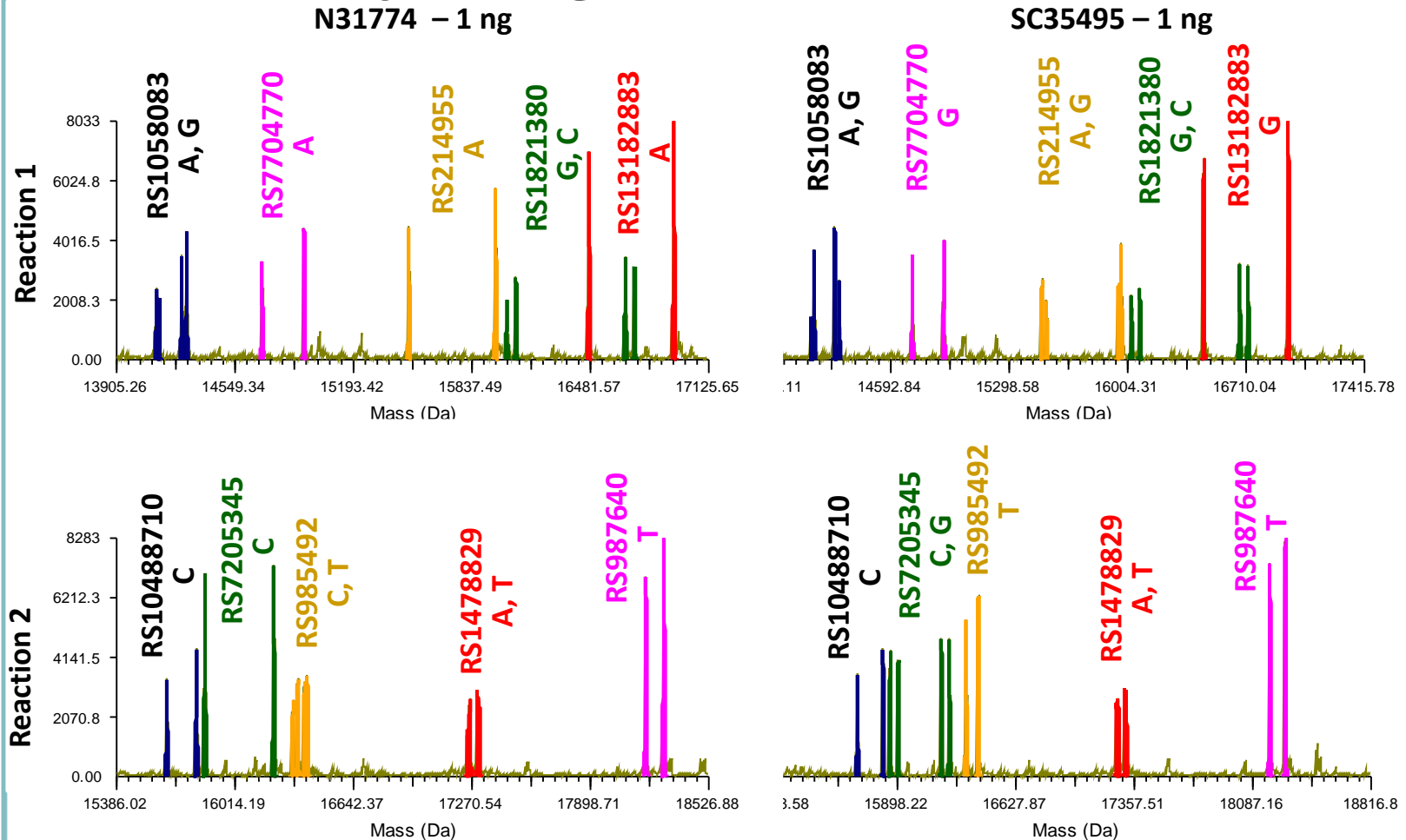
Add 5 ml template to each well of a plate and thermocycle

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



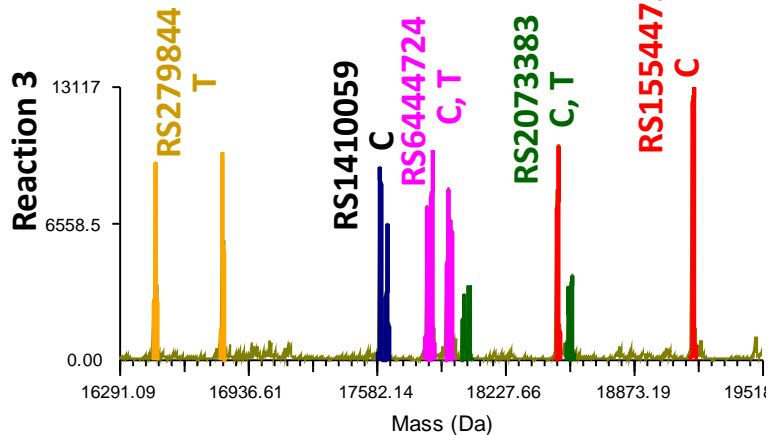
Each sample is distributed across one column of an assay plate

Initial Multiplexing Results

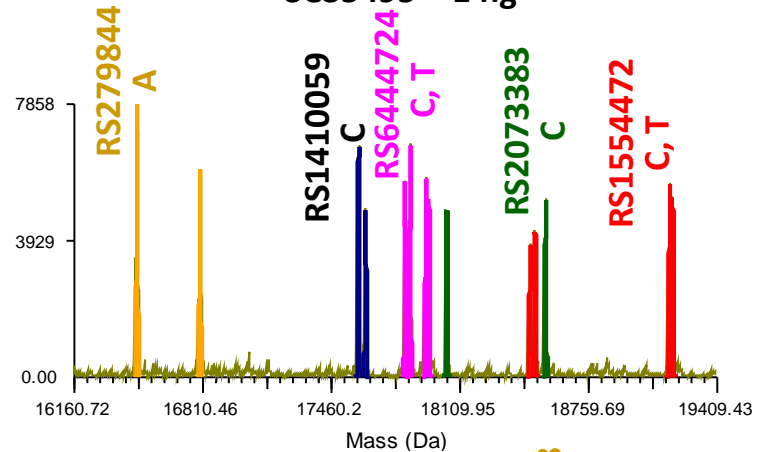


Initial Multiplexing Results

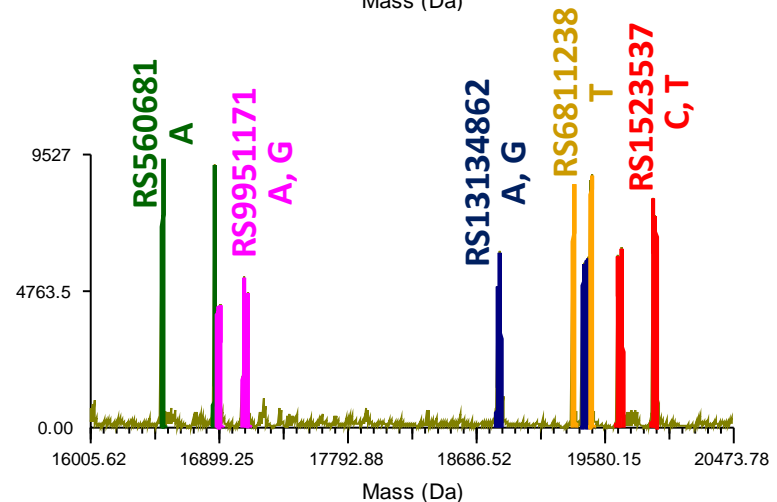
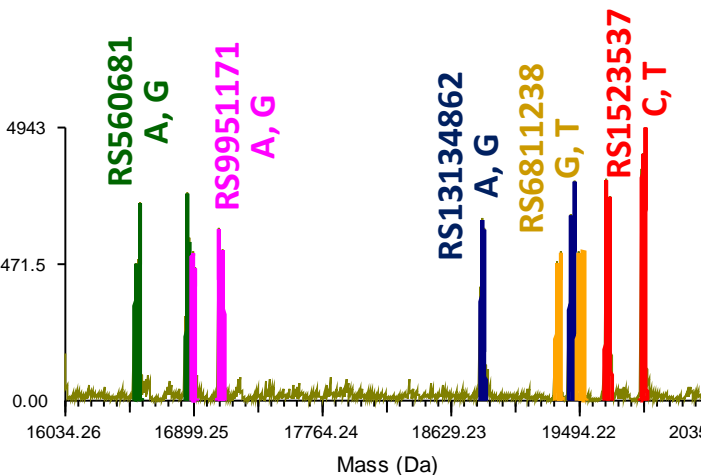
N31774 - 1 ng



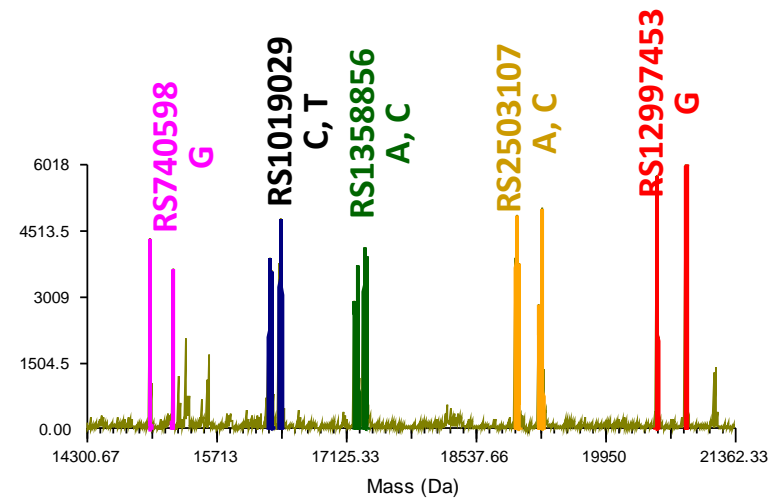
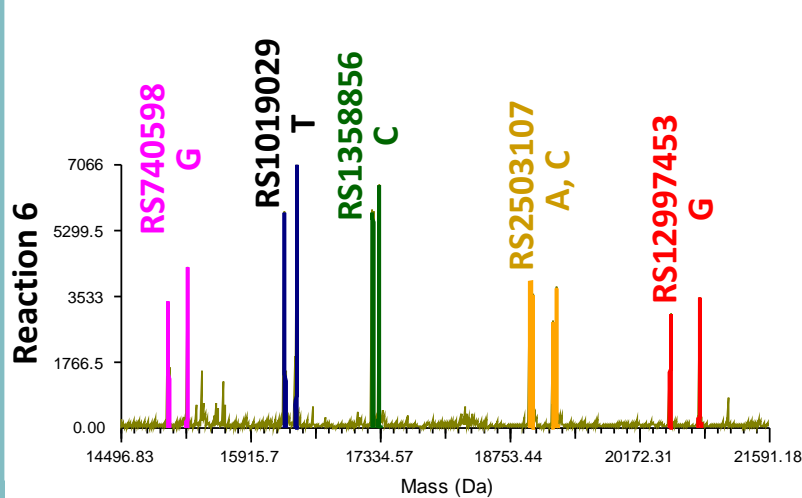
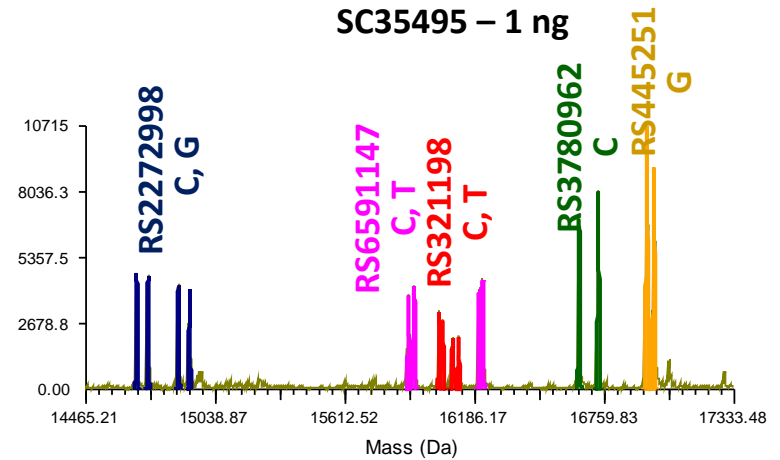
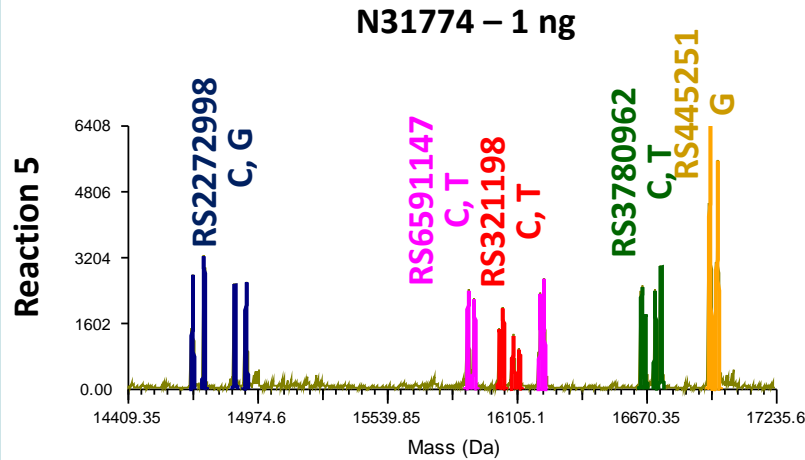
SC35495 - 1 ng



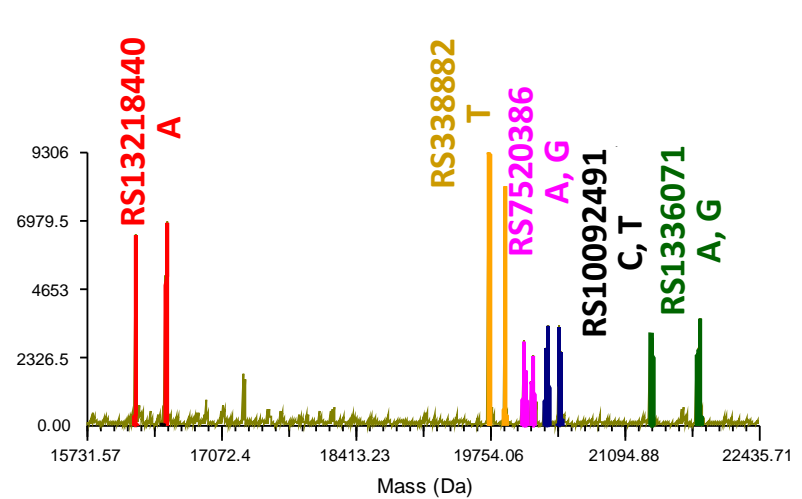
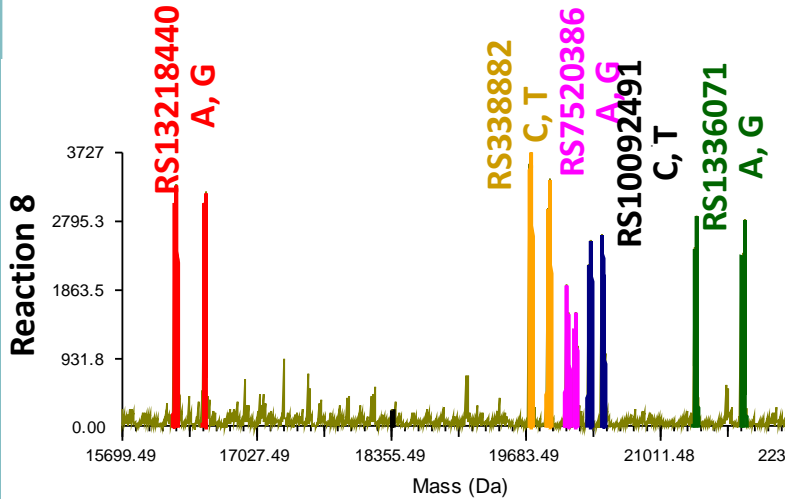
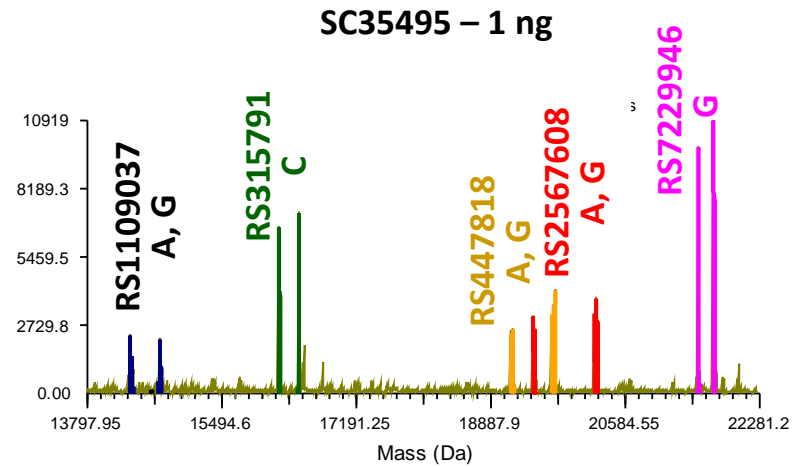
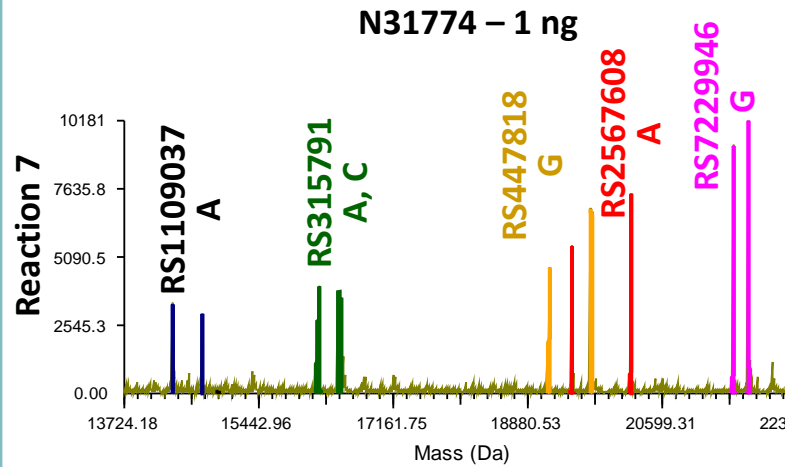
Reaction 4



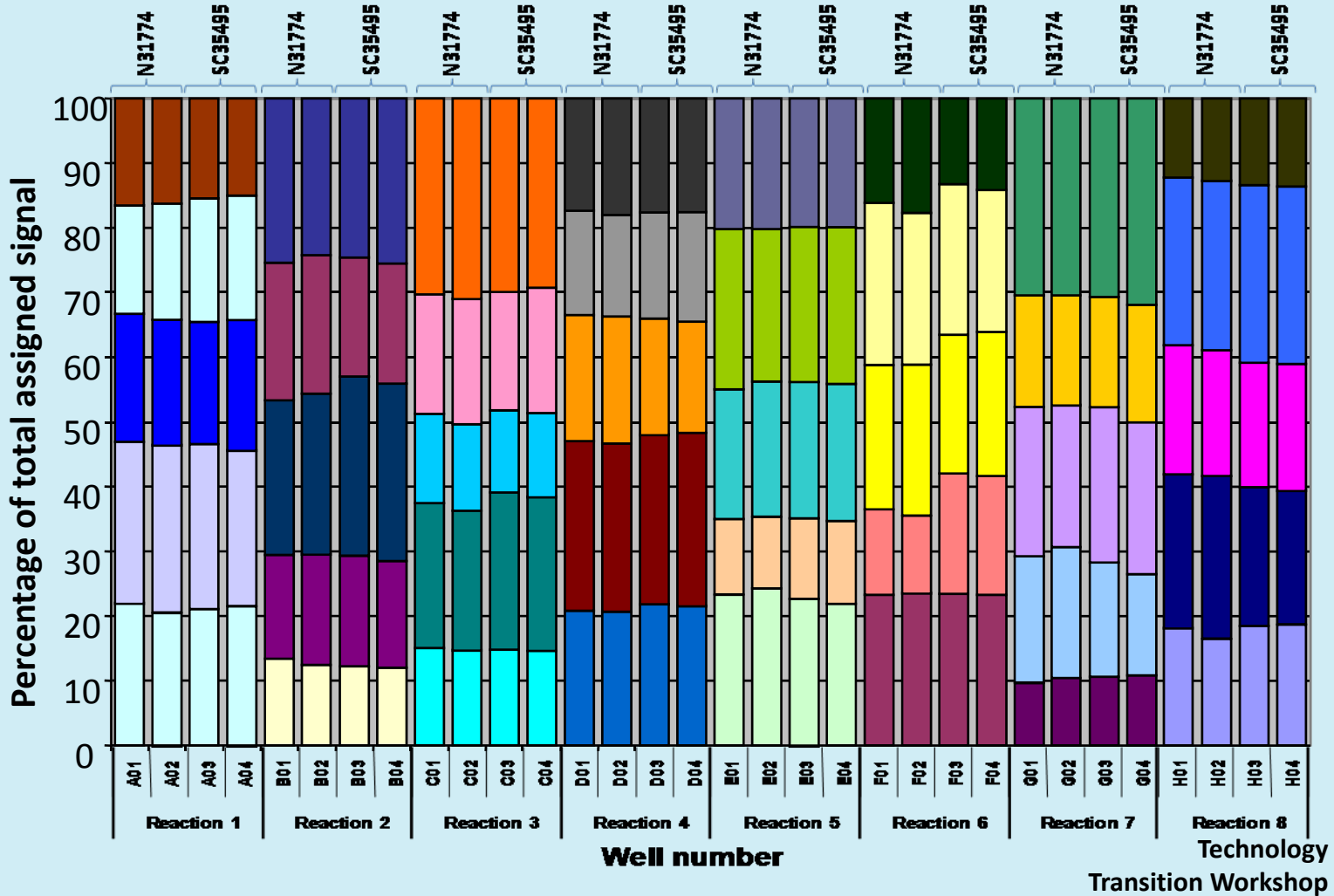
Initial Multiplexing Results



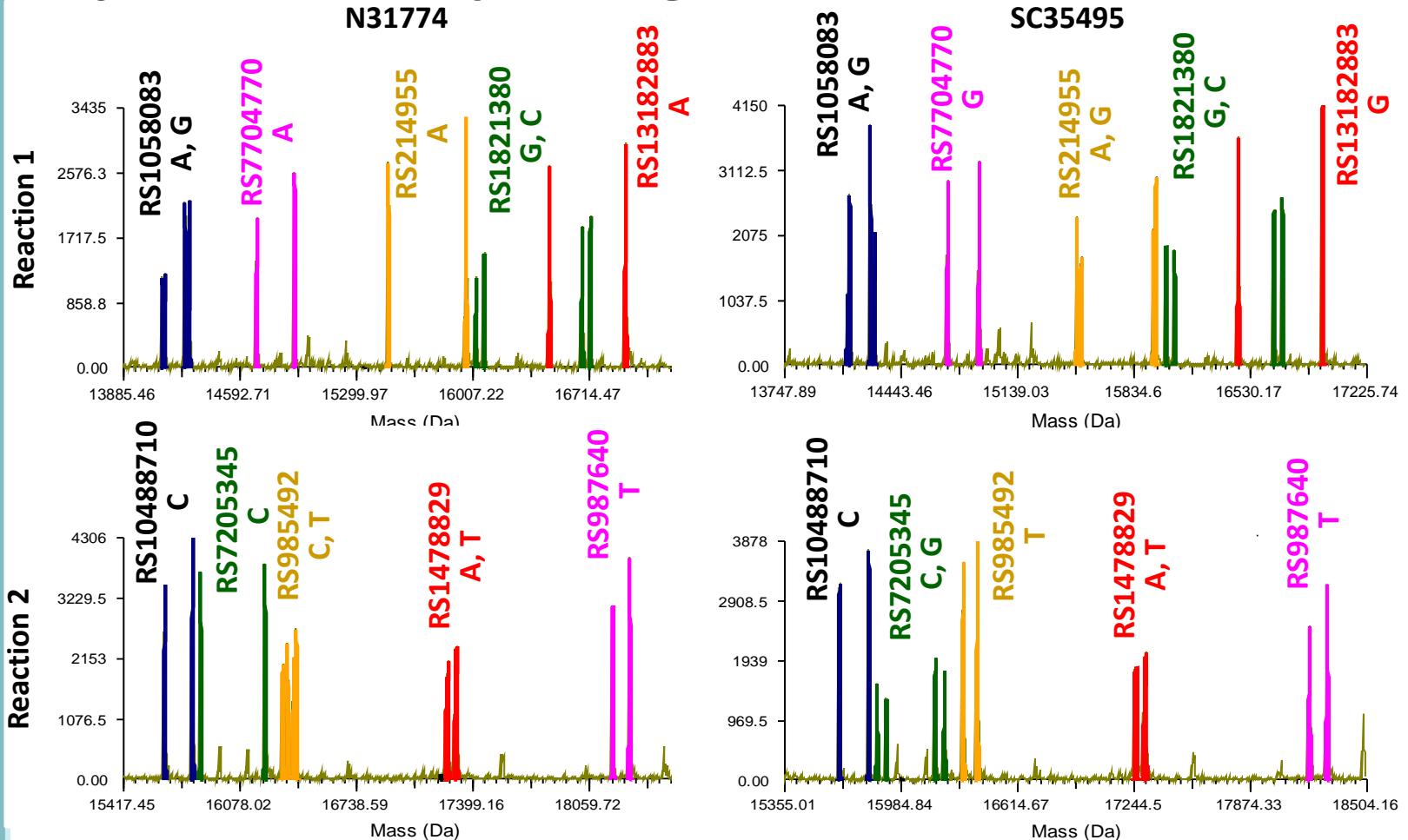
Initial Multiplexing Results



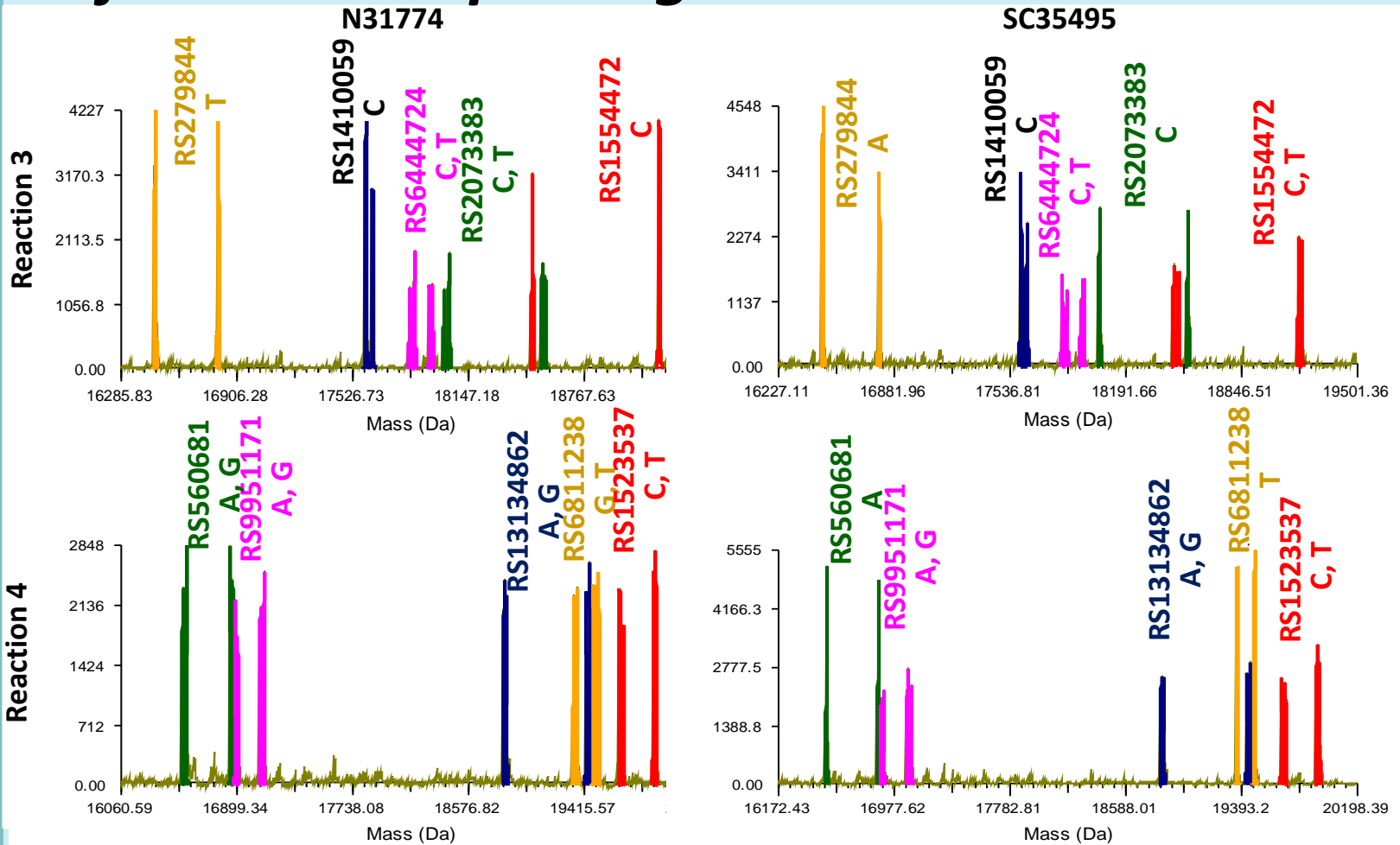
Initial Intra-locus Strand Balance



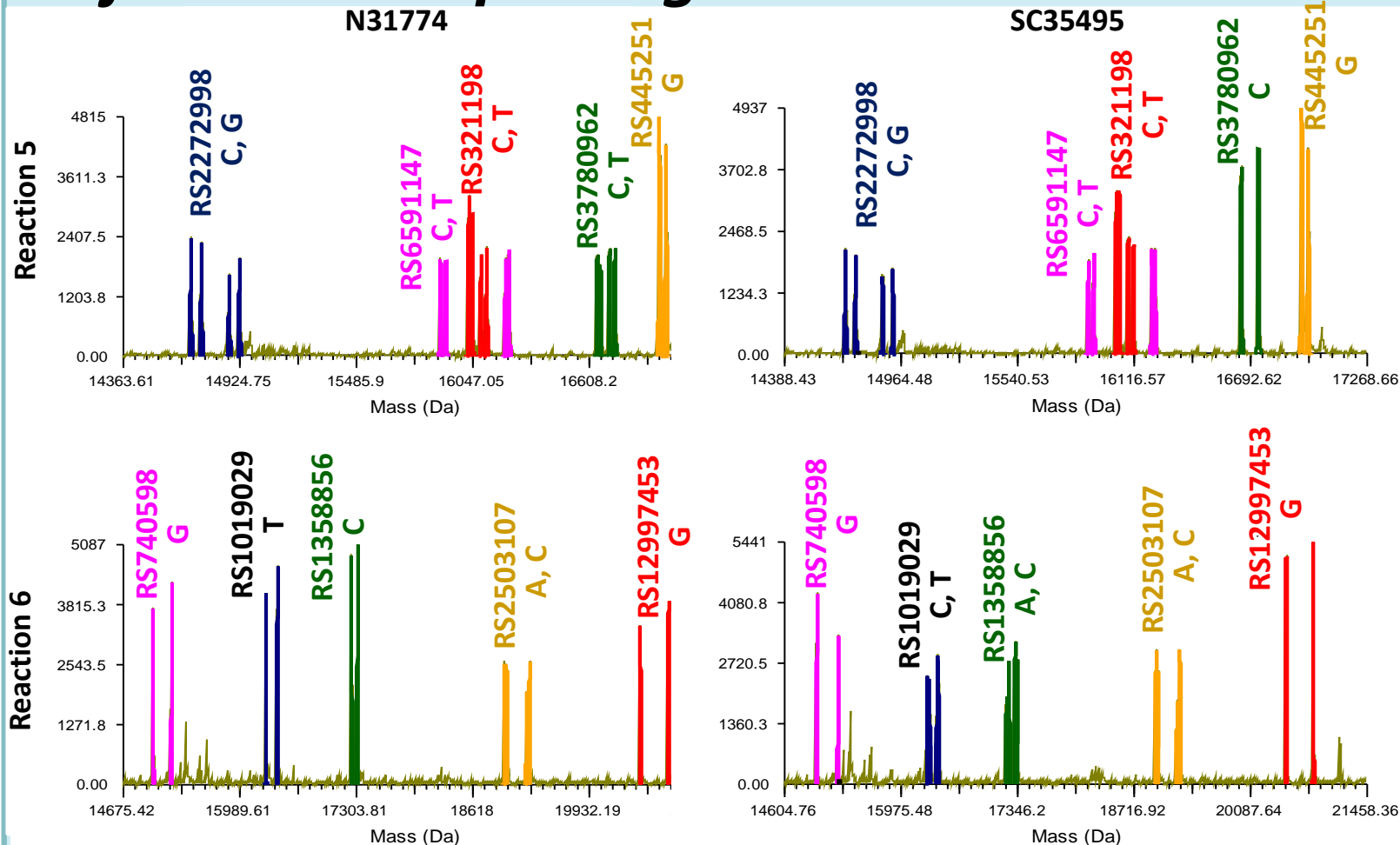
Adjusted Multiplexing Results



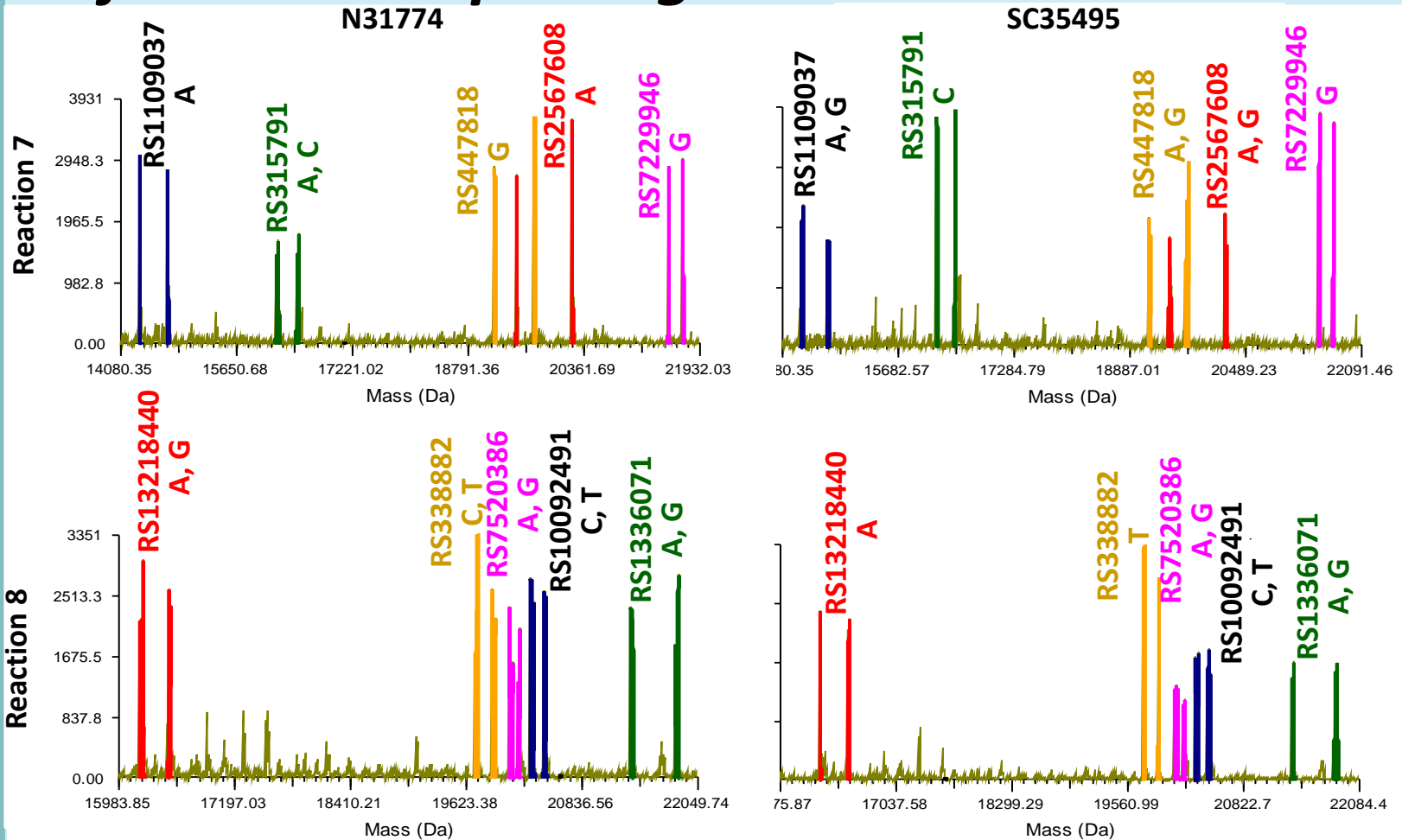
Adjusted Multiplexing Results



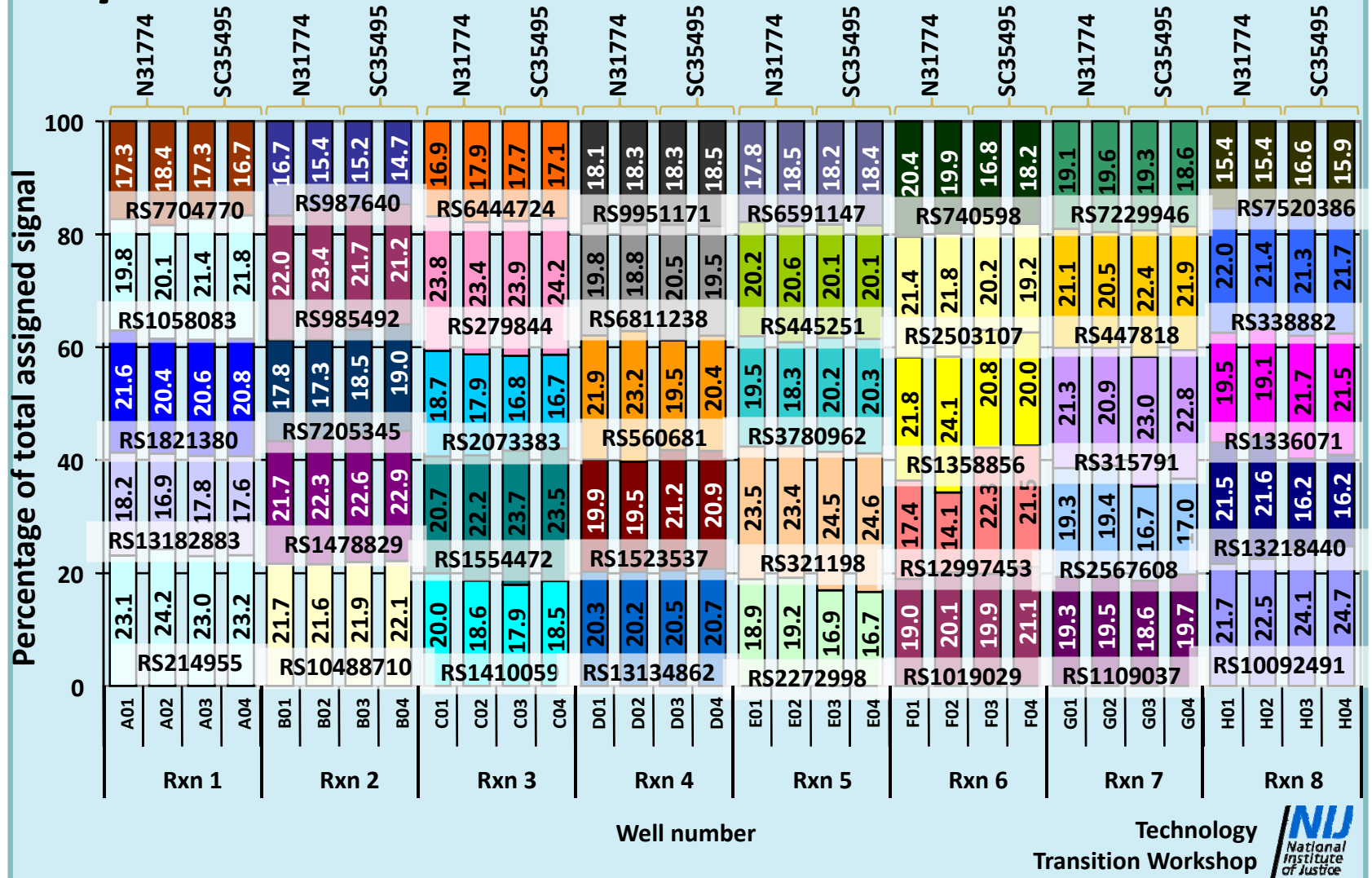
Adjusted Multiplexing Results



Adjusted Multiplexing Results



Improved Intra-locus Strand Balance



Sensitivity – 5-plexes

Full profile
obtained at
63 pg / reaction

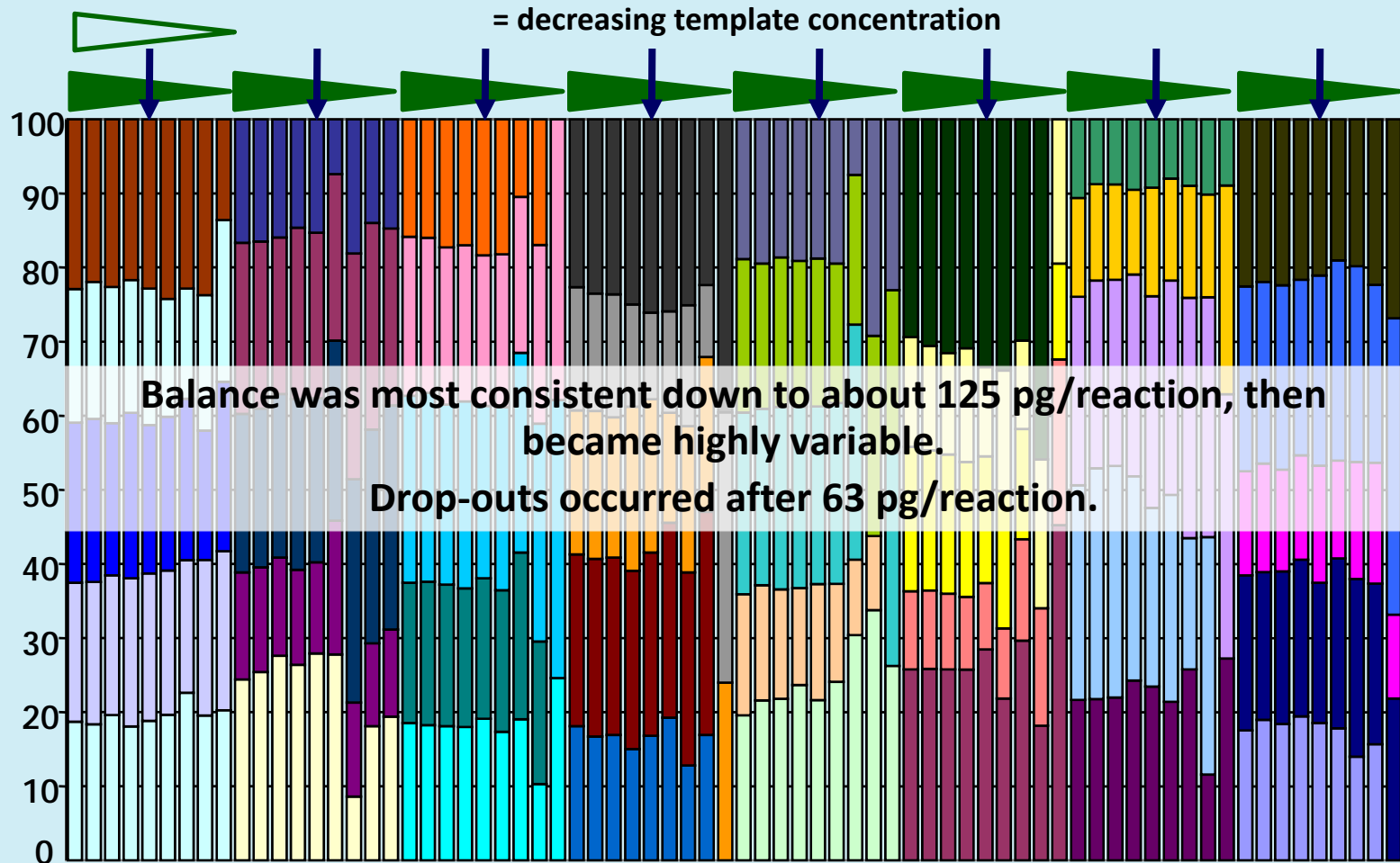
Reaction quality
degraded at
125 pg and
below

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

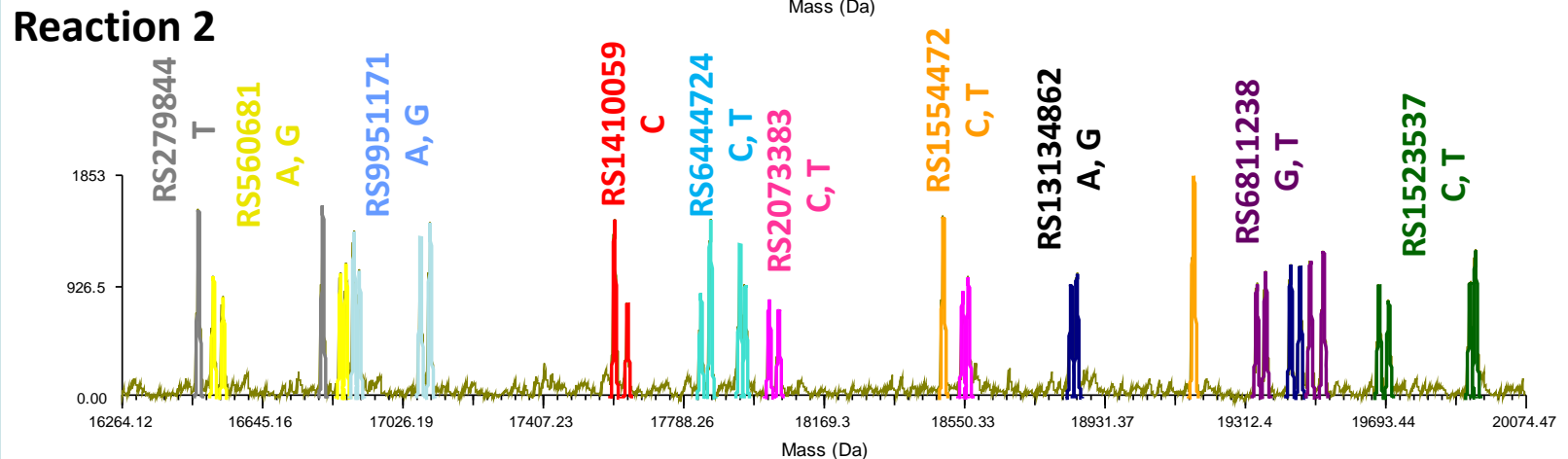
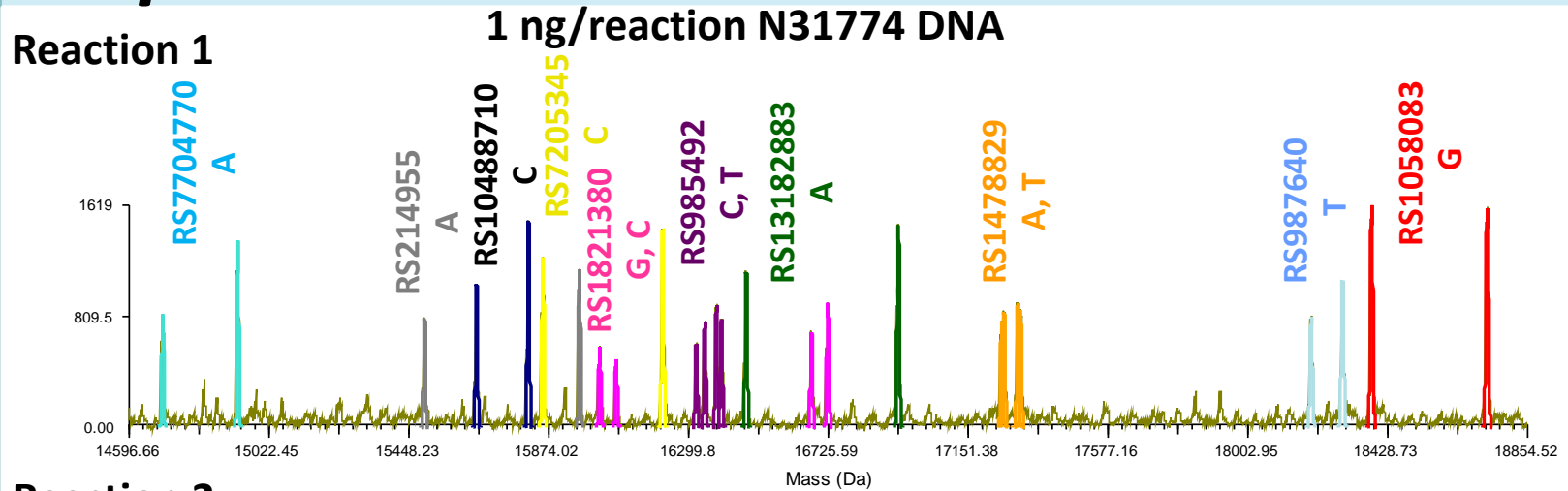
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Inter-Locus 5-plex Balance in Dilutions

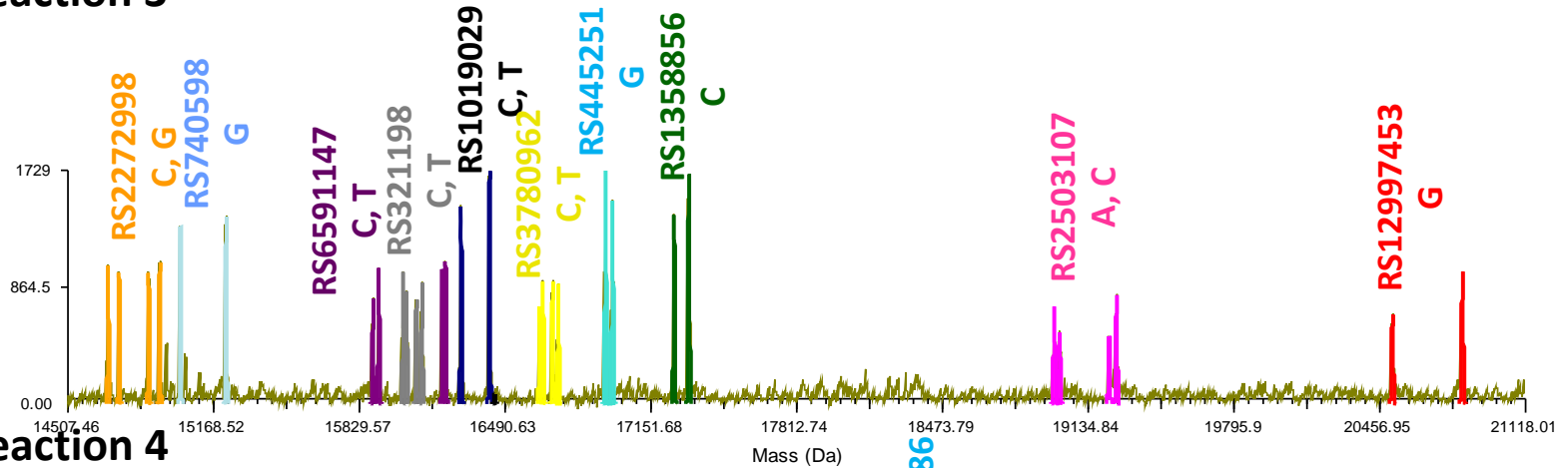


10-plex Reactions

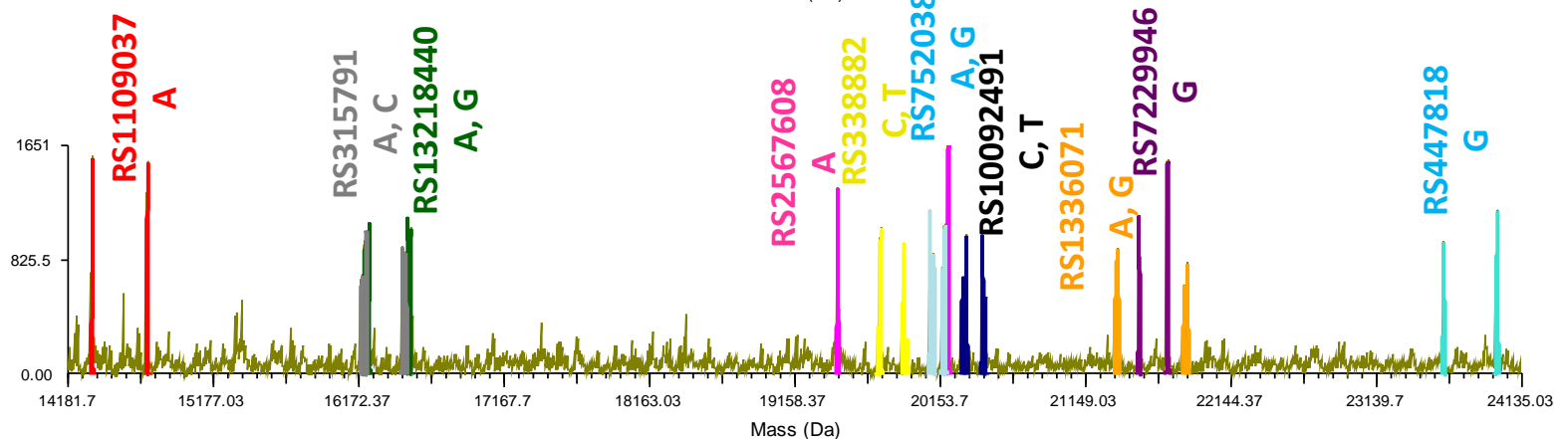


10-plex Reactions

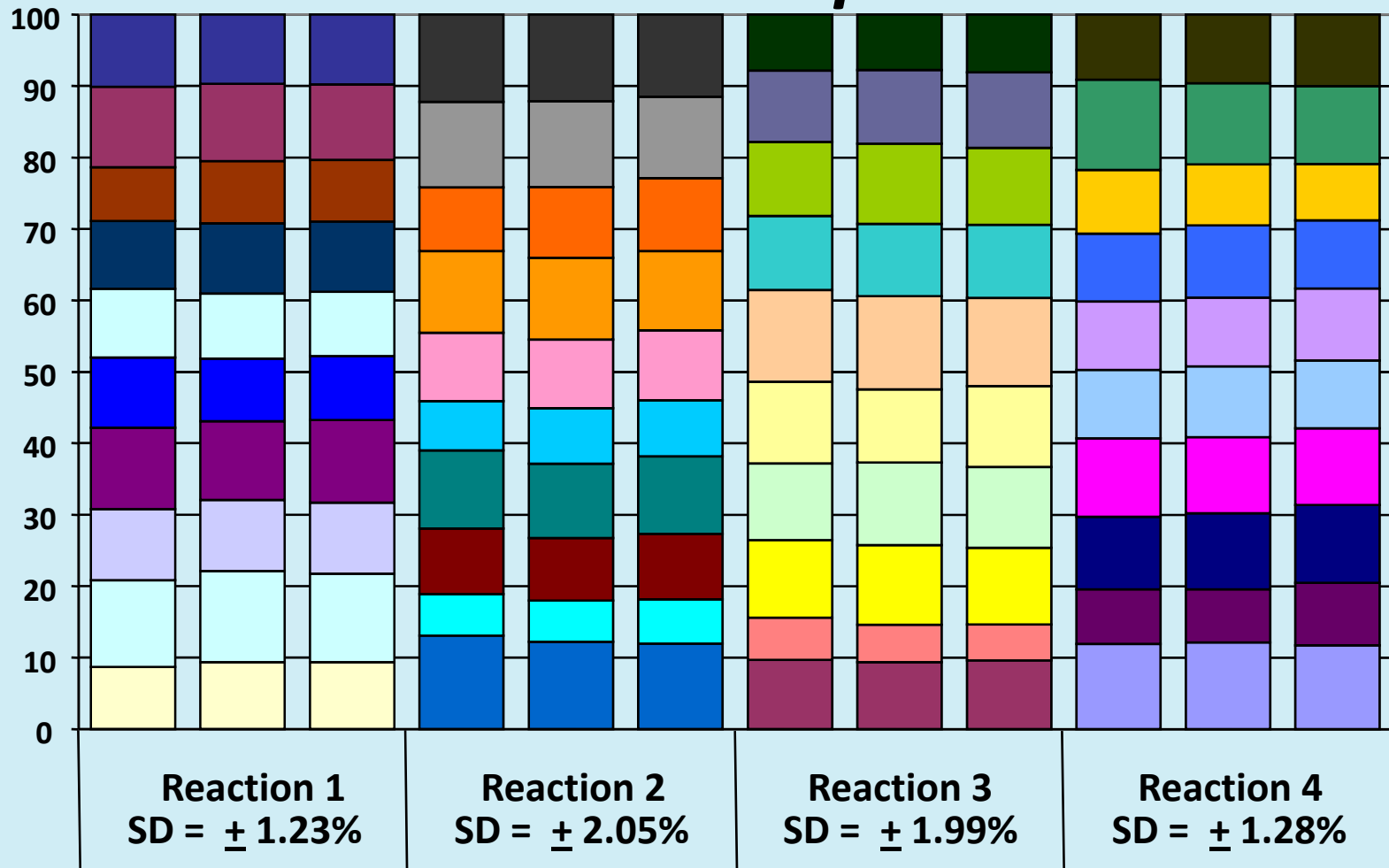
Reaction 3 1 ng/reaction N31774 DNA



Reaction 4

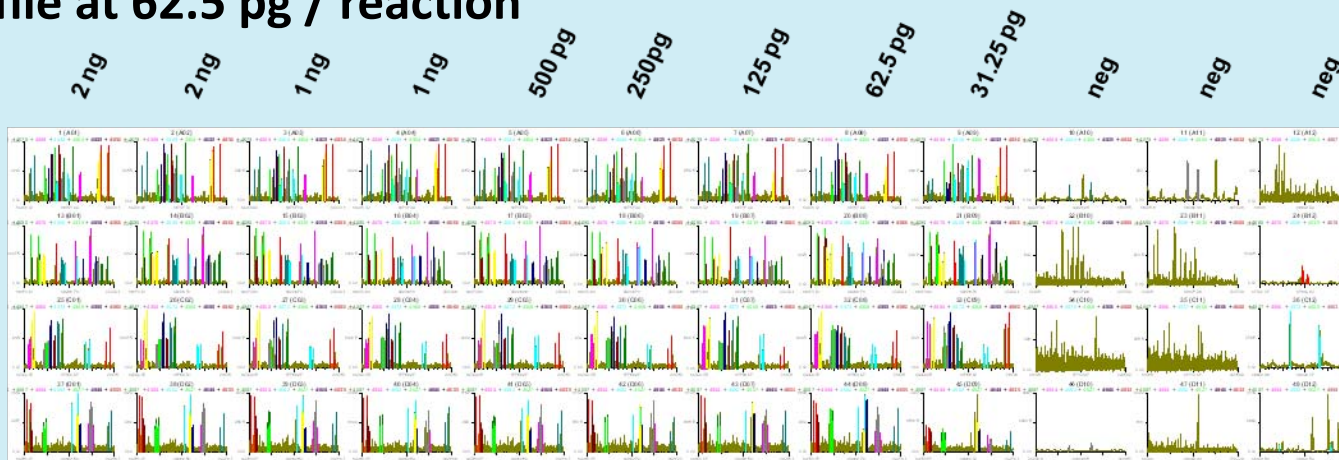


Inter-Locus Balance in 10-plex Reactions



Sensitivity of 10-plex Reactions

Full profile at 62.5 pg / reaction



RUB10292491	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	---	---	---
RUB1019329	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	---	---	---
RUB10489710	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	C, G	---	---
RUB1096009	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	---	---	---
RUB1100000	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	---	---	---
RUB12697453	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	---	---	---
RUB13134862	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	G, ---	---	---
RUB13166968	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	---	---	---
RUB13218140	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	---	---
RUB1338071	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, ---	---	---
RUB1366996	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	C, ---	---	---
RUB1412059	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	---	---
RUB1418829	A, T	A, T	A, T	A, T	A, T	A, T	A, T	A, T	A, T	T, ---	---	---
RUB1529630	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	---	---
RUB1554492	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	---	---	---
RUB1621980	G, G	G, G	G, G	G, G	G, G	G, G	G, G	G, G	G, G	G, G	---	---
RUB2073063	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	---	---
RUB211095	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	---	---	---
RUB212280	G, G	G, G	G, G	G, G	G, G	G, G	G, G	G, G	G, G	G, G	---	---
RUB252107	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	C, ---	---	---
RUB2591606	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	G, ---	---	---
RUB279844	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	---	---	---
RUB310791	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	---	---
RUB321198	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	---	---
RUB339862	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	C, ---	---	---
RUB440261	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	---	---
RUB441818	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	---	---	---
RUB493891	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, ---	---	---
RUB444924	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	---	---
RUB5291149	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	---	---
RUB6011238	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	---	---	---
RUB7209245	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	---	---	---
RUB7229346	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	---	---
RUB742998	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	G, ---	---	---	---
RUB7520386	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, ---	---	---
RUB7704970	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	A, ---	G, ---	---	---
RUB806462	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	G, T	---	---
RUB876440	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	T, ---	---	---
RUB9261191	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	A, G	---	---	---

Technology
Transition Workshop



Species Specificity

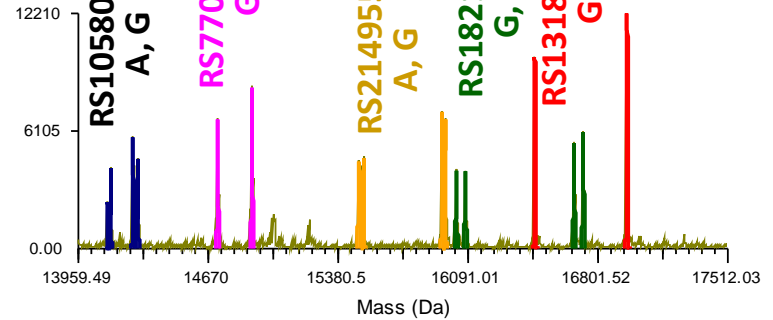
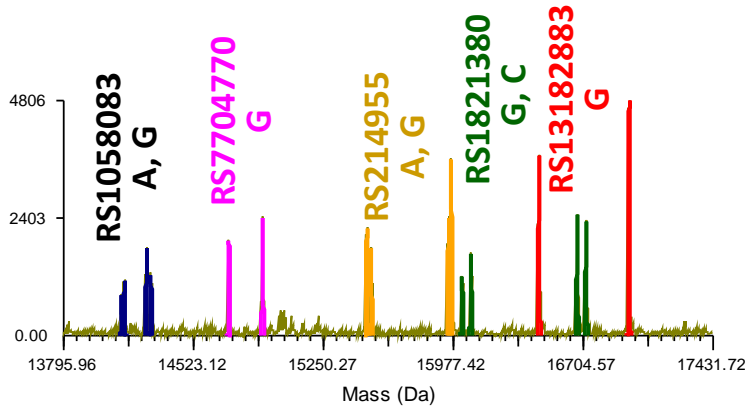
- **Human blood-derived DNA sample was tested in duplicate in the presence of 10-fold excess of exogenous DNA**
- **1 ng of human DNA per reaction**
- **10 ng exogenous DNA**
 - **Dog (male American Eskimo – buccal swab)**
 - **Cat (male long-hair, buccal swab)**
 - **Candida albicans (yeast)**
 - **Aspergillus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

Species Specificity

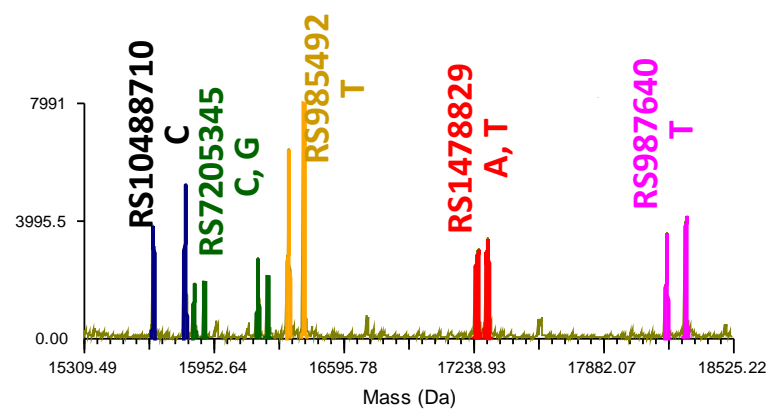
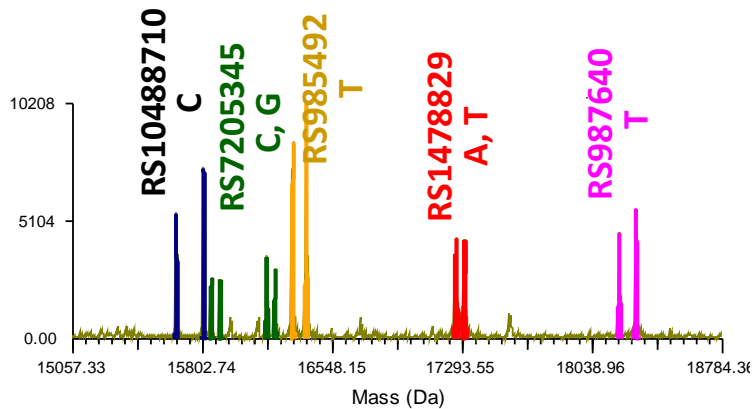
With dog DNA

With *Aspergillus oryzae* DNA

Reaction 1



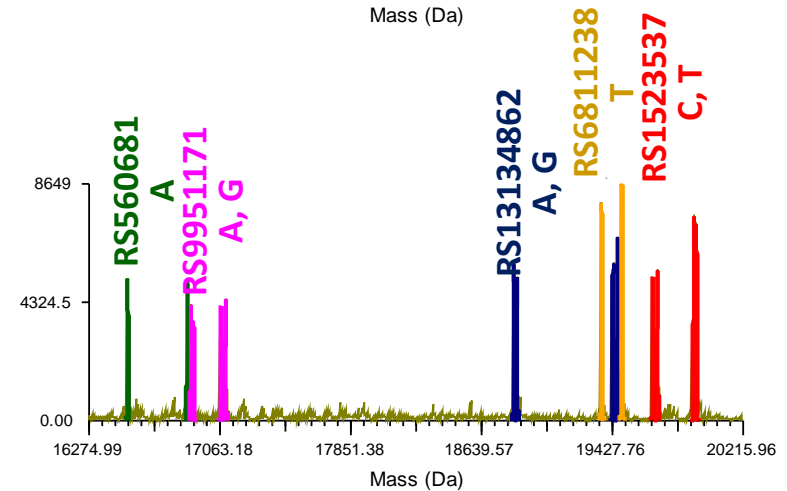
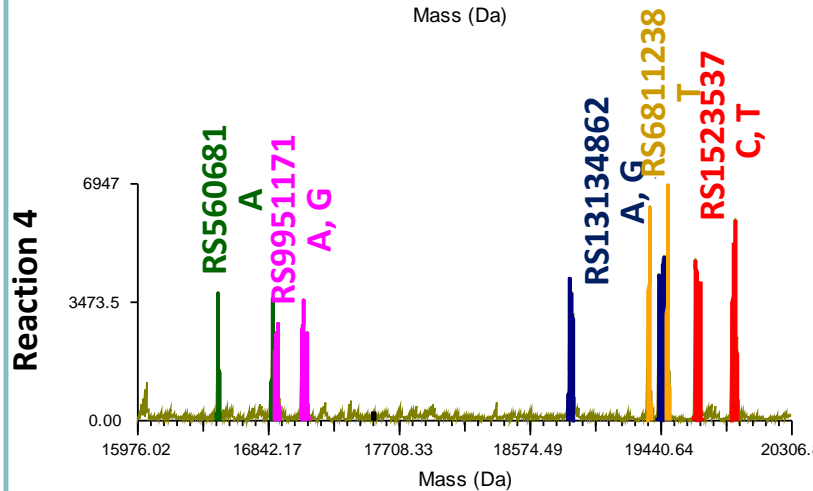
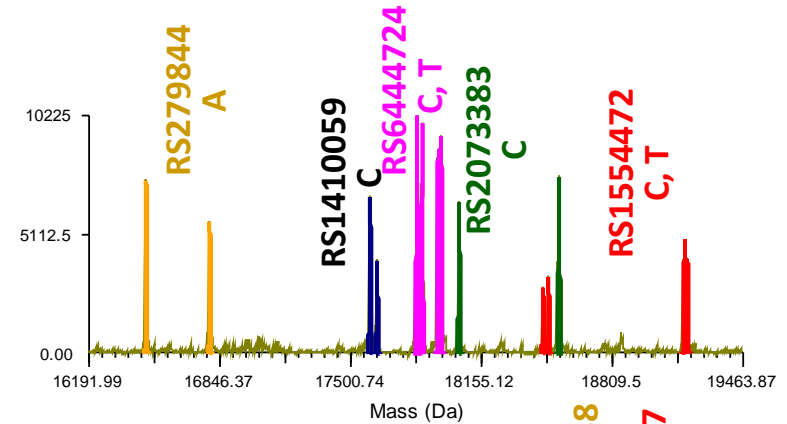
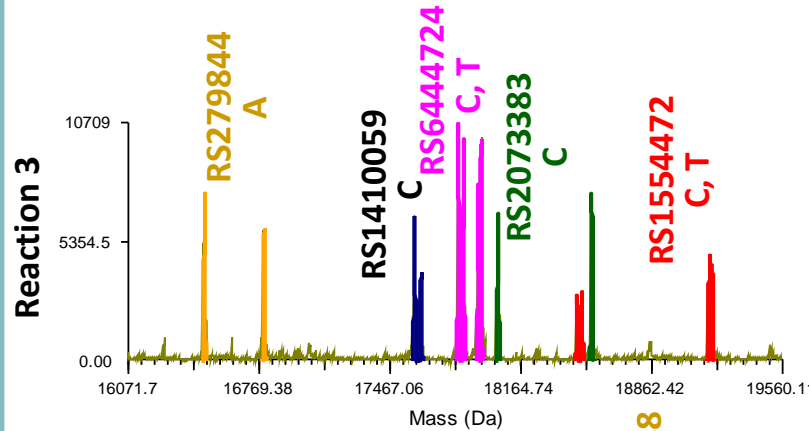
Reaction 2



Species Specificity

With dog DNA

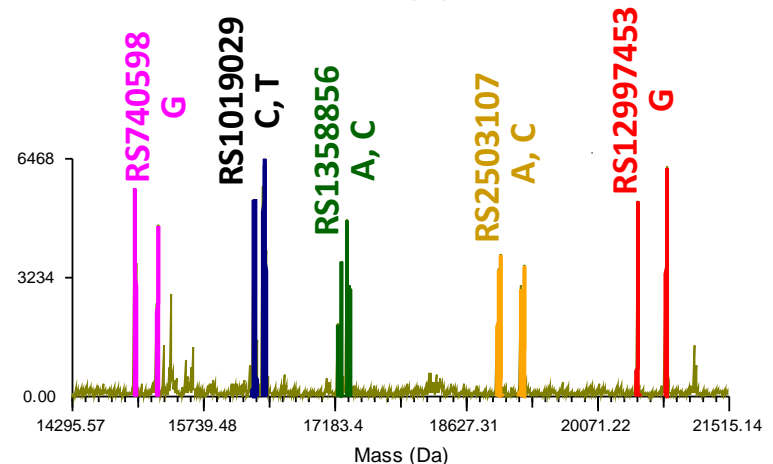
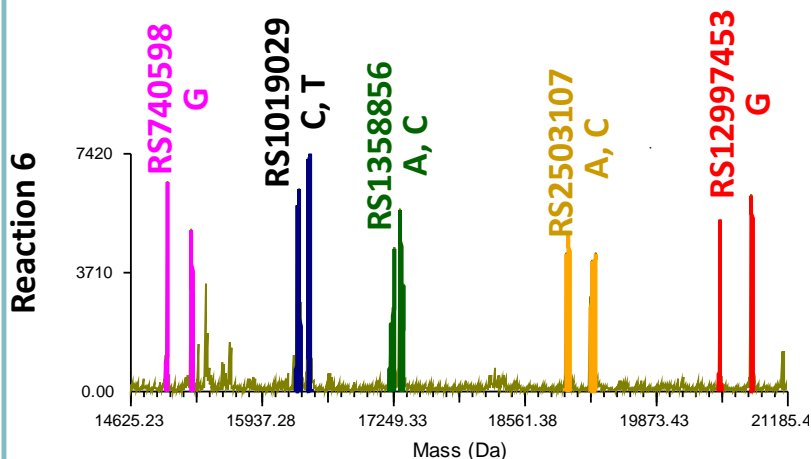
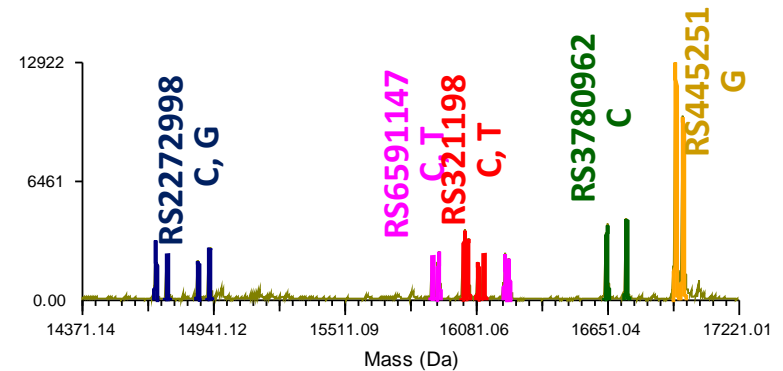
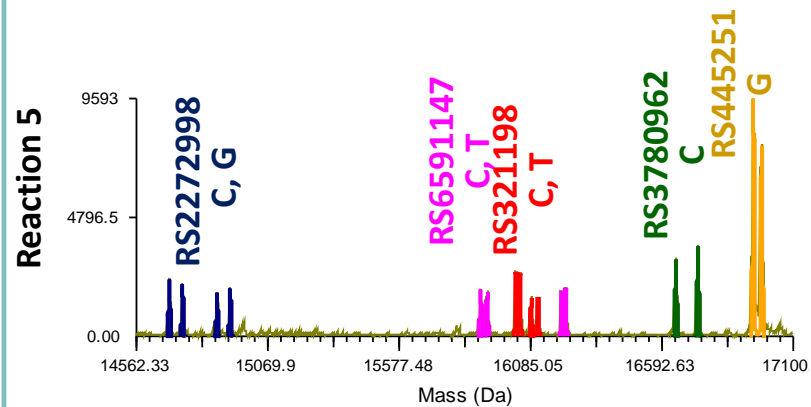
With *Aspergillus oryzae* DNA



Species Specificity

With dog DNA

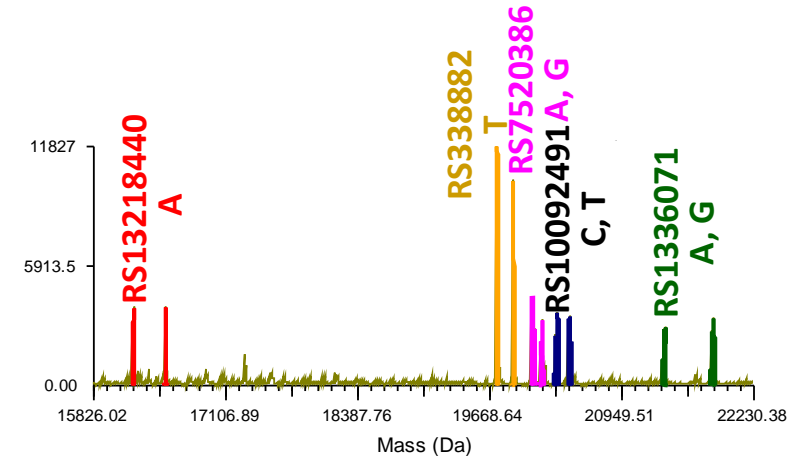
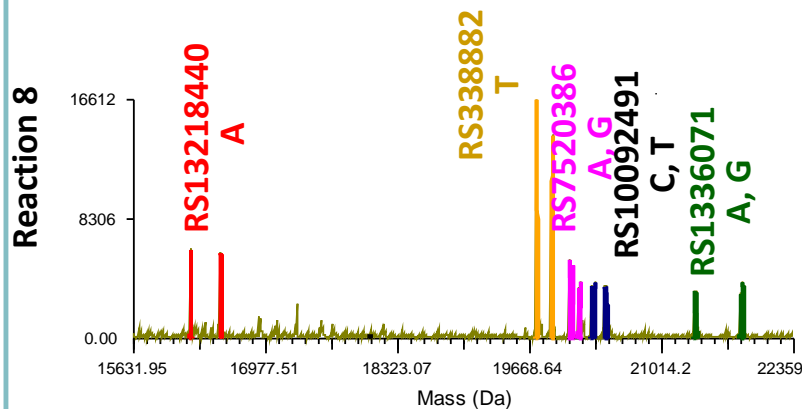
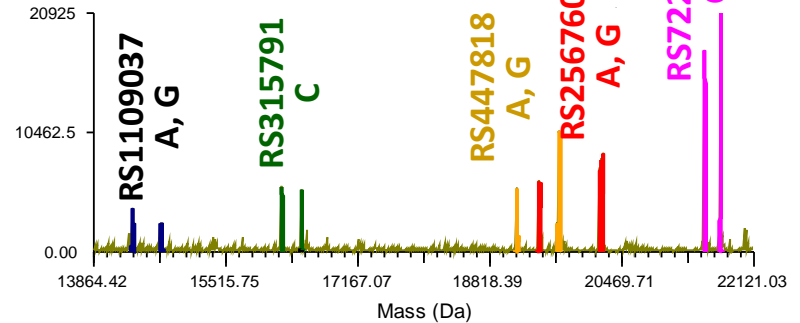
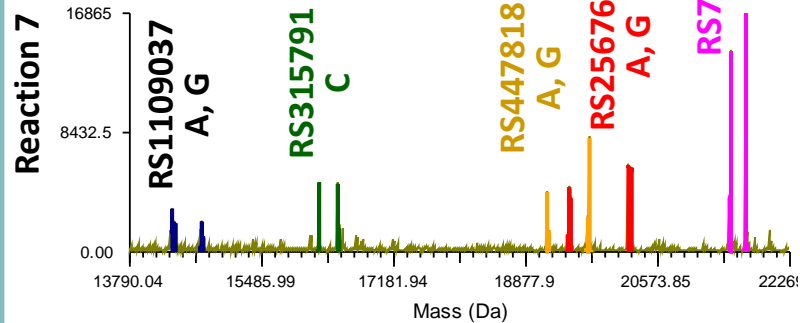
With *Aspergillus oryzae* DNA



Species Specificity

With dog DNA

With *Aspergillus oryzae* DNA



Species Specificity – 5-plex

10-fold excess of bacterial DNA

Pos

Pos

E. coli

E. coli

E. Coli + human

E. Coli + human

S. aureus

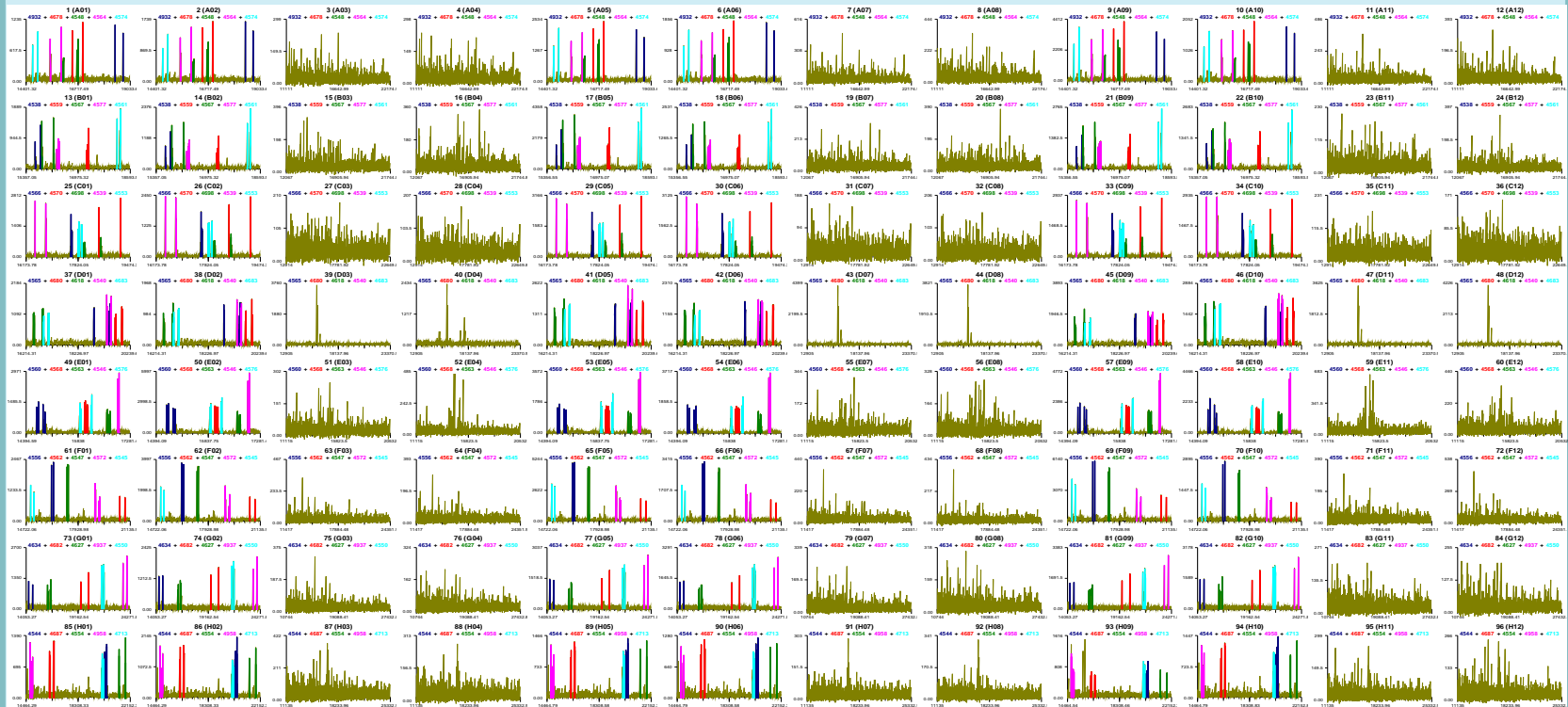
S. aureus

S. aureus + human

S. aureus + human

neg

neg



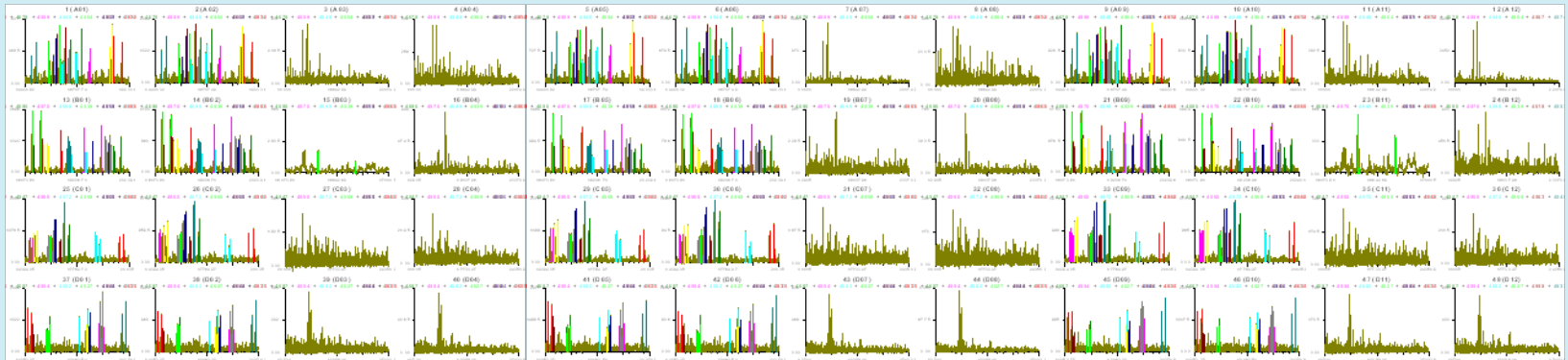
Technology
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Species Specificity – 10-plex

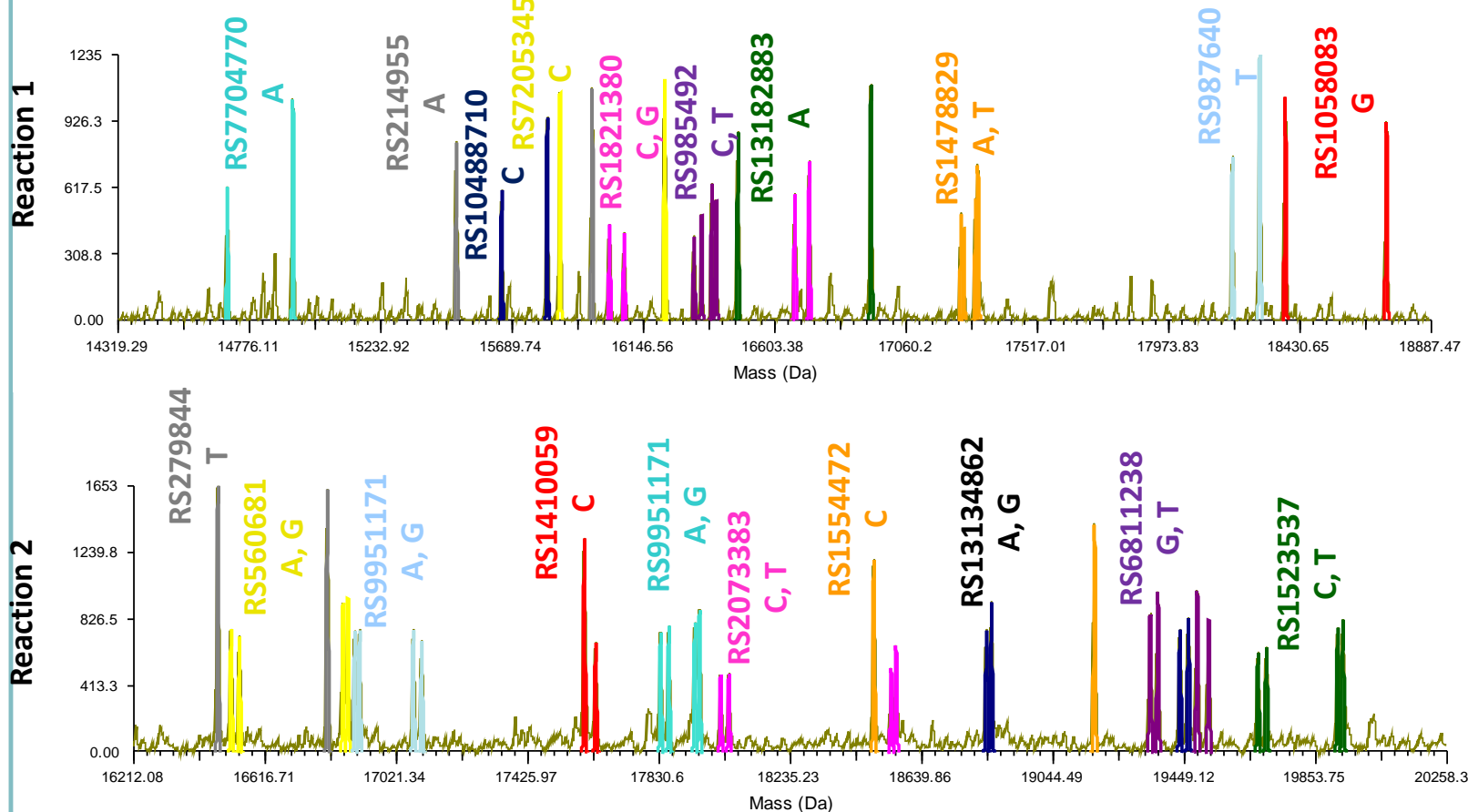
10-fold excess of
fungal DNA

Pos Pos *C. albicans* *C. albicans* *C. albicans* + human *C. albicans* + human *A. oryzae* *A. oryzae* *A. oryzae* + human *A. oryzae* + human neg neg



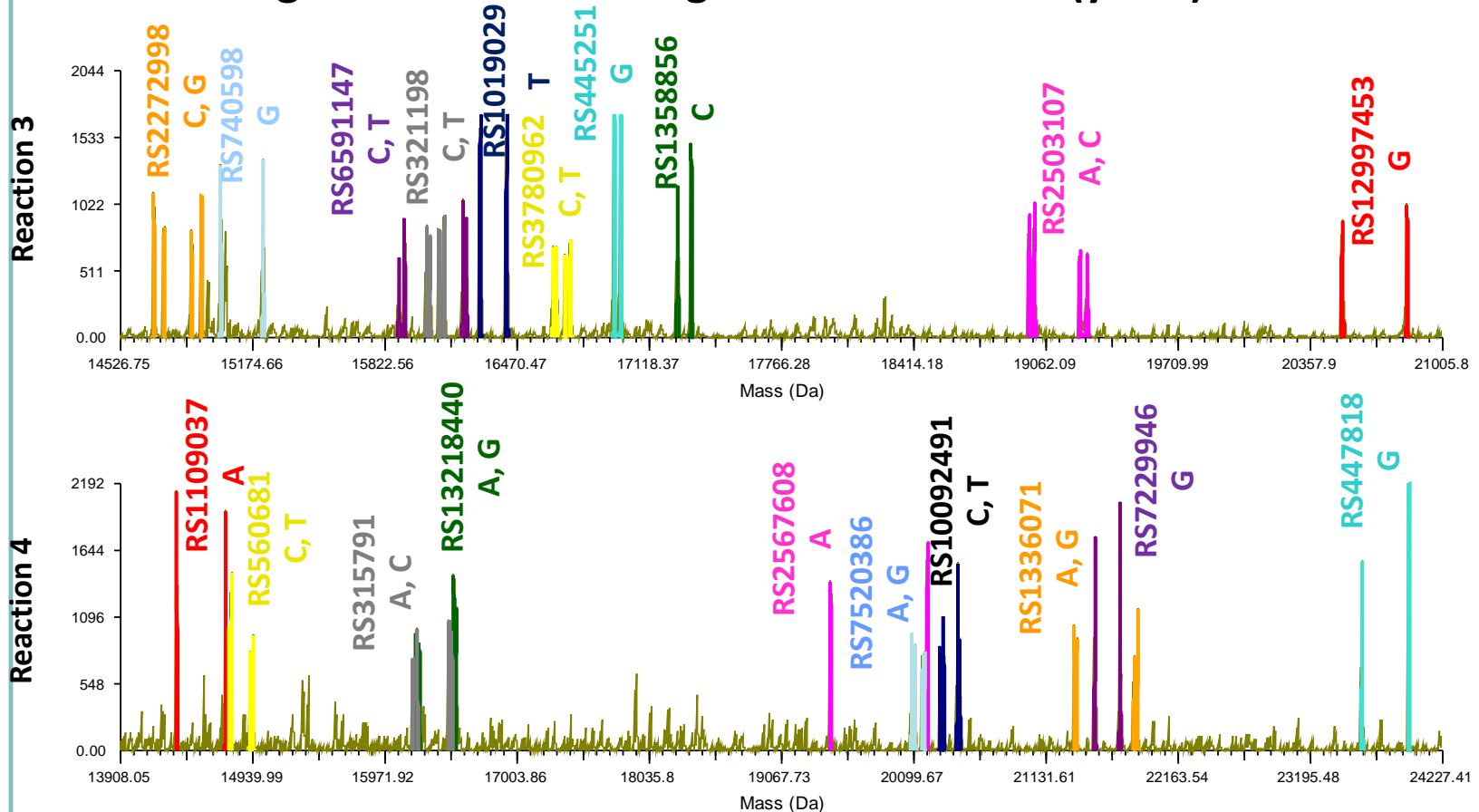
Species Specificity – 10-plex

1 ng human DNA + 10 ng *Candida albicans* (yeast) DNA



Species Specificity – 10-plex

1 ng human DNA + 10 ng *Candida albicans* (yeast) DNA



Species Specificity

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

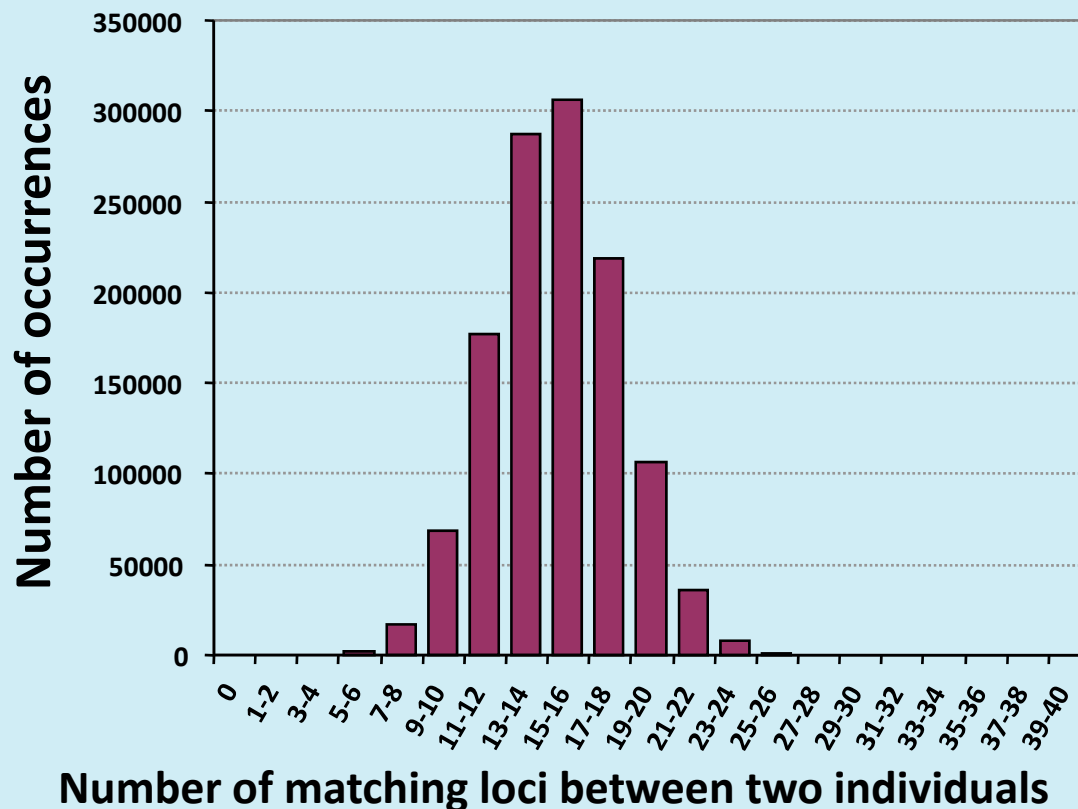
Locus	Dog	Cat	C. alb	A. ory	E. coli	S. aur	Dog	Cat	C. alb	A. ory	E. coli	S. aur
RS10092491	C,T	C,T	C,T	C,T	C,T	C,T	C,T	C,T	C,T	C,T	C,T	C,T
RS1019029	C,T	C,T	C,T	C,T	C,T	C,T	C,T	C,T	C,T	C,T	C,T	C,T
RS10488710	C,---	C,---	C,---	C,---	C,---	C,---	C,---	C,---	C,---	C,---	C,---	C,---
RS1058083	A,G	A,G	A,G	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

Profile comparisons

- **86 samples run with Ibis™ Kidd-40 SNP panel**
- **50 samples from UNTHSC**
- **26 blood samples**
- **10 buccal swab samples**
- **Cross comparisons of all pairs of samples:**
 - **Samples differ at an average of 24.7 ± 3.0 loci**
 - **3655 pair-wise cross-comparisons**

Resolving Power of Kidd SNP Panel

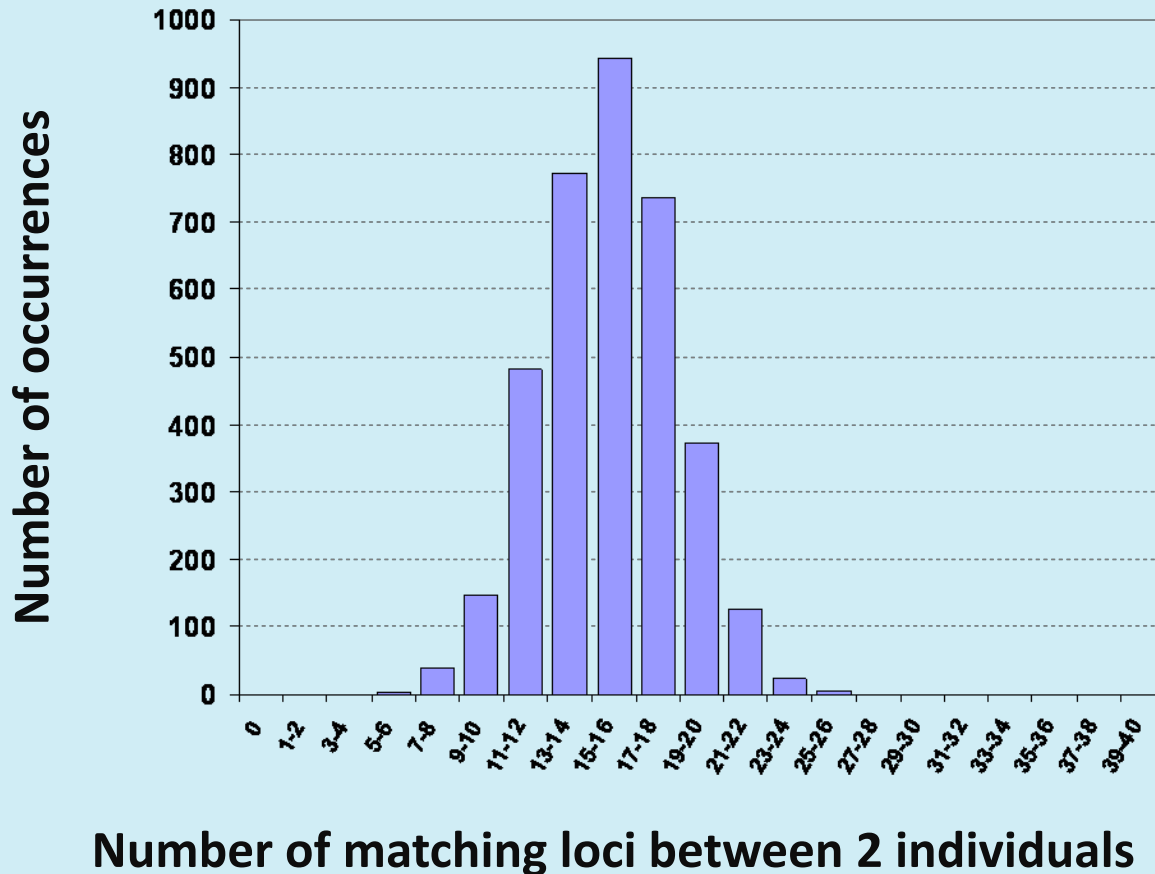
- ‘Perfect’ 40-SNP panel: random match with probability of 9.15×10^{-18}
- Kidd-40 panel has ave of about 1×10^{-15} match probability
- Within populations, ave match probability ranged from 10^{-12} to 10^{-16}



- 1,568 full profiles
- 40 populations
- All pairwise profile-to-profile comparisons
- 2,457,056 pairwise comparisons
- Most people will differ at 15 – 16 loci

Cross-Comparisons of Ibis™ Profiles

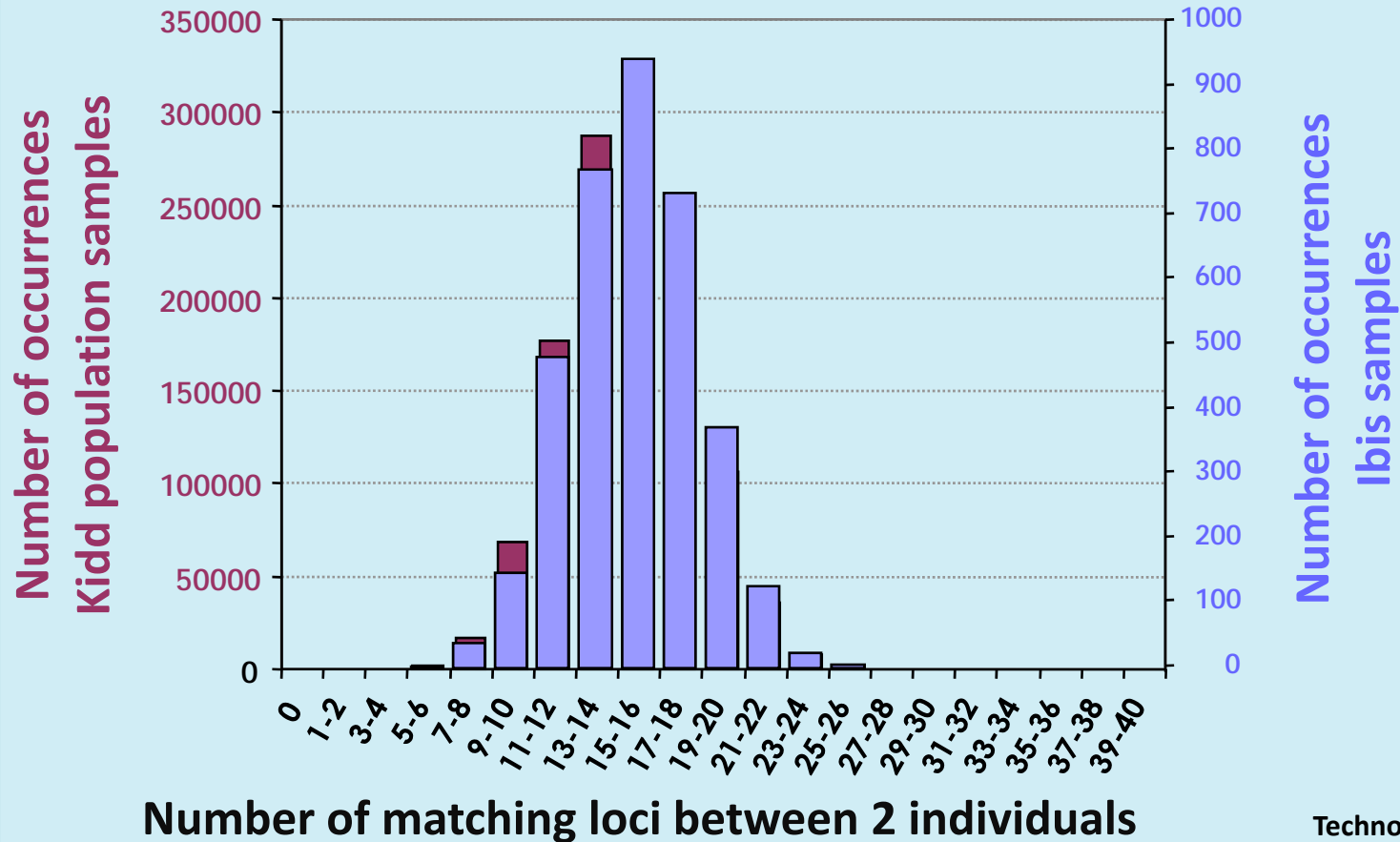
All pair-wise comparisons between
86 individuals



Distribution of comparisons for small set of 86 unrelated samples is virtually identical to 1568 samples taken from 40 global population groups

Cross-Comparisons of Ibis™ Profiles

All pair-wise comparisons between
1568 individuals



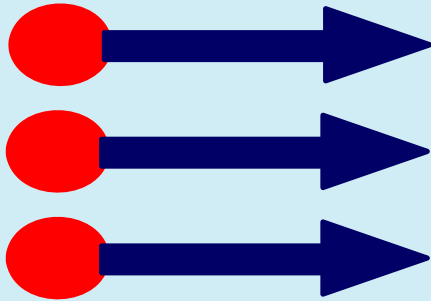
Reducing Spectral Complexity

- **Multiple PCR products congest an ESI mass spectrum**
- **Multiplexing capacity is limited both by PCR and mass spectrometry**
- **Multiplexing capacity may be able to be increased by removing one PCR product strand**
 - **Removing one DNA strand from each product would cut spectral complexity in half**
 - **It may be possible to effectively double multiplexing capacity**

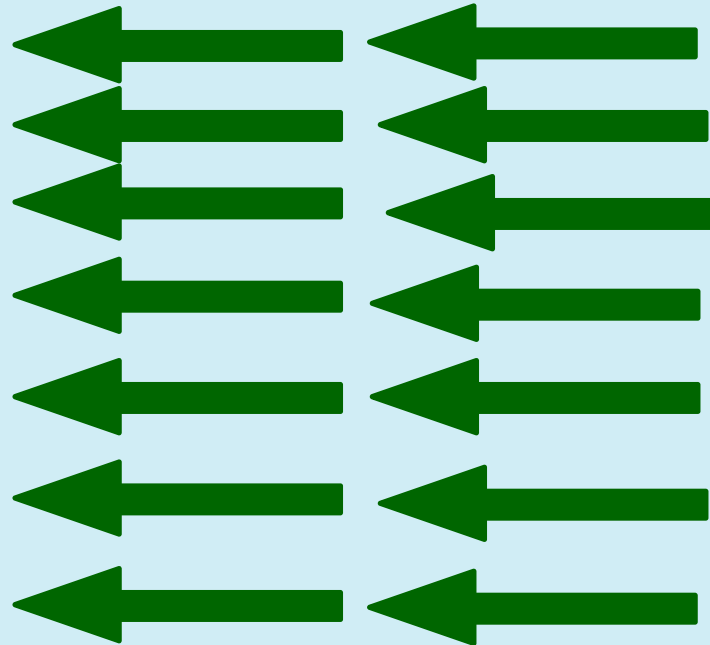
Reducing Spectral Complexity

By using one biotin-tagged forward primer in each primer pair ...

biotin



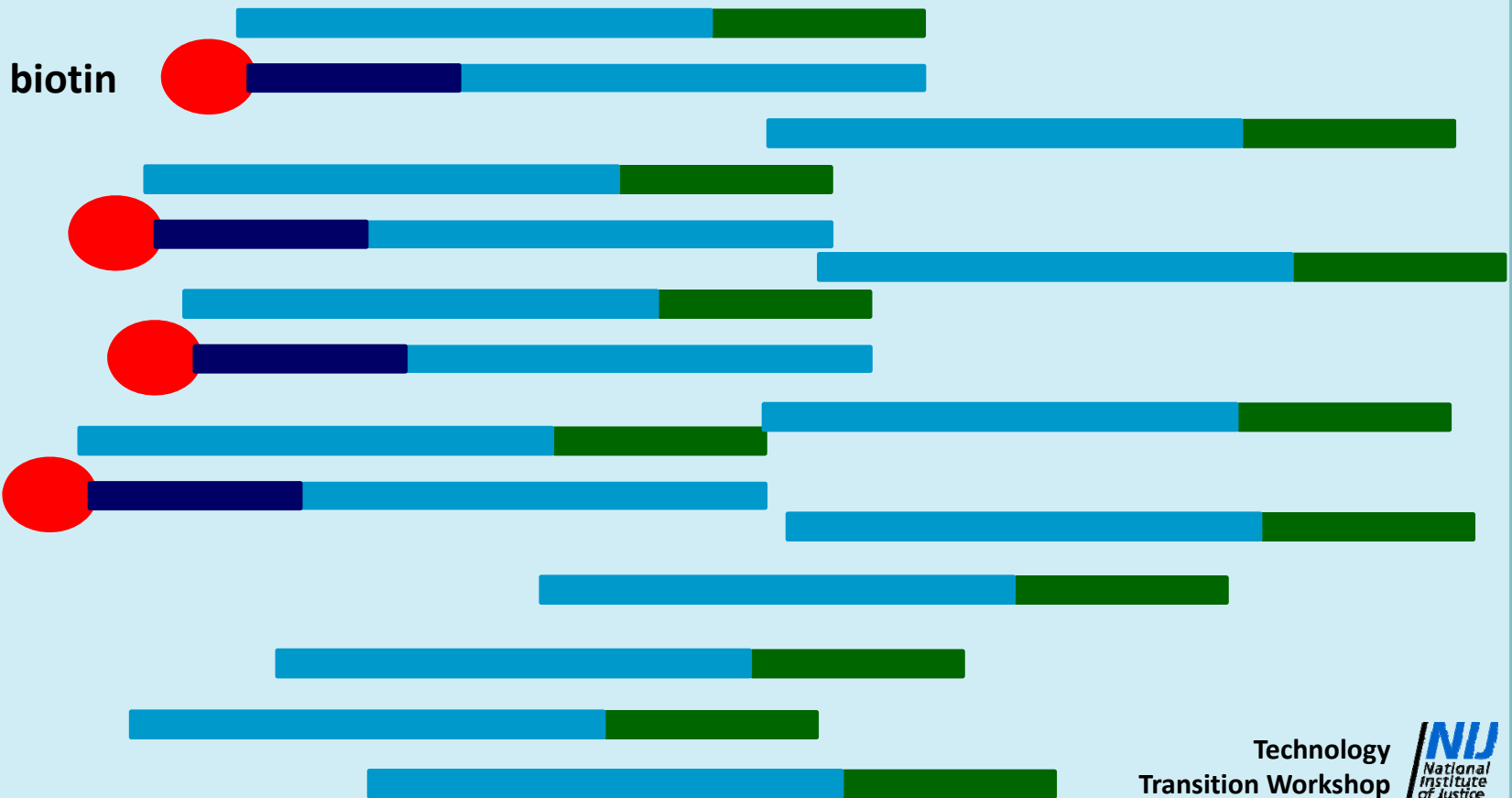
And an excess of non-tagged reverse primer



Template

Reducing Spectral Complexity

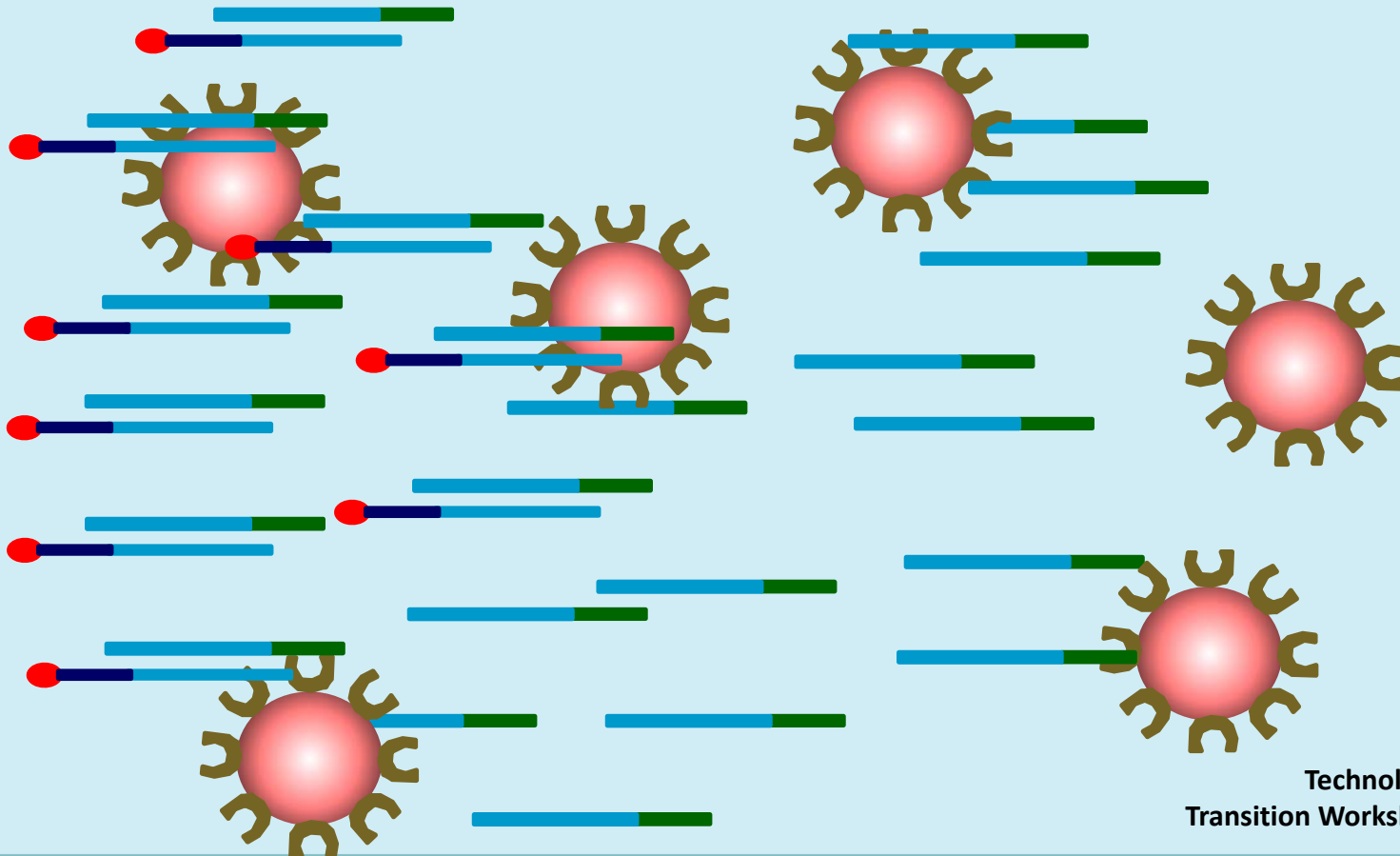
PCR products can be generated with an over-abundance of non-tagged reverse strands



Reducing Spectral Complexity

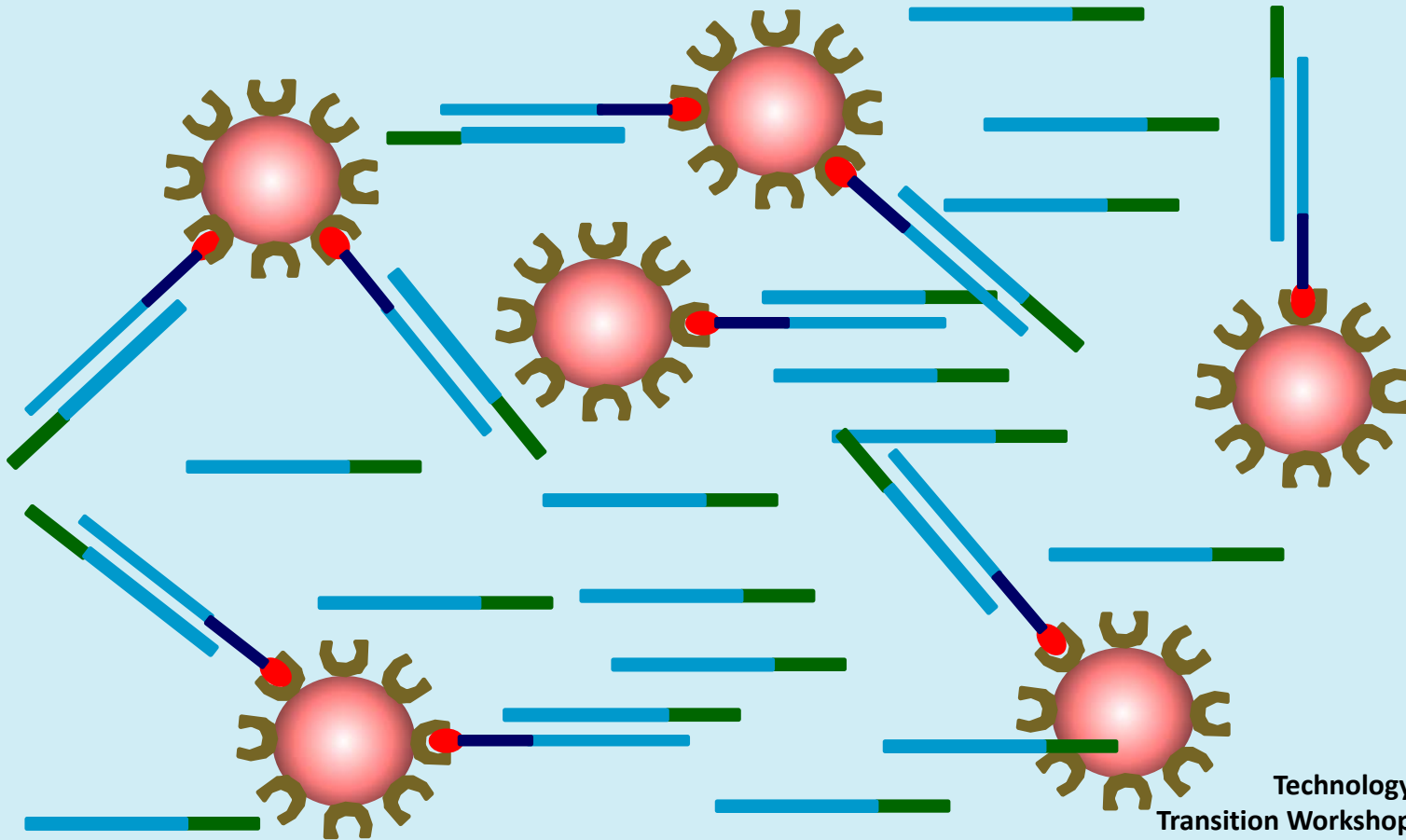
After running PCR ...

Streptavidin-coated magnetic beads are added



Reducing Spectral Complexity

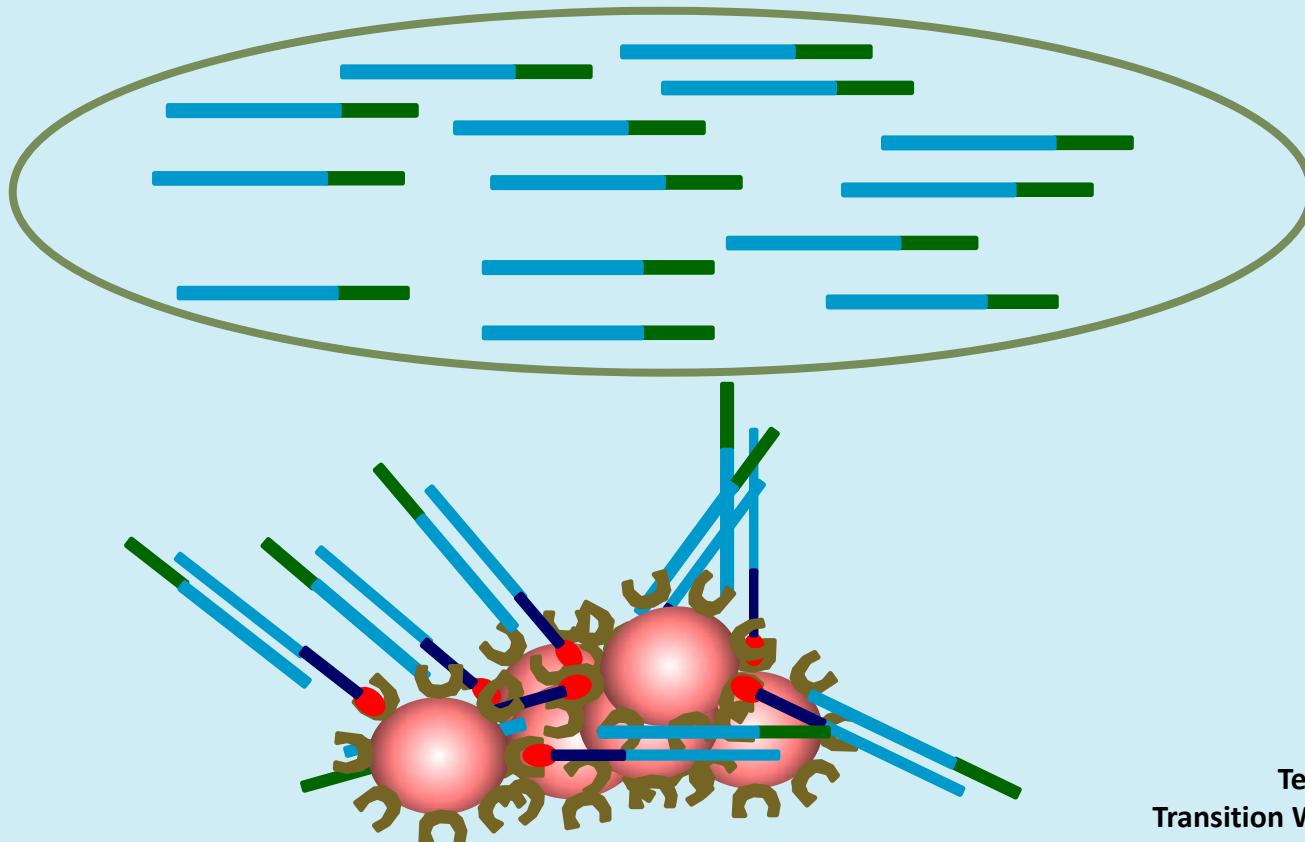
Biotin tags bind streptavidin beads after a short incubation



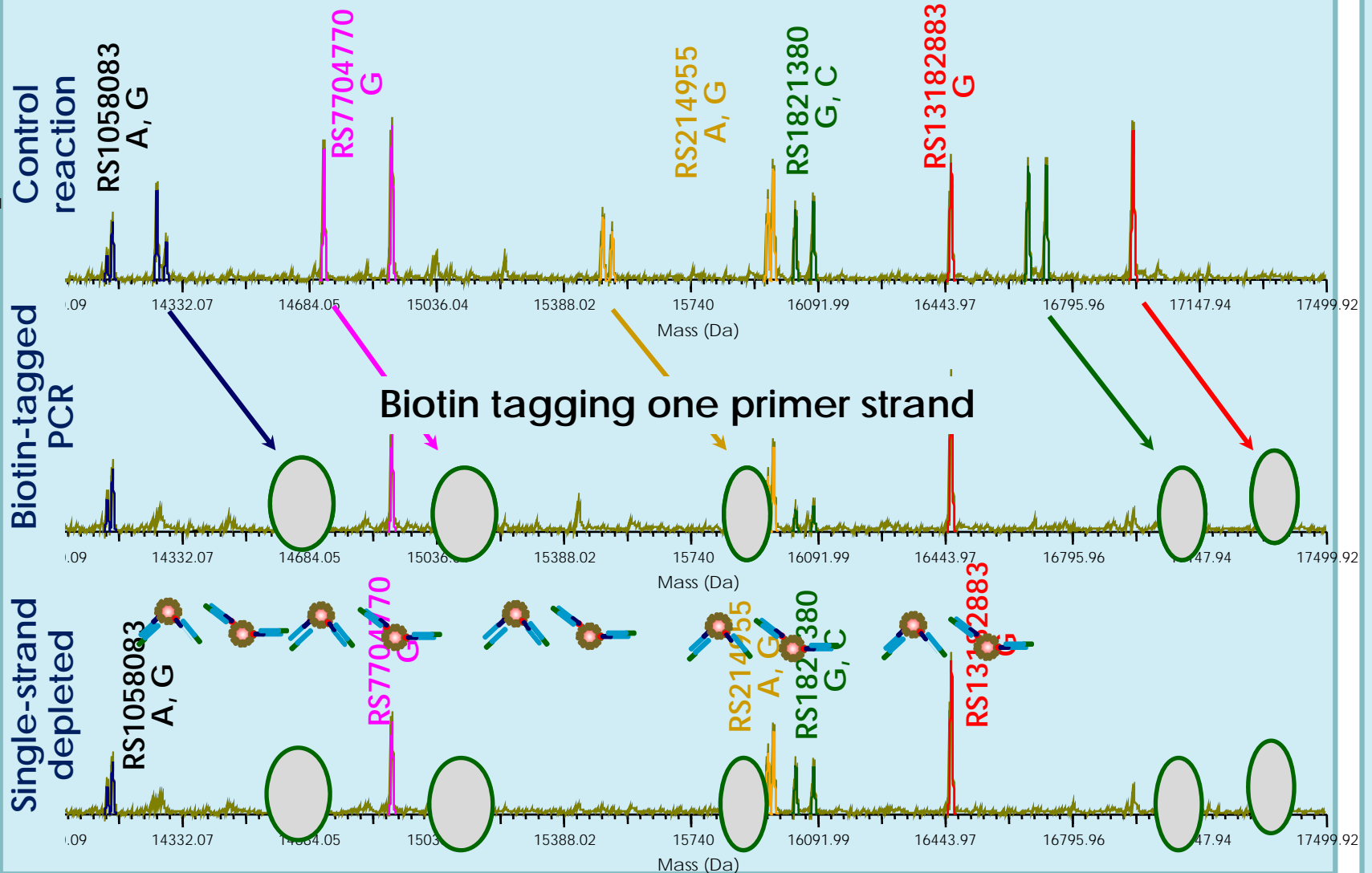
Reducing Spectral Complexity

Magnetic beads are pulled to bottom of wells with a magnet

Single DNA strands are left to be analyzed by MS



Single-stranded Multiplex Products, Rxn 1



Single-stranded Multiplex Profiles

Locus	No streptavidin beads						50 ug/well streptavidin beads					
	Normal primers		Biotin-tagged 1:7		Biotin-tagged 1:15		Normal primers		Biotin-tagged 1:7		Biotin-tagged 1:15	
	1	2	1	2	1	2	1	2	1	2	1	2
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	no data	no data	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, ---	no data	no data	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	no data	no data	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	no data	no data	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, ---	no data	no data	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

Double-stranded products produced

Single-stranded products produced

Technology Transition Workshop



Summary

- **Basic 5-plex and 10-plex, 40-Kidd-SNP assays defined and moving into validation**
- **Limit of sensitivity down to 63 – 125 pg / reaction**
- **Mammal, fungal or bacterial DNA does not appear to interfere with the assay**
- **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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