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Overview of the Ibis™ Y-STR Assay

Y-STR Markers

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

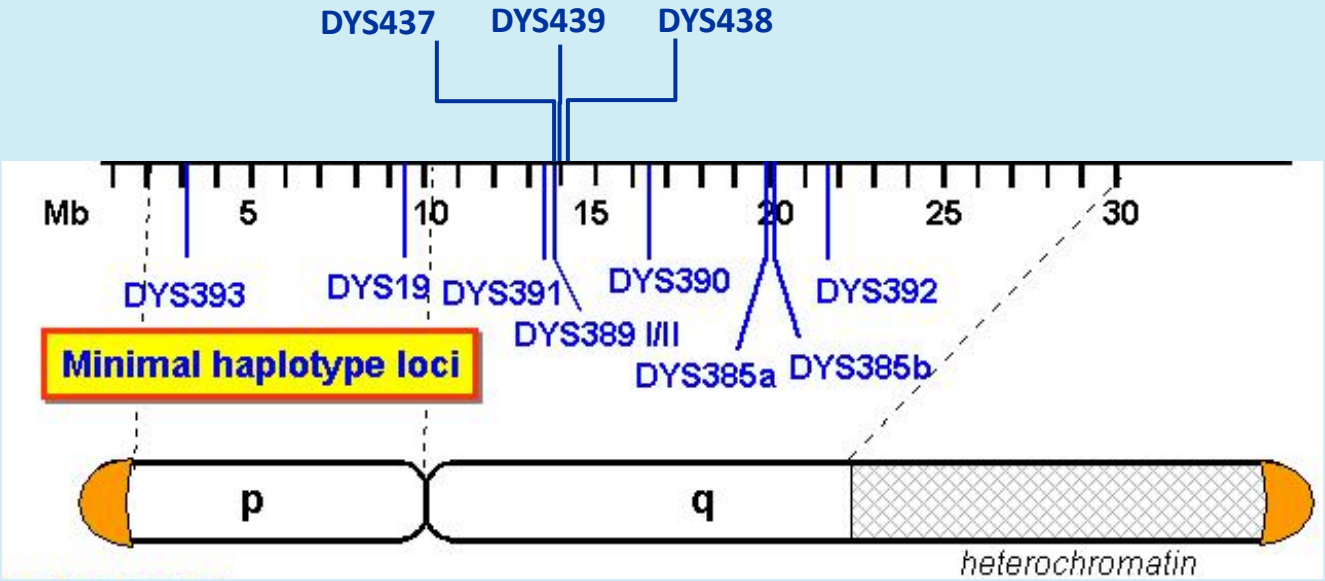

Image adapted from:
http://www.cstl.nist.gov/biotech/strbase/ystrpos1.htm

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Additional Y-STR Loci Being Considered

- DYS456
- DYS458

} added in current assay configuration

- DYS448
- DYS635
- Y-GATA-H4

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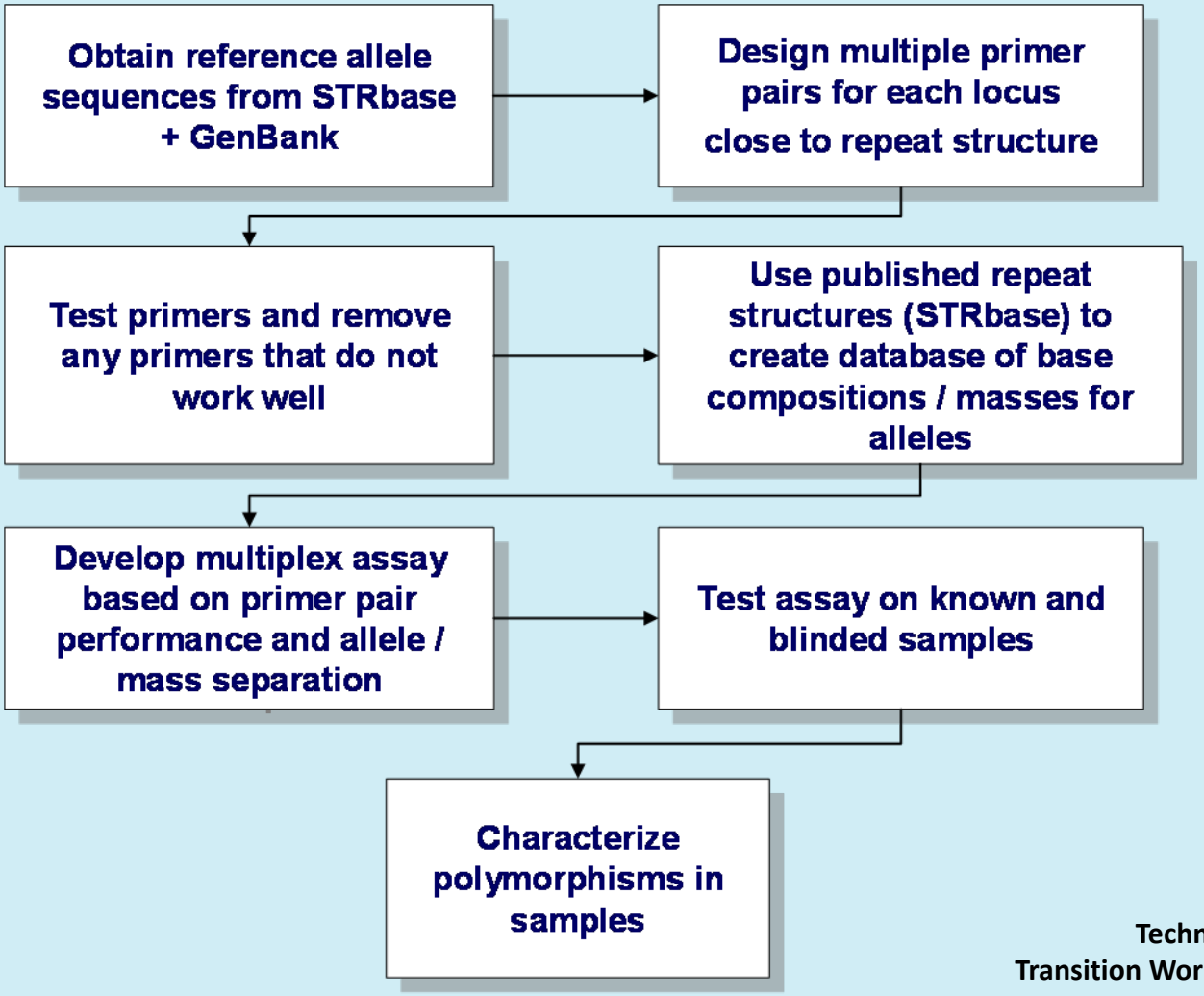
Approach to Y-STR Assay Development



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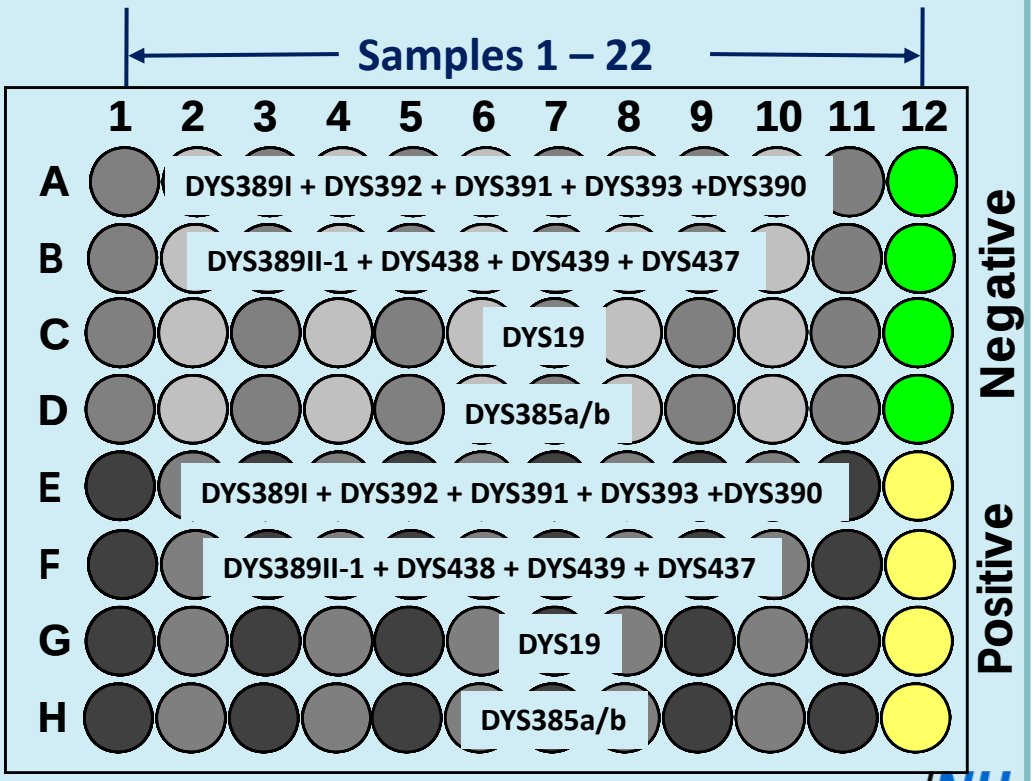
Original Y-STR Assay Layout

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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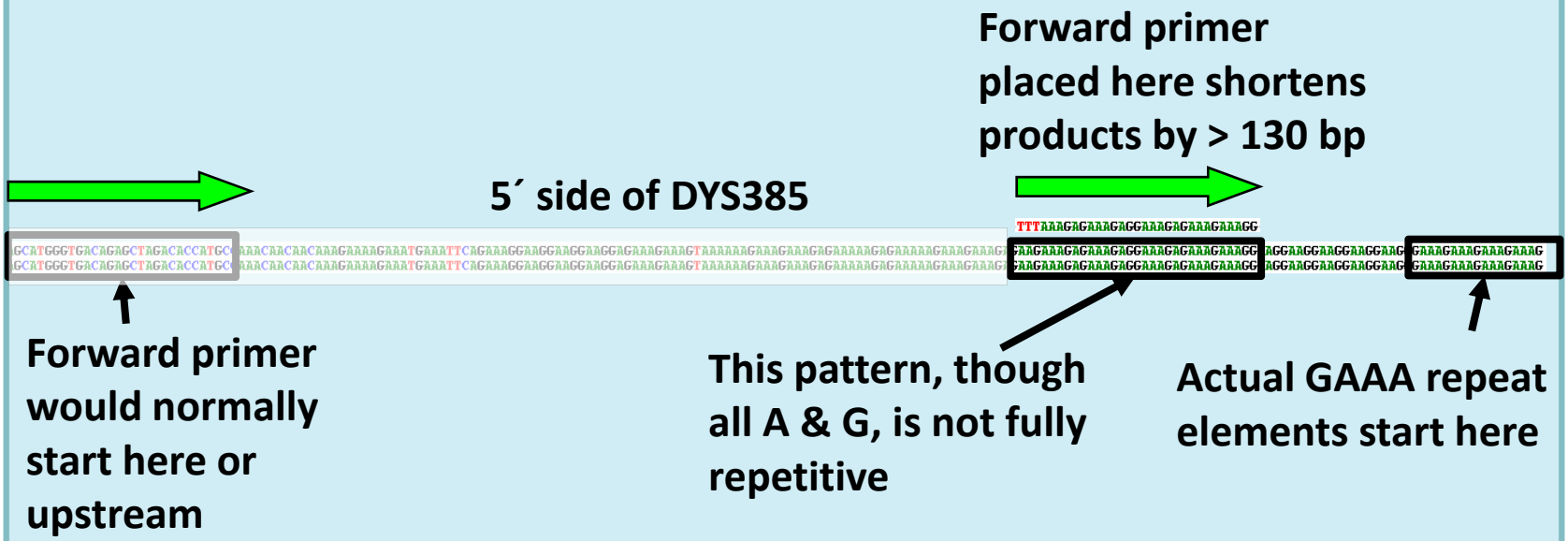
Reducing the Size of DYS385a/b

- 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 99 samples

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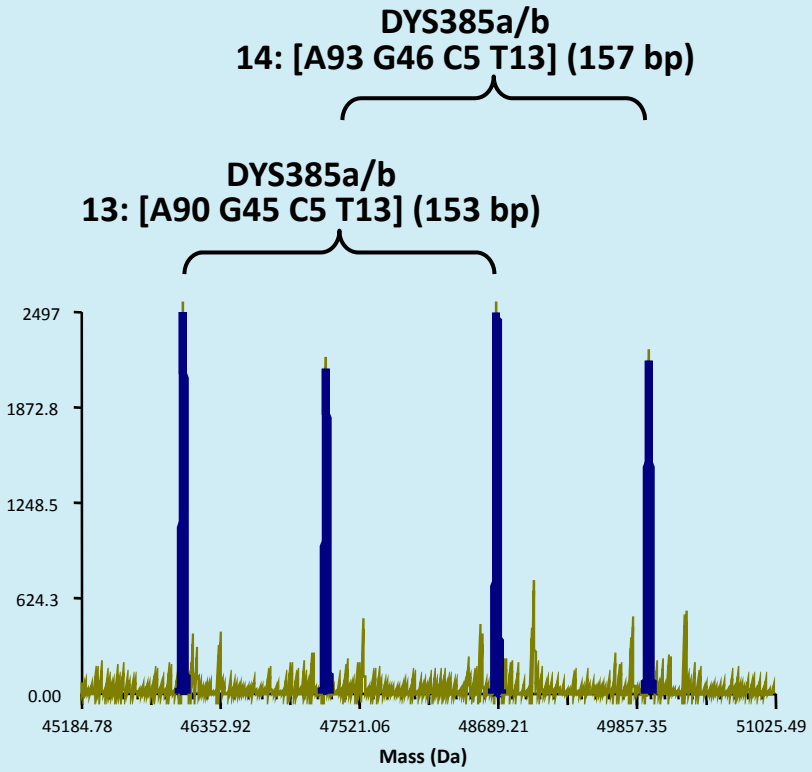
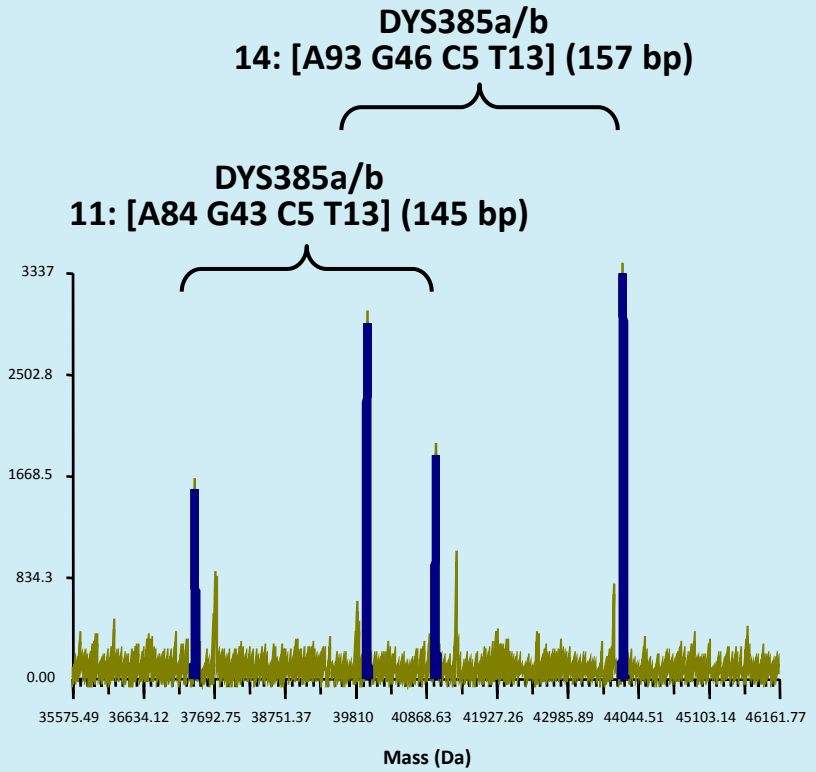
Reducing the Size of DYS385a/b

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

072109C



SC35495



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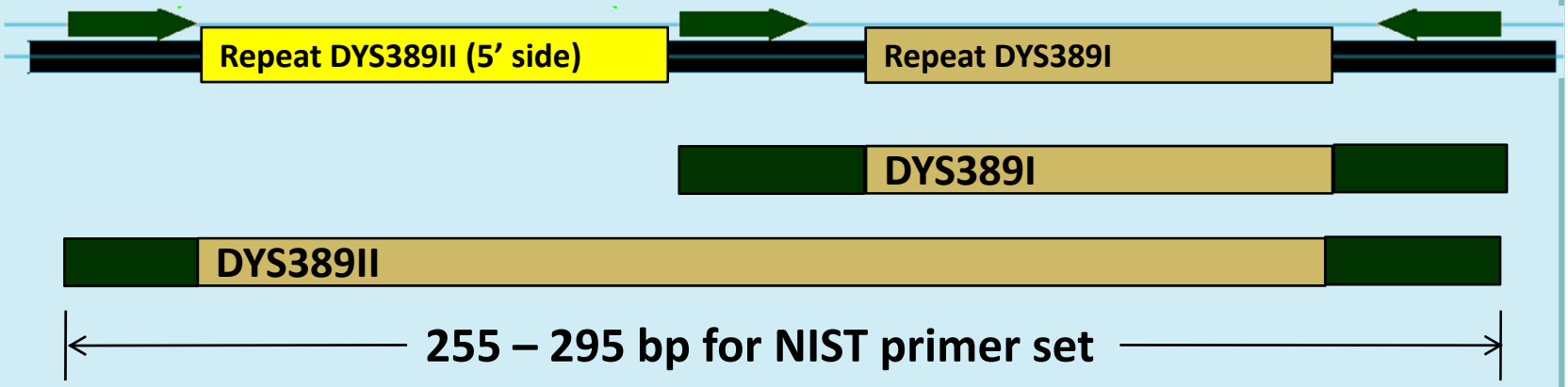
Splitting DYS389I/II into Separate Products

- 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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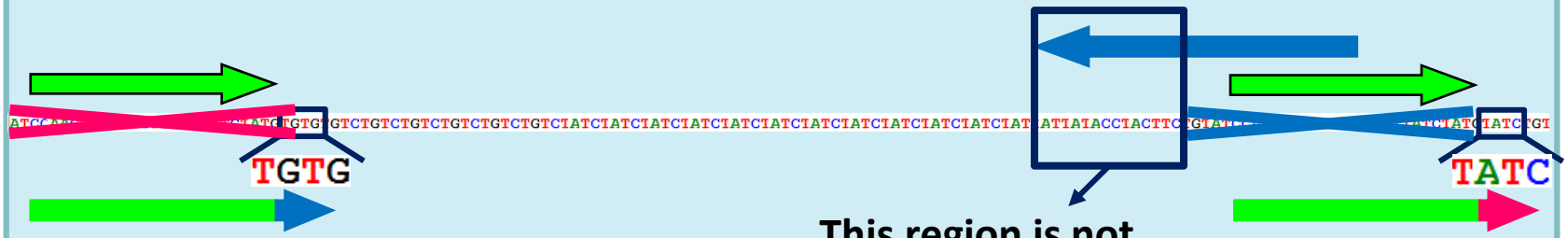
Splitting DYS389I/II into Separate Products

- **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**



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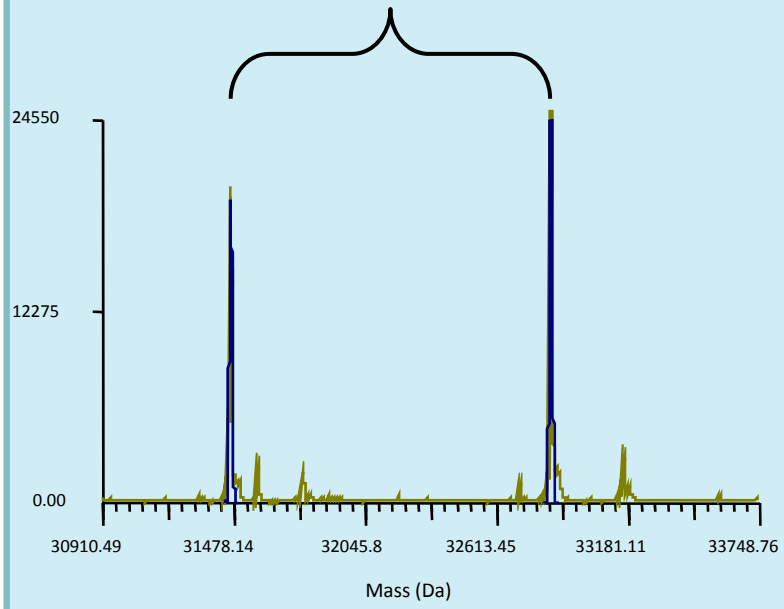
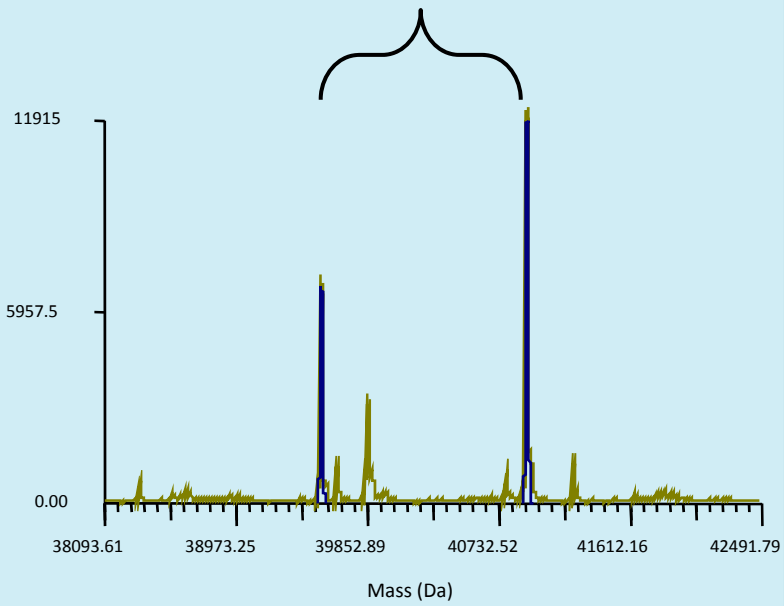
Splitting DYS389I/II into Separate Products

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**
- **92 samples had previous Y-STR truth data**
- **All loci were concordant**
- **One locus (DYS393) also primed X-chromosome and has been replaced**
  - **A primer pair has been developed for this locus that does not prime the X-chromosome**

Locus	Number of different alleles with SNPs seen	Percentage of samples with variant alleles
DYS385a/b	3	3.2
DYS389II	5	33.7
DYS390	6	7.4
DYS437	3	25.3
DYS438	1	1.1

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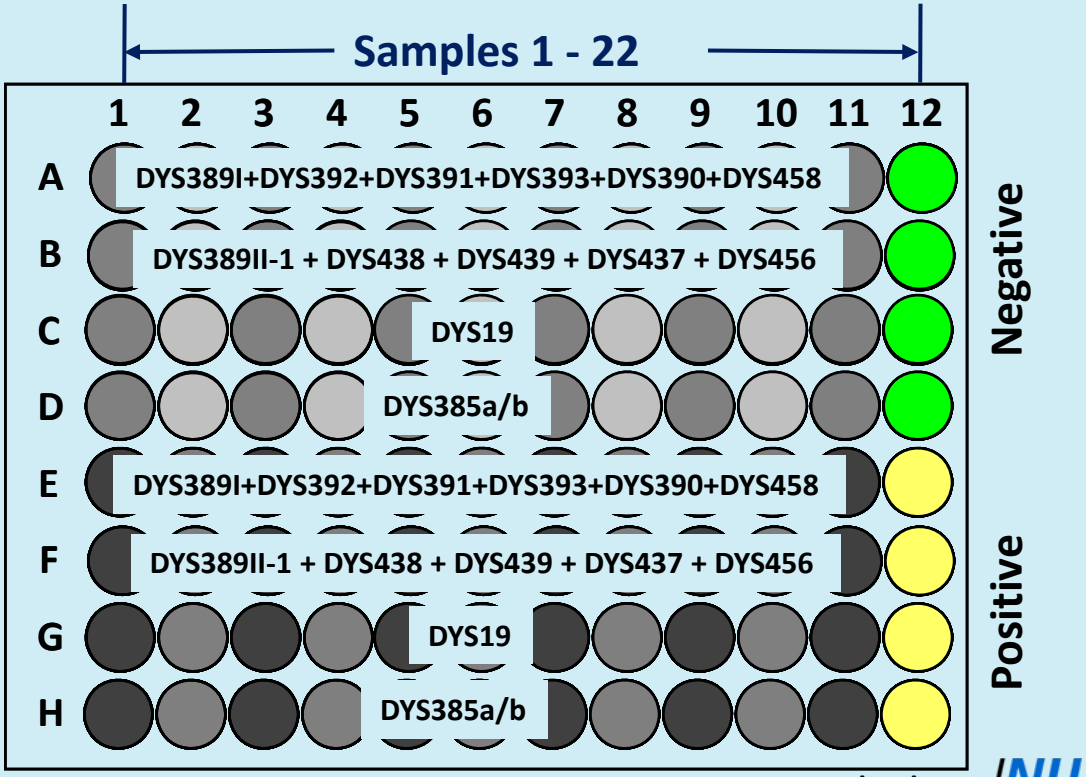
Updated Y-STR Assay Layout

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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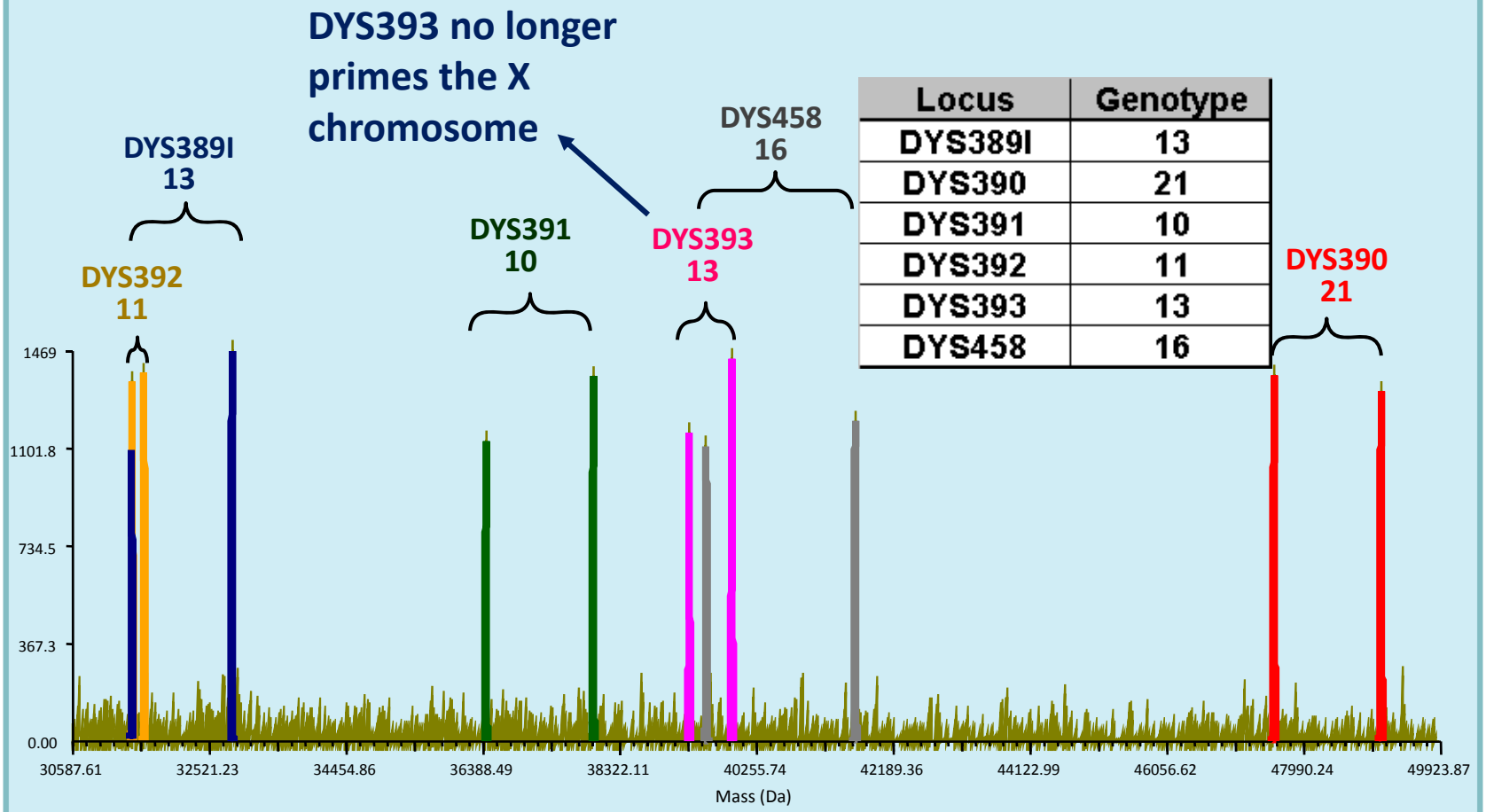
6-plex Reaction 1: Sample N31773



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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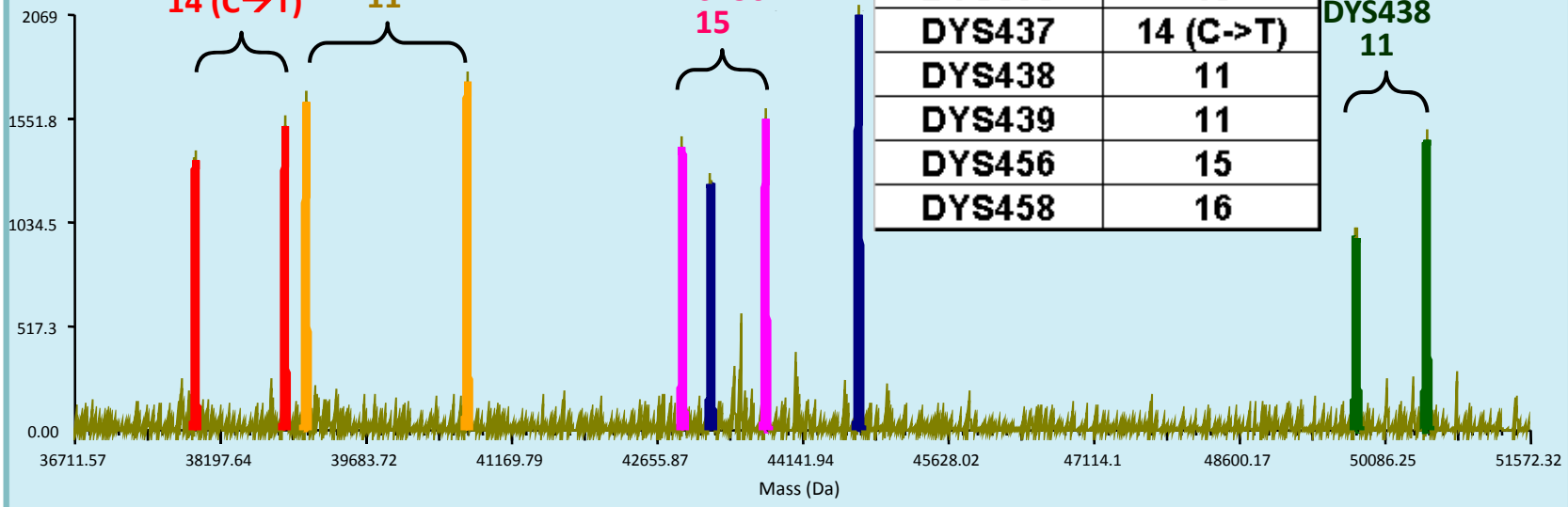

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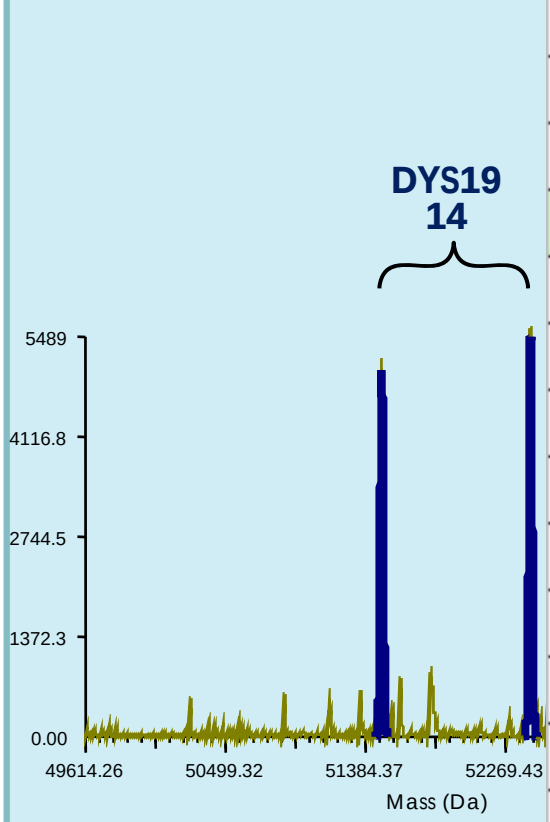
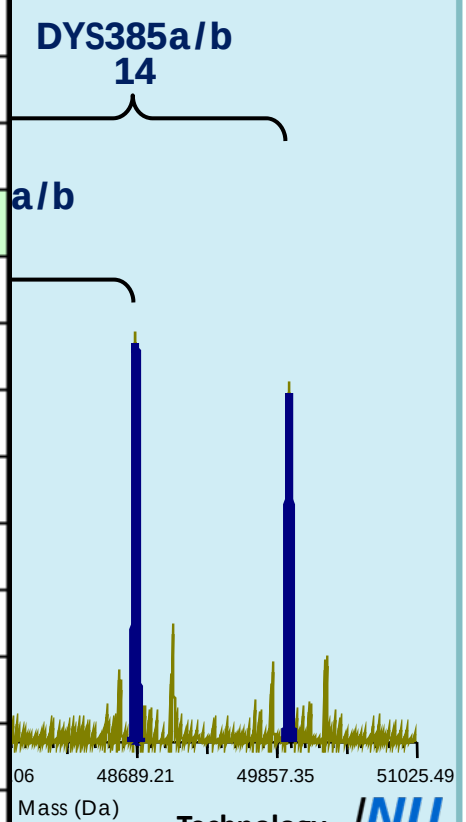
Single Reactions 3 and 4: Sample N31773

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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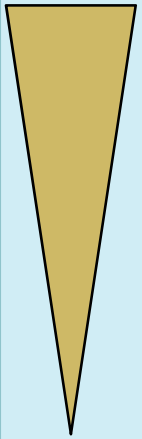
Sensitivity

- **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
31.25	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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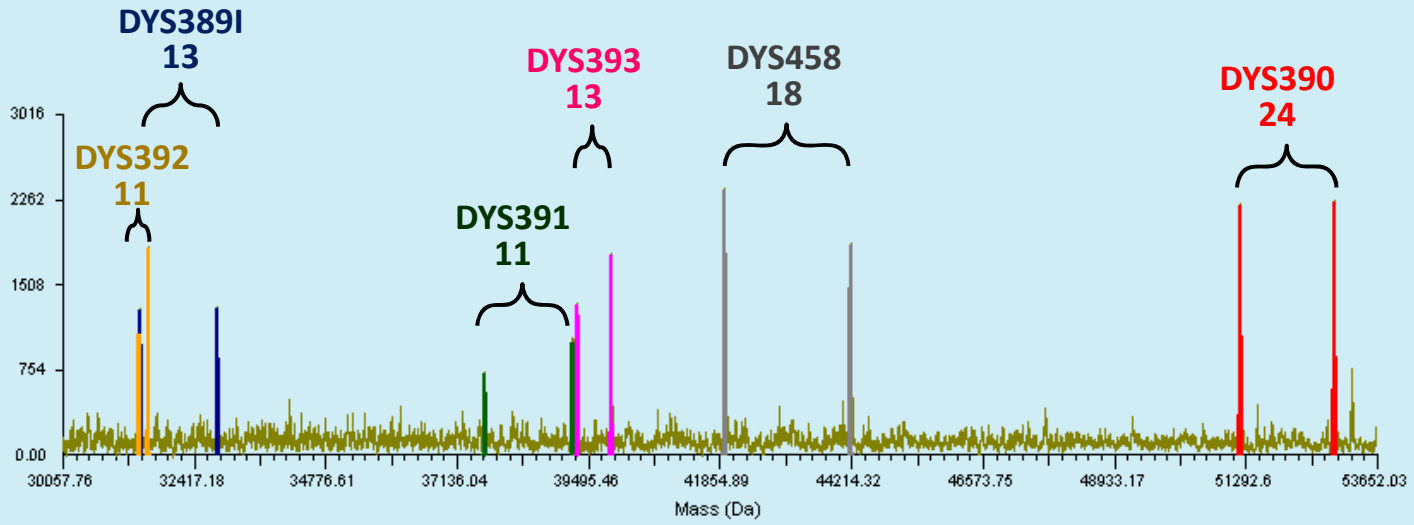
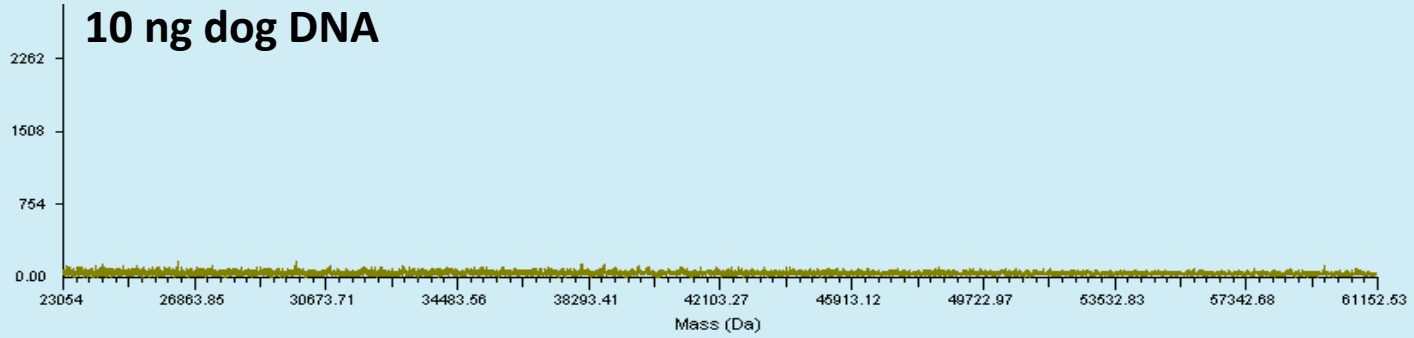
Species Specificity – 6-plex Reaction 1

1 ng human DNA + 10 ng dog DNA





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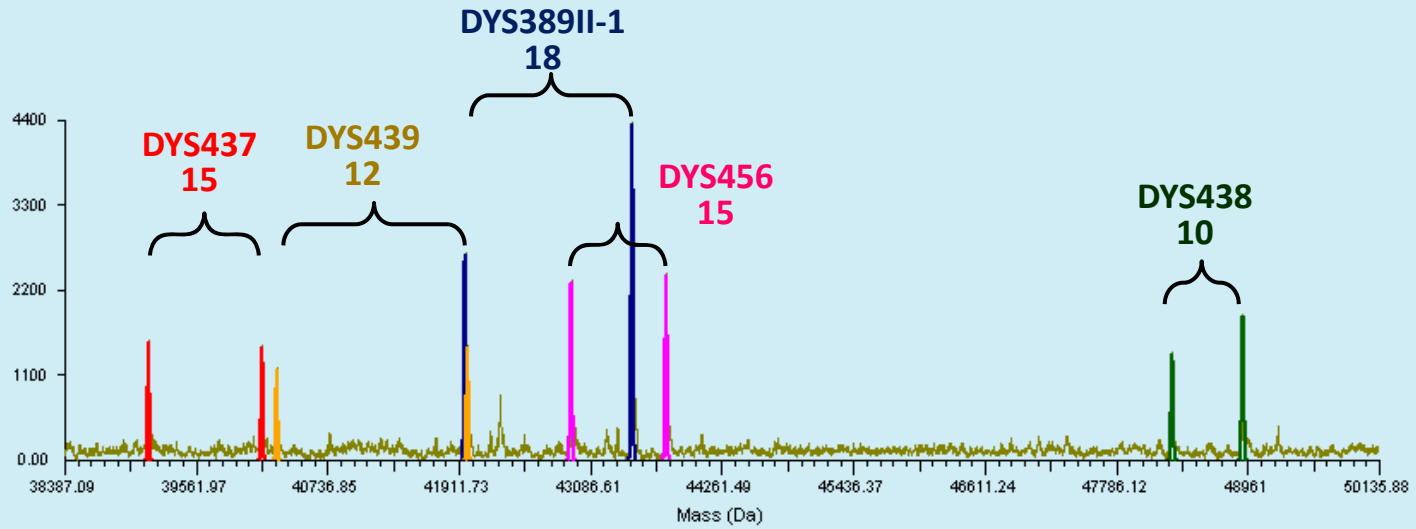
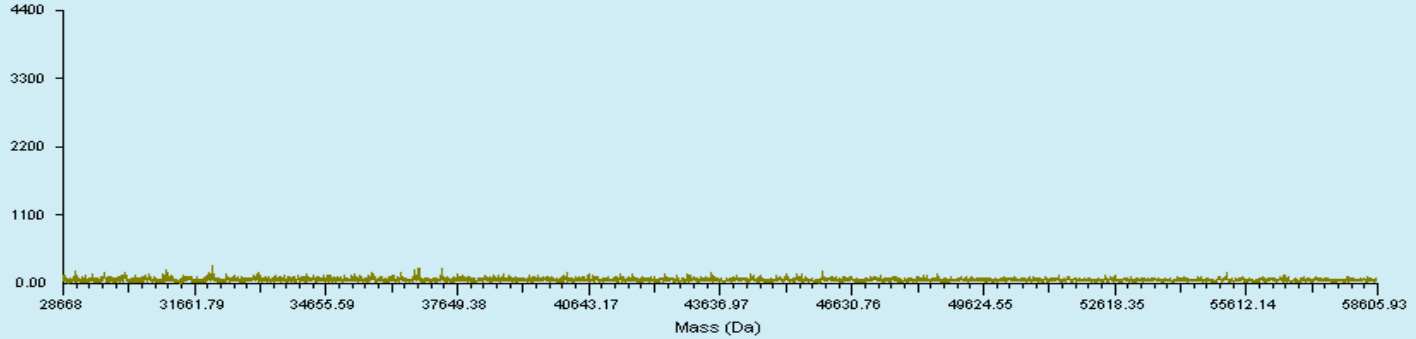
Species Specificity – 5-plex Reaction 2

1 ng human DNA + 10 ng dog DNA



10 ng dog DNA



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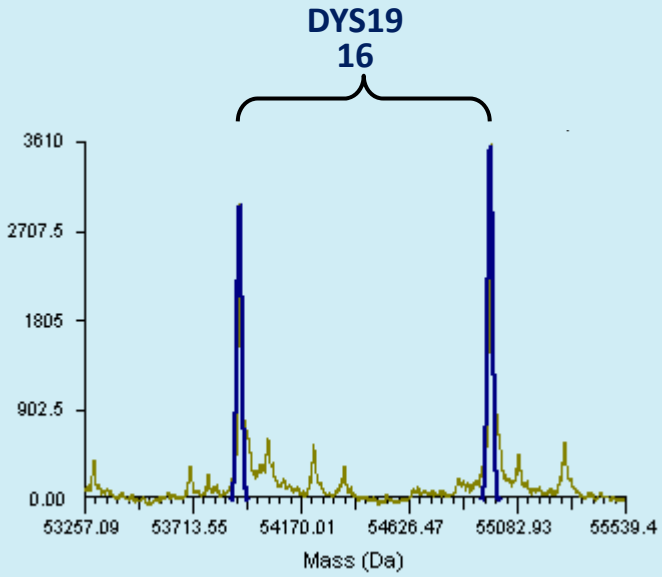
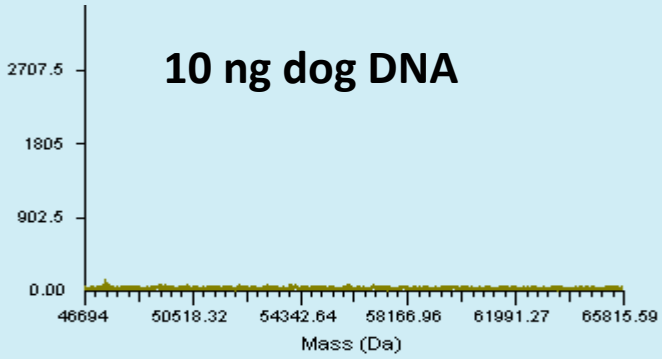
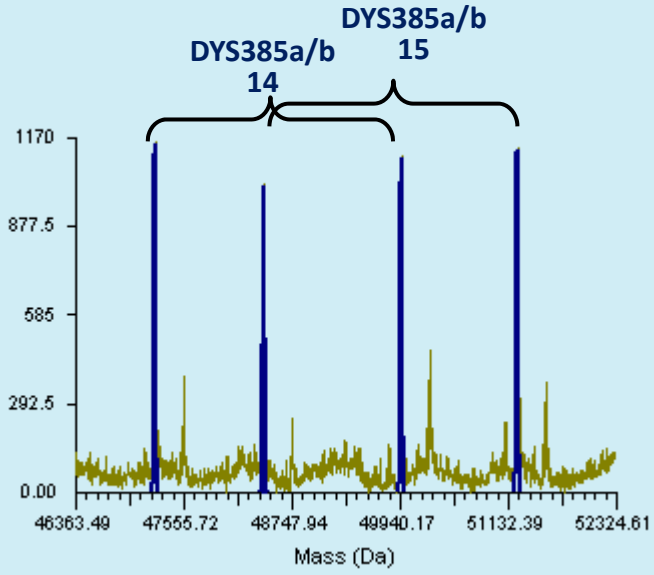
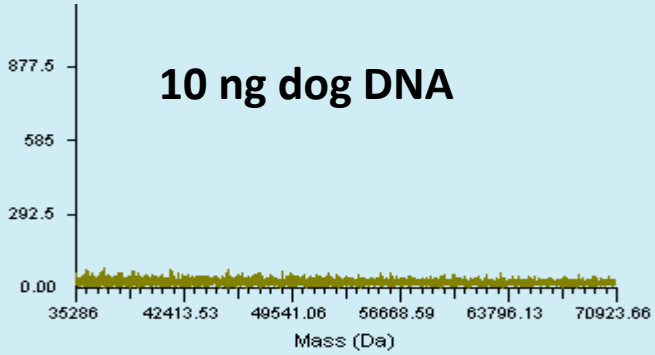
Species Specificity – Single Reactions 3 and 4

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
	16	14, 15	13	18	24	11	11	13	15	10	12	15	18
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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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Exogenous DNAs did not interfere with full profile detection

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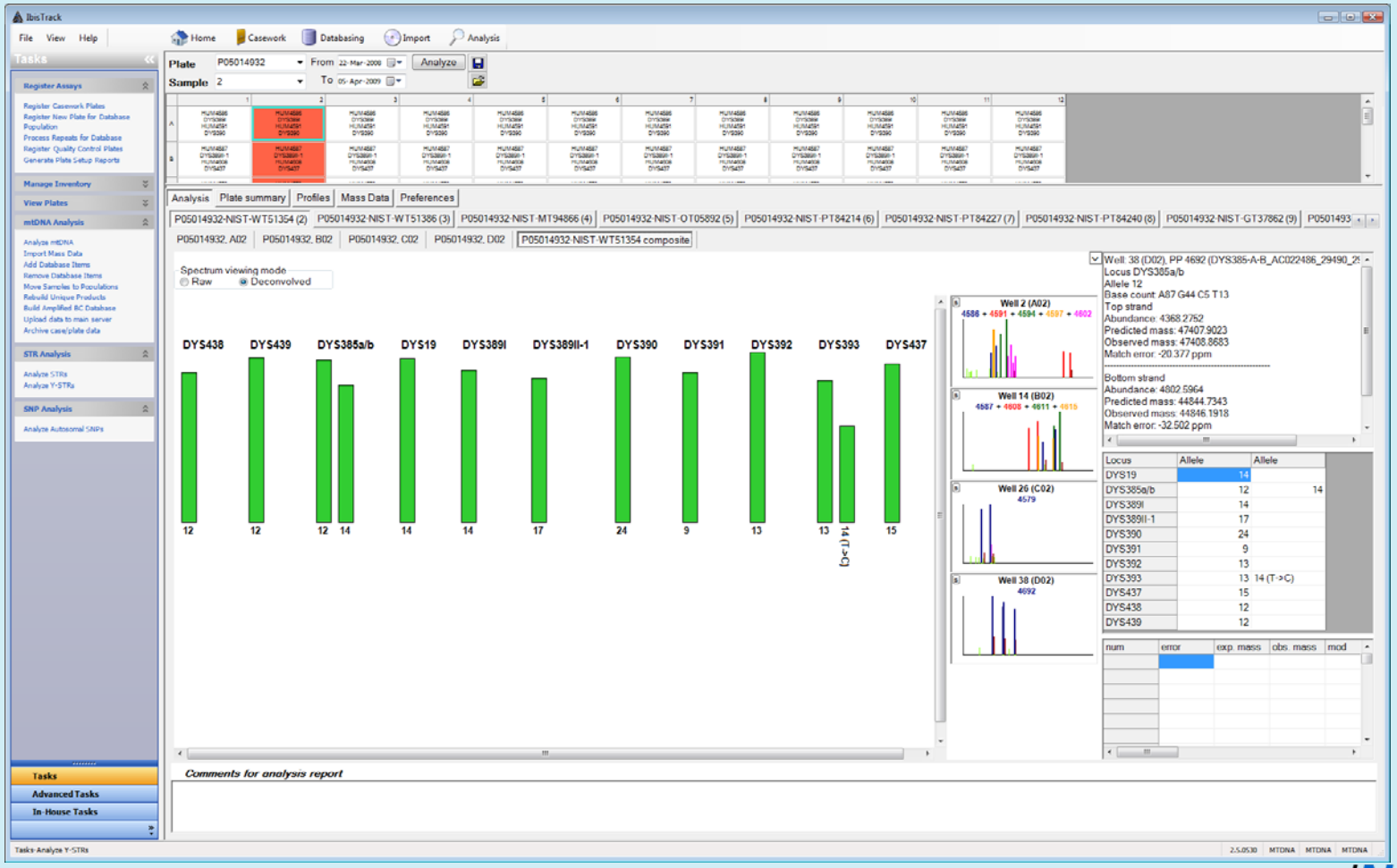
Software and Database in Place for Y-STRs



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Moving Forward

- **Work DYS635 and Y-GATA-4H into multiplex reactions**
- **Refine product balance in multiplexes**
- **Further concordance studies**
- **Pilot kit testing**
- **Sensitivity with multiple templates**
- **Reproducibility studies**
- **Kit stability**
- **Continued refinement of software interface**

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Questions?

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Contact Information

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thall@ibisbio.com

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

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