



## B87 Validation and Implementation of Y-Plex™ 6 for Forensic Casework

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The goal of this presentation is to discuss the validation of the Y-PLEX™ 6 (ReliaGene Technologies, Inc.) and its implementation for forensic casework.

Many sexual assault cases require the testing of intimate samples that are a mixture of male and female body fluids. In some cases, only the female profile is detected using standard autosomal STR systems even when sperm/semens are identified.

A Y-chromosome STR system significantly overcomes the problems associated with large female to male DNA ratios that can lead to poor/no amplification of the perpetrator's DNA. The ability to selectively target the male contributor(s) can be a substantial tool for the DNA analyst in these cases.

ReliaGene Technologies, Inc., has developed a multiplexed Y-chromosome STR system (Y-PLEX™ 6). This system amplifies 6 Y-STR markers (DYS393, DYS19, DYS389, DYS390, DYS391, and DYS385) in a single multiplexed reaction.

DNA from blood samples (CT Sex Offender Database) was extracted using the QIAmp Blood Kit (QIAGEN). The extracts were quantitated using the QuantiBlot Kit (Applied Biosystems, Inc.). Optimally, ~1 ng of DNA was amplified for 30 cycles according to the manufacturer's protocols. The amplification reaction (total volume 25 ul) consisted of DNA (50pg->100ng), 5.0 ul of primer set mix (ReliaGene Technologies, Inc.), and 0.5 ul of AmpliTaq Gold (Applied Biosystems, Inc.). Reducing the reaction volume by 1/2 did not significantly affect the performance of the kit. The addition of BSA (1 ul of 1.6 mg/ml) improved the amplification efficiency of many field samples, especially mixtures. BSA was generally not necessary for the amplification of known samples. Amplifications were performed using standard thermocyclers (Applied Biosystems, Inc., models 2400 and 9700). The amplified products were separated on a 377 DNA Sequencer (Applied Biosystems, Inc) and analyzed using Genescan and Genotyper software. A custom Genotyper macro is supplied with the Y-PLEX™ 6 kit.

To evaluate the discrimination power of the Y-PLEX™ 6 STR markers, a population database was generated for standard Connecticut populations (Caucasian, African American, and Hispanic). Overall, out of 787 males, 55% of the haplotypes (435) were unique, with the most common haplotype detected in 32 males (4.10%). Within populations, 54%-74% of the haplotypes were unique (African American-74%, Caucasian-65%, Hispanic-54%). The most common haplotypes ranged from ~2%-6% (African Americans-1.87%, Caucasians-6.17%, Hispanics-5.78%—see tables below).

The Y-PLEX™ 6 validation study also examined the following issues: species specificity (common domestic animals (males & females), reproducibility, the effects of environmental degradation,

DYS 19	12	1	0.41
	13	23	9.47
	14	144	59.26
	14.2	1	0.41
	15	43	17.70
	16	21	8.64
	17	10	4.12
		243	100.00
DYS 389	27	3	1.23
	28	38	15.64
	29	104	42.80
	30	70	28.81
	31	19	7.82
	32	9	3.70
			243
DYS 390	21	2	0.82
	22	30	12.35
	23	68	27.98
	24	100	41.15
	25	41	16.87
	26	2	0.82
			243
DYS 391	9	10	4.12
	10	112	46.09
	11	115	47.33
	12	5	2.06
	13	1	0.41
			243
DYS 385	8	1	0.21
	10	5	1.03
	11	130	26.80
	12	35	7.22
	11,12,14	1	0.21
	13	42	8.66
	14	150	30.93
	15	51	10.52
	16	32	6.60
	17	20	4.12
	17.3	1	0.21
	18	11	2.27
	18.3	1	0.21
	19	4	0.82
	20	1	0.21
		485 <sup>1</sup>	100.00

### AFRICAN AMERICAN

	ALLELE	# OF TIMES	%
DYS393	12	13	4.87
	13	134	50.19
	14	89	33.33
	15	29	10.86
	16	1	0.37
	17	1	0.37
		267	100.00
DYS 19	12	0	0.00
	13	8	3.00
	14	58	21.72
	14.2	0	0.00
	15	96	35.96
	16	61	22.85
	17	43	16.10
18	1	0.37	
		267	100.00

### CAUCASIAN

	ALLELE	# OF TIMES	%
DYS393	12	30	12.35
	13	175	72.02
	14	31	12.76
	15	7	2.88
		243	100.00

sample mixtures, stutter, peak balance at DYS385, kit sensitivity, and nonprobative casework samples. These results along with the success of the method with casework samples are discussed.



# Criminalistics Section – 2004

<b>DYS 389</b>	27	5	1.87
	28	21	7.87
	29	53	19.85
	30	<b>102</b>	38.20
	31	63	23.60
	32	19	7.12
	33	4	1.50
		267	100.00

<b>DYS 390</b>	20	2	0.75
	21	<b>156</b>	58.43
	22	30	11.24
	23	21	7.87
	24	32	11.99
	25	23	8.61
	26	3	1.12
		267	100.00

<b>DYS 391</b>	9	10	3.75
	10	198	74.16
	11	59	22.10
	12	0	0.00
	13	0	0.00
		267	100

<b>DYS 385</b>	8	0	0.00
	10	2	0.37
	11	37	6.93
	12	9	1.69
	13	16	3.00
	13.2	1	0.19
	14	70	13.11
	15	76	14.23
	16	<b>118</b>	22.10
	17	115	21.54
	18	58	10.86
	19	26	4.87
	20	6	1.12
		534	100.00

## HISPANIC

<b>DYS393</b>	<b>ALLELE</b>	<b># OF TIMES</b>	<b>%</b>
	12	31	11.19
	13	<b>202</b>	72.92
	14	34	12.27
	15	10	3.61
		277	100.0

<b>DYS 19</b>	12	0	0.00
	13	46	16.61
	14	<b>153</b>	55.23
	15	45	16.25
	15/16	3	1.08
	16	16	5.78
	17	14	5.05
		277	100.0

<b>DYS 389</b>	26	1	0.36
	27	5	1.81
	28	30	10.83
	29	94	33.94
	30	<b>99</b>	35.74
	31	37	13.36
	32	11	3.97
	33	0	0.00
		277	100.0

<b>DYS 390</b>	20	1	0.36
	21	37	13.36
	22	33	11.91
	23	59	21.30
	24	<b>121</b>	43.68
	25	24	8.66
	26	1	0.36
	27	1	0.36
		277	100.0

<b>DYS 391</b>	8	1	0.36
	9	30	10.83
	10	145	52.35
	11	97	35.02
	12	4	1.44
		277	100.0

<b>DYS 385</b>	8	0	0.00
	10	5	0.90
	11	100	18.05
	12	28	5.05
	13	90	16.25
	14	<b>146</b>	26.35
	15	55	9.93
	16	44	7.94
	17	33	5.96
	18	39	7.04
	18.3	1	0.18
	19	11	1.99
	20	2	0.36
		554	100.0

1. Three band haplotype detected in one individual-considered a single allele for statistical purposes.

### Most Common Haplotypes (Overall 787 )

	DYS 393	DYS 19	DYS 389	DYS 390	DYS 391	DYS 385	%	N
1	13	14	29	24	11	11,14	4.10	32
2	13	14	29	23	11	11,14	2.16	17
3	13	13	30	24	9	13,14	1.91	15
4	13	14	30	24	11	11,14	1.52	12
5	13	14	29	24	10	11,14	1.40	11

### Most Common Haplotypes (African American - 267)

	DYS 393	DYS 19	DYS 389	DYS 390	DYS 391	DYS 385	%	N	
1	13	15	31	21	10	16,17	1	.87	5
2	13	14	29	24	10	11,14	1.50	4	4
3	14	15	30	21	10	16,17	1.12	3	3
4	13	14	28	25	11	14	1.12	3	3
5	14	17	30	21	10	18	1.12	3	3

\*3 other haplotypes were detected three times each in CT African American males.

### Most Common Haplotypes (Caucasian - 243)

	DYS 393	DYS 19	DYS 389	DYS 390	DYS 391	DYS 385	%	N
1	13	14	29	24	11	11,14	6.17	15
2	13	14	29	23	11	11,14	3.70	9
3	13	14	29	24	10	11,14	1.65	4
4	13	14	29	25	11	11,13	1.65	4
5	13	15	29	24	11	11,15	1.23	3

\*\*5 other haplotypes were detected three times each in CT Caucasian males.

### Most Common Haplotypes (Hispanic - 277)

	DYS 393	DYS 19	DYS 389	DYS 390	DYS 391	DYS 385	%	N
1	13	14	29	24	11	11,14	5.78	16
2	13	13	30	24	9	13,14	4.69	13
3	13	14	30	24	11	11,14	2.89	8
4	13	14	29	23	11	11,14	2.17	6
5	13	14	31	21	11	16,18	2.17	6

### Y-PLEX 6, STRs, PCR