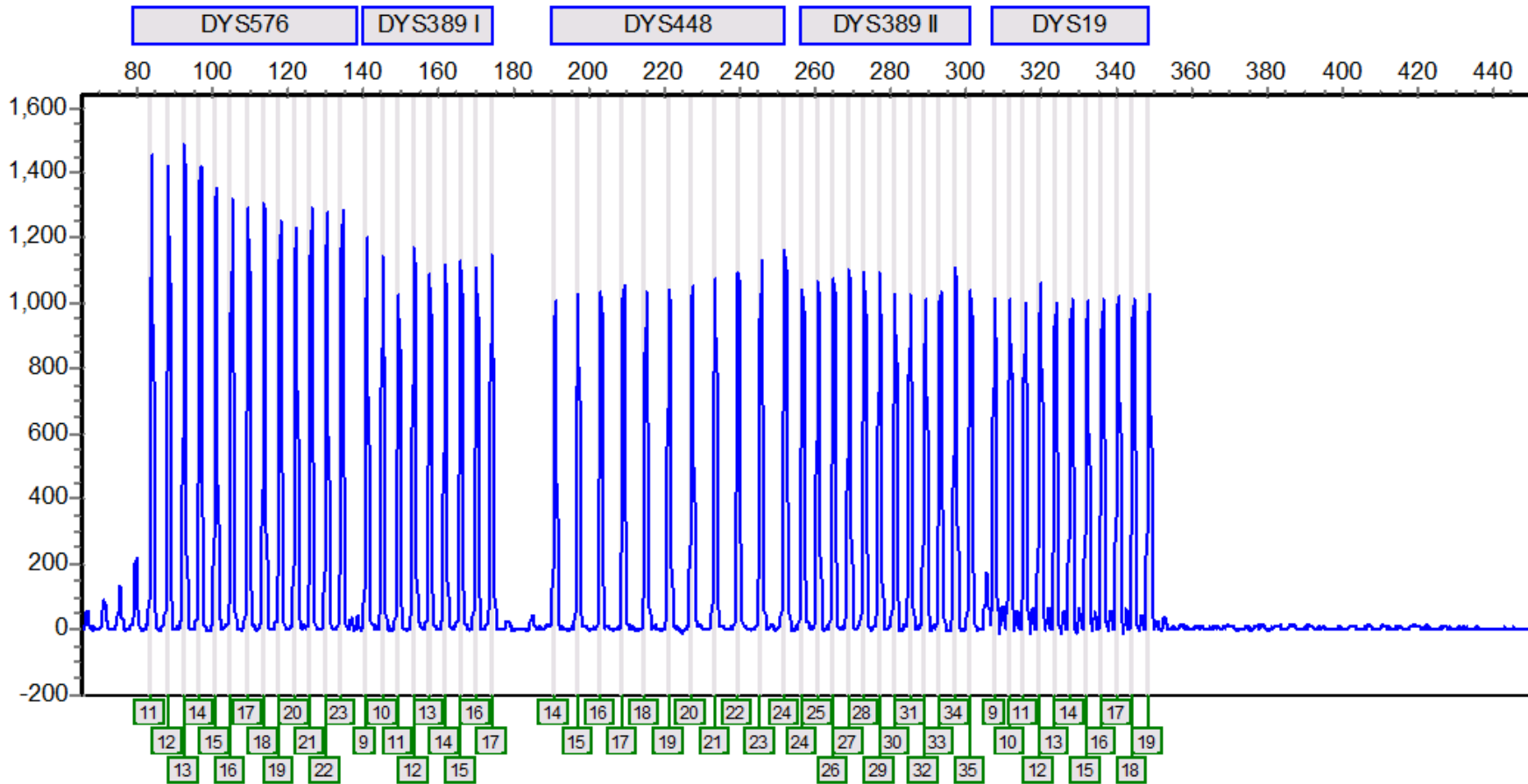


# FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

## Manual Appendix for PowerPlex Y23 on 3500xL

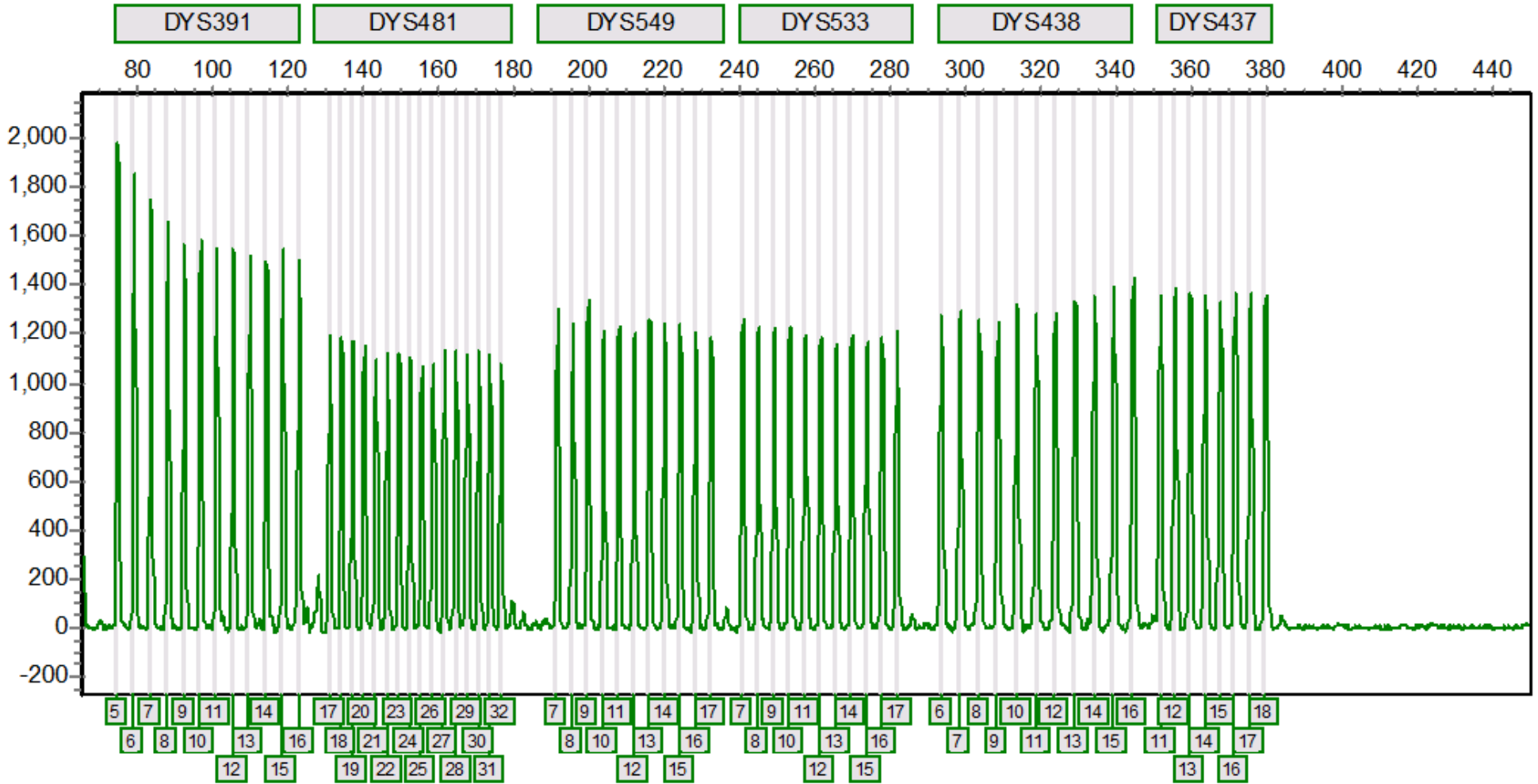
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### 1 a) Allelic Ladder for PowerPlex® Y23



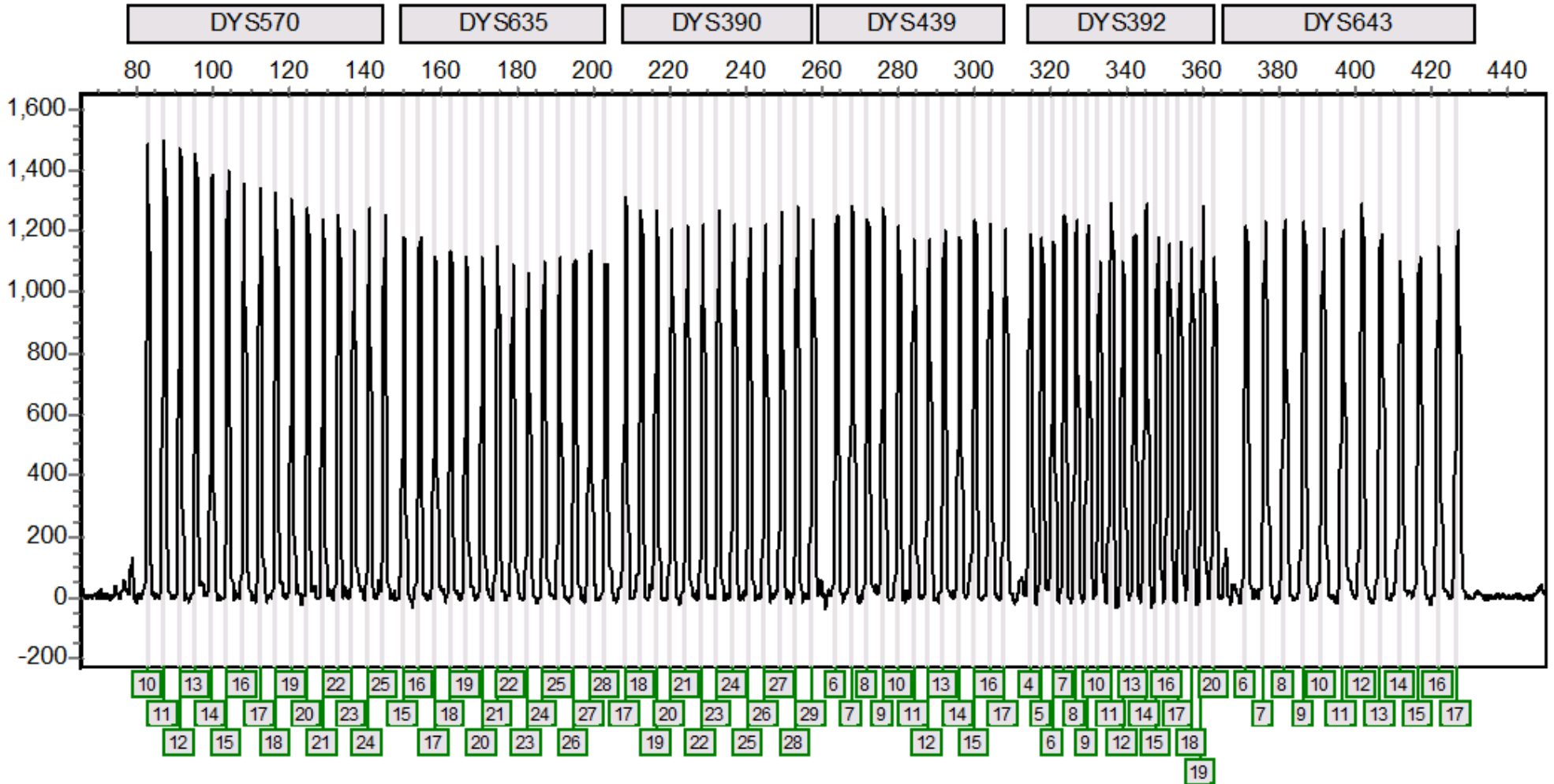
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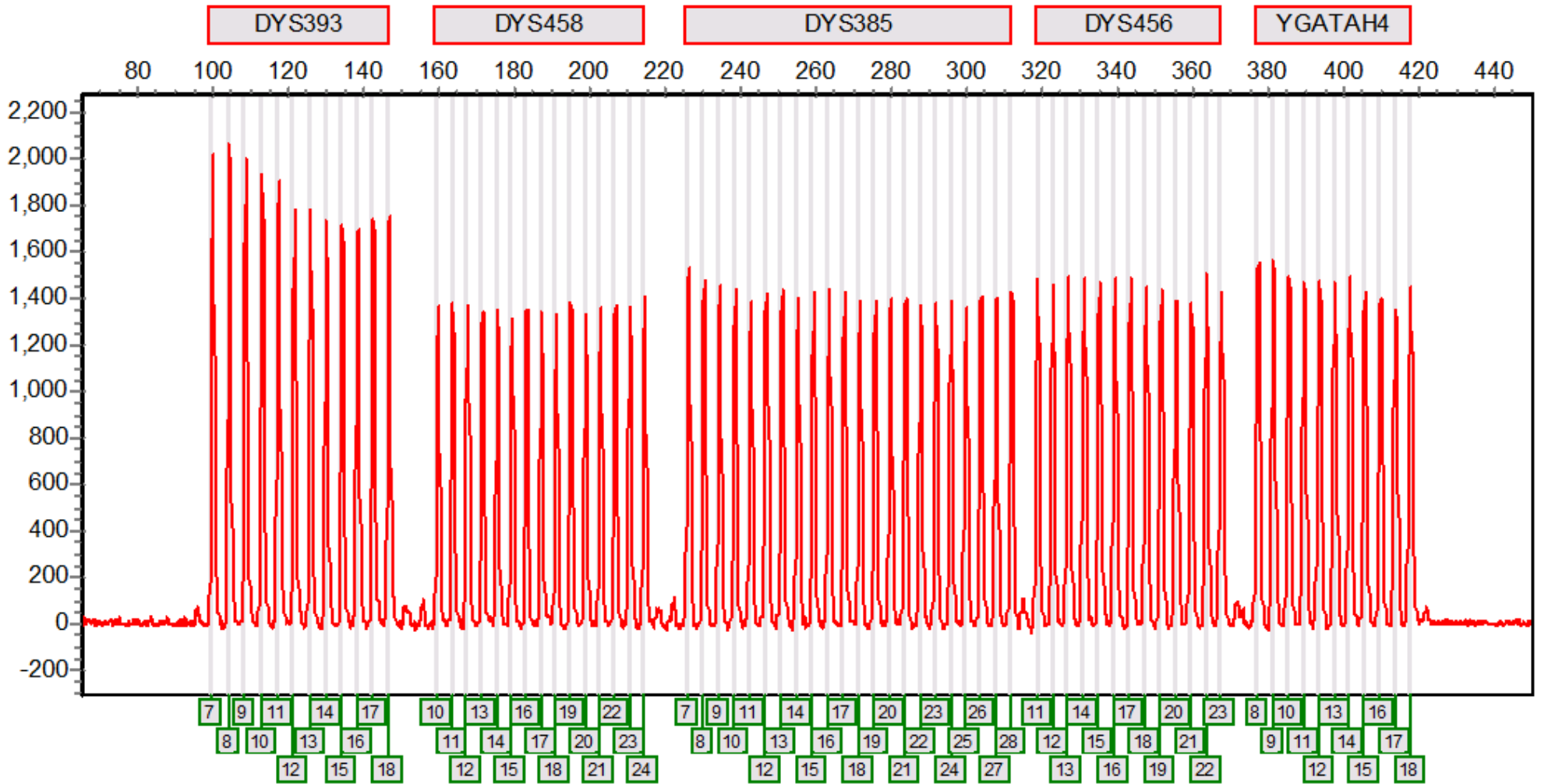
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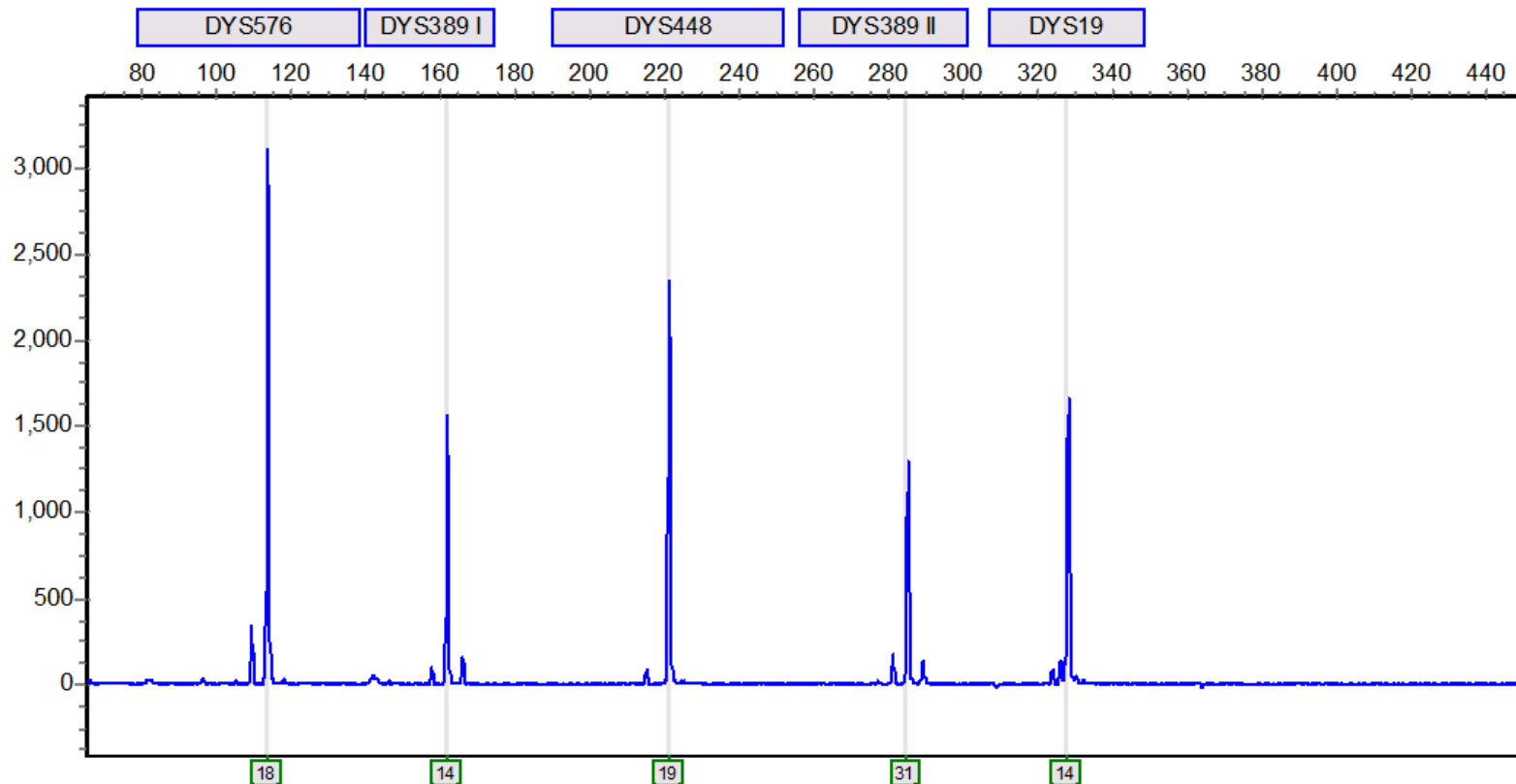


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### b) Positive Control for PowerPlex® Y23

DYS576	DYS389 I	DYS448	DYS389 II	DYS19	DYS391	DYS481	DYS549	DYS533	DYS438	DYS437
18	14	19	31	14	10	22	13	12	9	14
DYS570	DYS635	DYS390	DYS439	DYS392	DYS643	DYS393	DYS458	DYS385	DYS456	YGATAH4
17	21	24	12	13	10	13	17	13, 16	17	11



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## Manual Appendix for PowerPlex Y23 on 3500xL

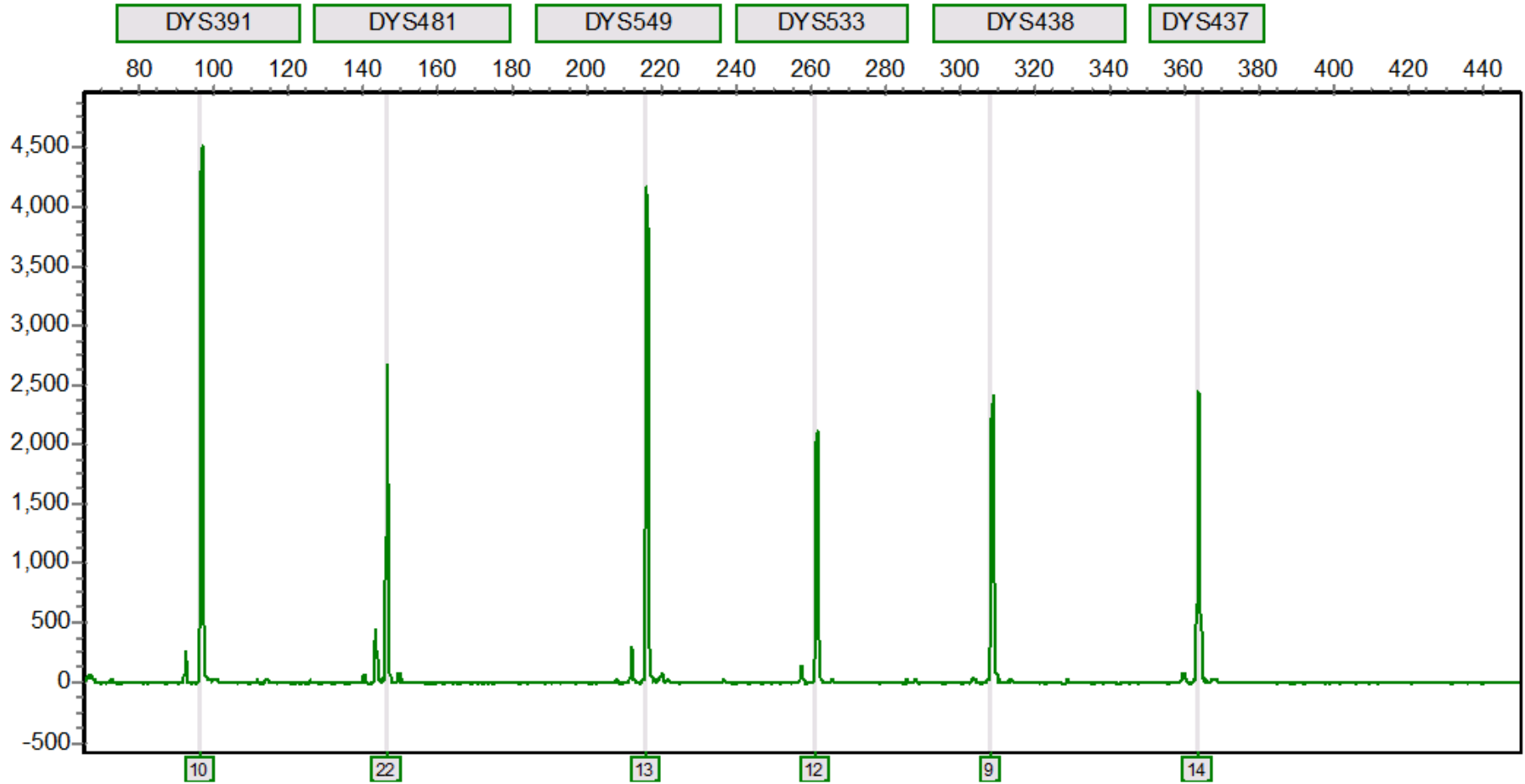
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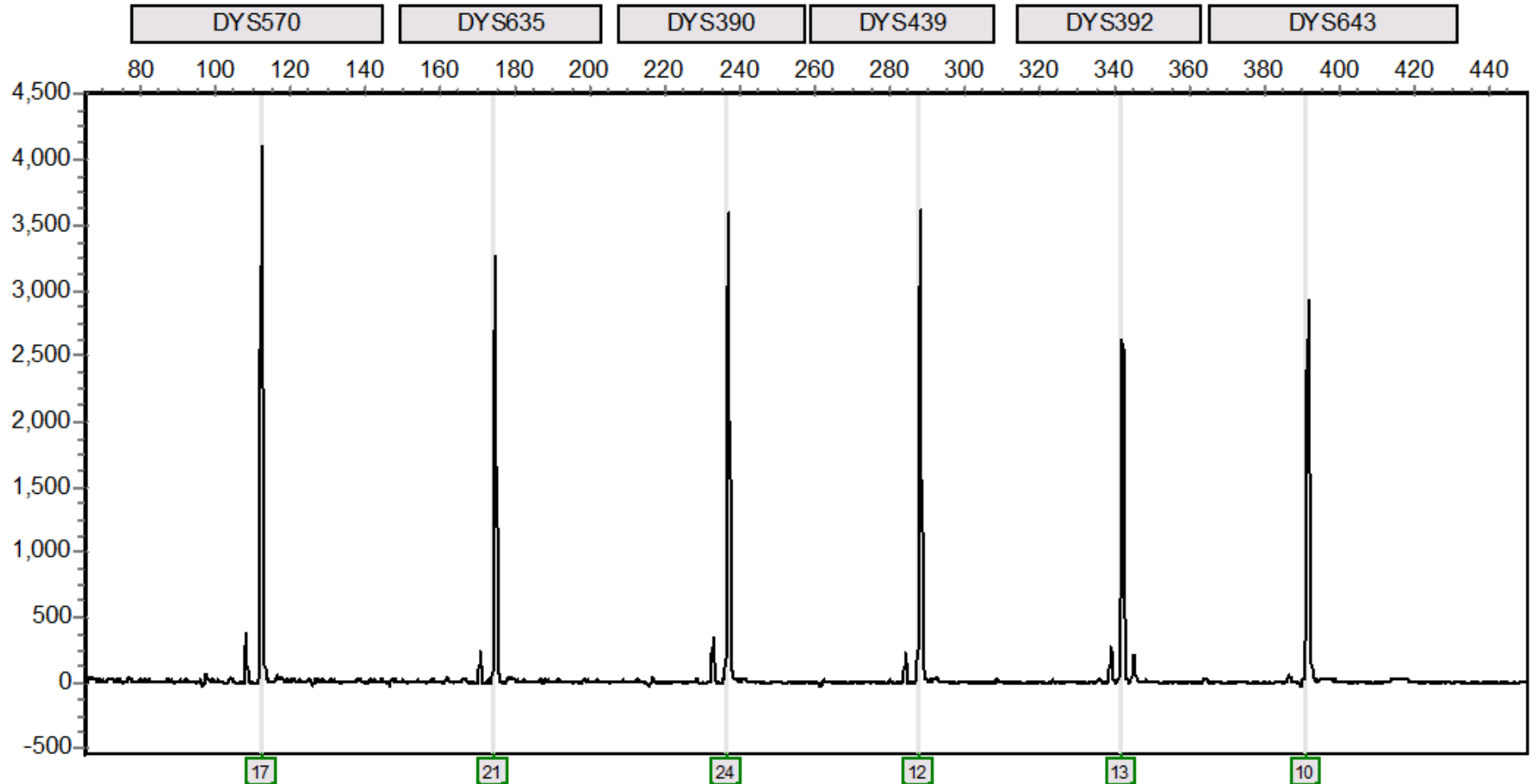
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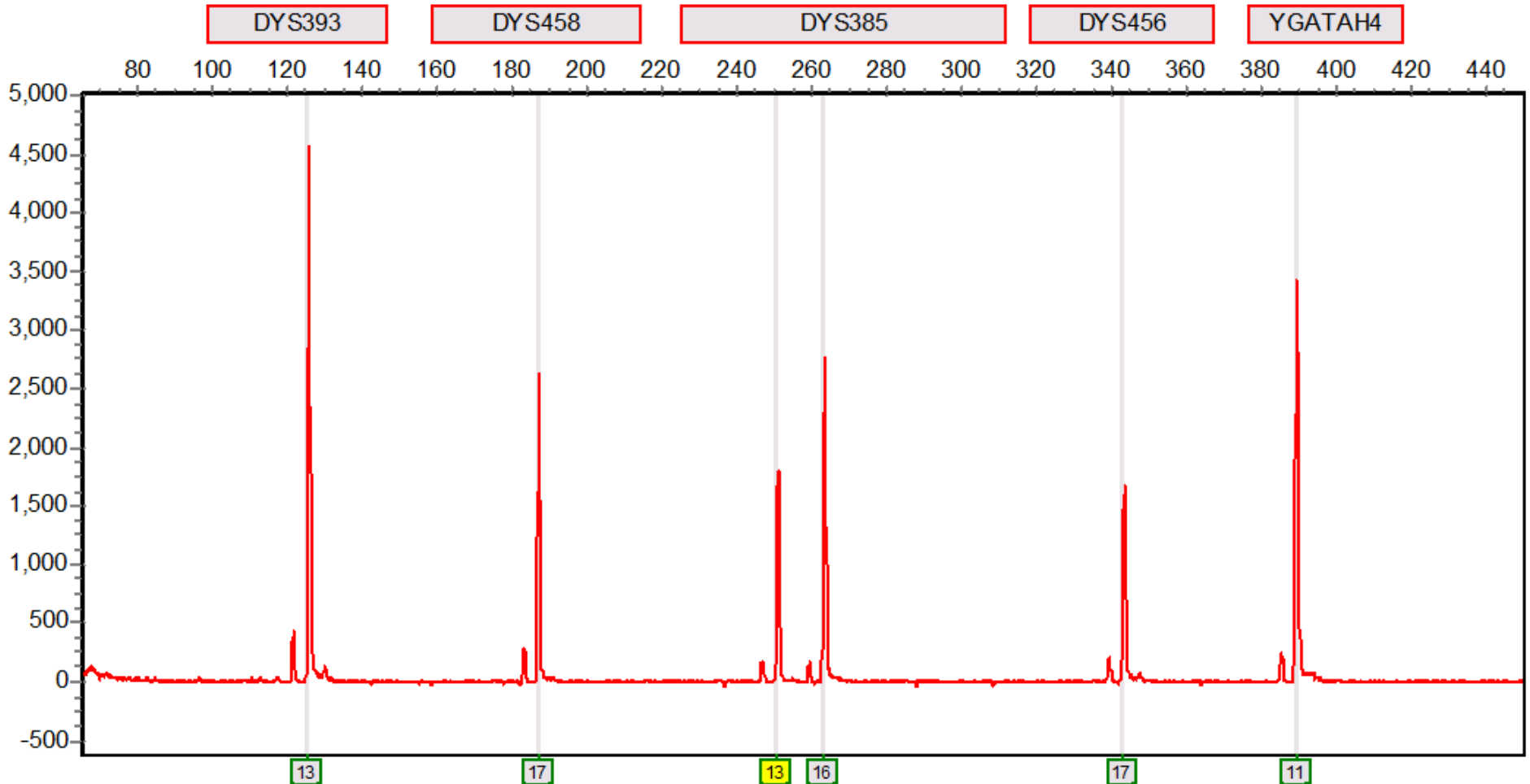
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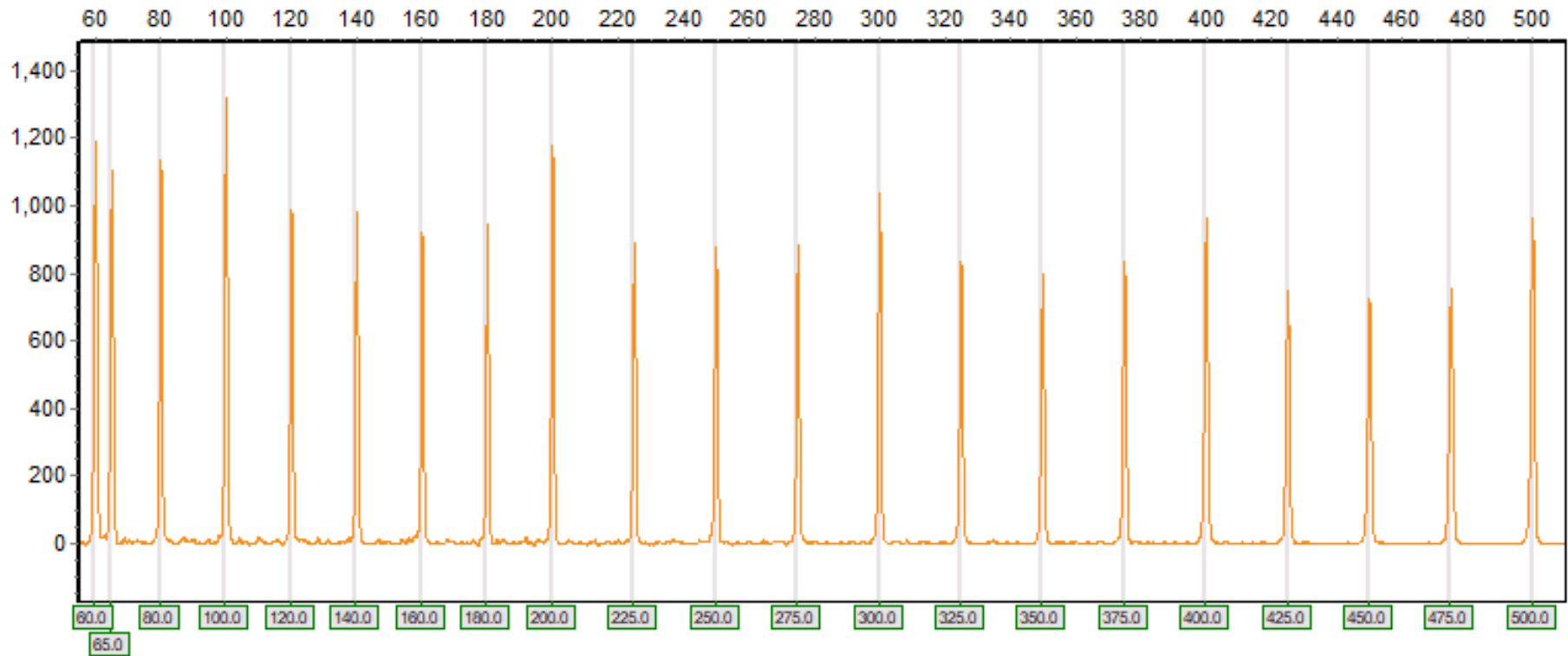


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### c) Size Standard for PowerPlex® Y23



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### 2 Known artifacts

#### 2.1 Known artifacts that are independent of DNA amount, from Promega Technical Manual (4)

Dye	Artifact <sup>1</sup>
Fluorescein	~60–65 bases <sup>2</sup>
	~58–63 bases <sup>2</sup>
	~80–84 bases
	~136–147 bases <sup>3</sup>
JOE	~61–67 bases
	~69–73 bases
	~136–144 bases <sup>3</sup>

<sup>1</sup>Artifact sizes may vary depending on CE instrumentation and environmental conditions in the laboratory.

<sup>2</sup>The signal strength of these artifacts increases with storage of the amplification plate at 4°C, sometimes in as short a time period as overnight but more commonly when plates are left at 4°C for a few days. We recommend storing amplification products at –20°C.

<sup>3</sup>Artifact may appear as a dye blob or a peak in sample reaction and negative control reaction.

#### 2.2 Known artifacts that are dependent of DNA amount, from Promega Technical Manual (4)

Dye	Artifact
Fluorescein	DYS448 n–7 to n–15 <sup>1,2</sup>
	DYS19 n–2; n+2 <sup>3</sup>
JOE	~163 bases <sup>4</sup>
	~187 bases <sup>4</sup>
	~253 bases <sup>4</sup>
	~272 bases <sup>4</sup>
TMR-ET	~159 bases <sup>4</sup>
	~428 bases <sup>4</sup>
	~441 bases <sup>4</sup>
CXR-ET	~201 bases <sup>4</sup>

<sup>1</sup>These variably sized peaks on the Applied Biosystems® 3130 and 3500 Genetic Analyzers may represent double-stranded DNA derived from the DYS448 amplicon. Double-stranded DNA is known to migrate faster than single-stranded DNA on capillary electrophoresis (CE) instruments.

<sup>2</sup>The low-level, DNA-dependent artifact is noticeable only with high input template amounts and allele peak heights.

<sup>3</sup>Two bases below and above the true allele peak, respectively.

<sup>4</sup>Artifact is observed more often with samples that contain relatively higher levels of female DNA.

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### 2.3 Known/observed artifacts continued

- 2.3.1 Low-level products can be seen in the -2bp and +2bp positions at some loci such as DYS391, DYS448 ( $\pm 3$ ), DYS456, DYS385a/b, DYS533, DYS570, DYS576, DYS635, and YGATAH4. Editing and removing labels for peaks that can be attributed to half back/forward stutters can be performed.
- 2.3.2 A shift in the base pair length for DYS481 can occur resulting in peaks that may fall under out-of-bin (OB) or N.1 positions (**1**).
- 2.3.3 A wide range of low-level artifacts (-8 ~ -10bp) were also observed for DYS448 (hexanucleotide) within validation consistent with Promega's findings (refer to 4.2).
- 2.3.4 Per the Promega technical manual (**4**), increased forward stutter (i.e., n+4 and n+8) can be observed at DYS389I and DYS389II loci when using cell-line derived 2800M positive control DNA which is used for the positive control. This increased forward stutter ratio is inherent to this cell line DNA and is not seen in amplification of DNA from human blood, body fluid or other human-derived samples. DYS389I and DYS389II for the positive control were not used in forward stutter ratio calculations. Any instance of forward stutter in the positive control at these locations can be edited.
- 2.3.5 Many regions of the Y-chromosome may be duplicated or triplicated in some individuals. This then would result in duplication or triplication by having more than one allele amplified with a single primer pair. The common loci where duplicated sequences can occur are DYS437, DYS439 and/or DYS389I/II (**5**). Additionally, triplication of Y-STR loci have been previously reported in YHRD, release 64 for the following loci: DYS385a/b, DYS19, DYS390, DYS439, DYS448 and DYS481.

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### 2.4 Common artifacts observed in samples with high amount of female DNA (\* were only seen in stutter positions)

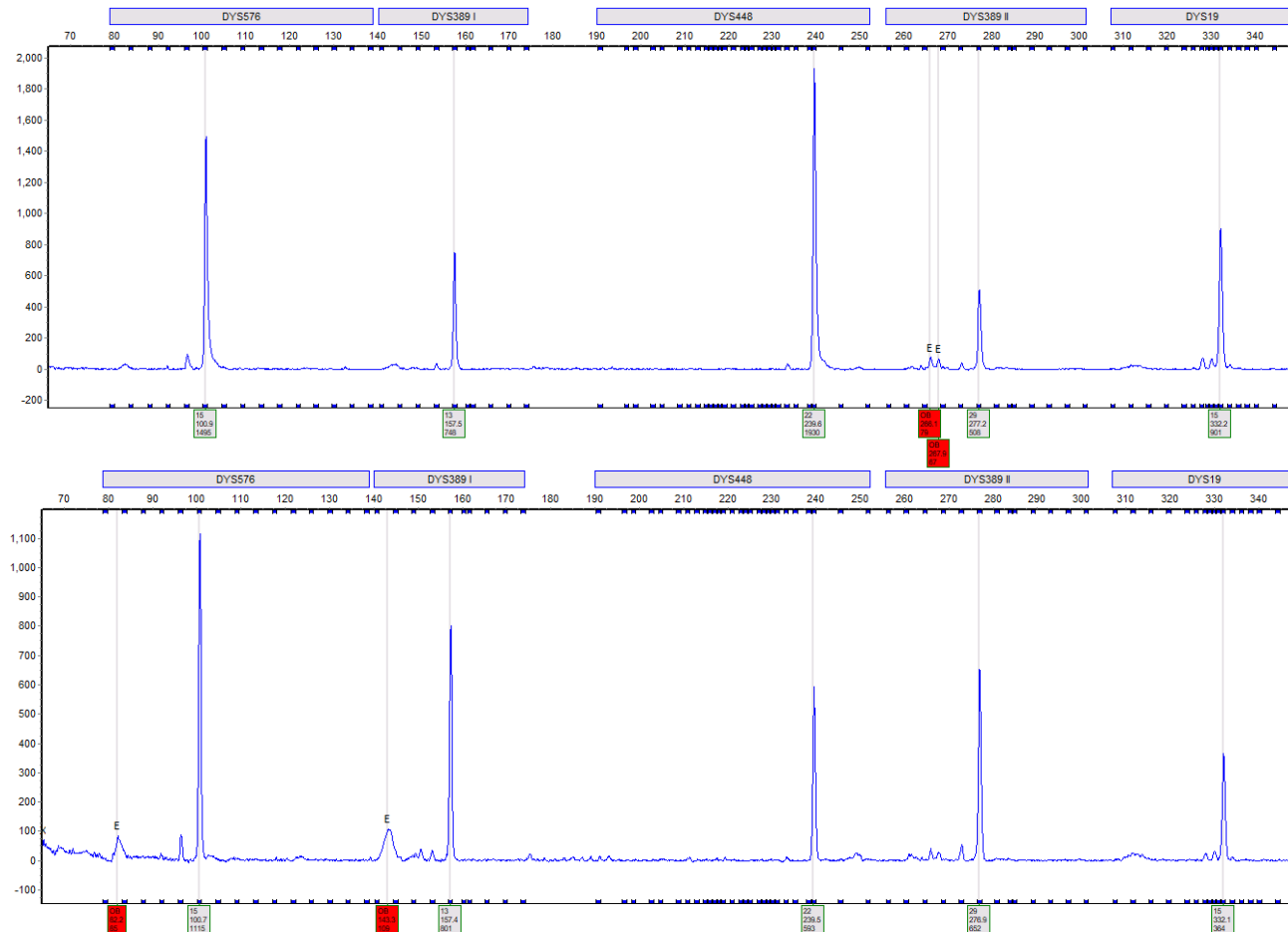
Artifacts Reported by (~ bp)				
Dye	Promega Technical Manual (4)	Moore et al. (2)	OCME Internal Validation	Common labels
Blue		143-147	143	OB
		152.5		OB
		242		OB
			262-264	Below AT
			266-267	OB
			308-315	308-315
Green	163	164.5	163	OB/27.1
	187	187.5	187	OL/OB/6
	253	253.2	253	10
		264.2		OB
	272	272.5	272.6-272.7	OB
		354.7		12
Yellow		387.7		OL
	159		159.4	OB
		283	283.5-283.8*	11*
		394	395	OB
		413-419	413-419	Below AT
	428	426.7	428	OL
Red	441	440.5	441.5	OL
	201			OB
			436	OL

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## 2.5 Common examples of artifacts observed in samples with high female DNA (labeled with **E** in electropherogram)

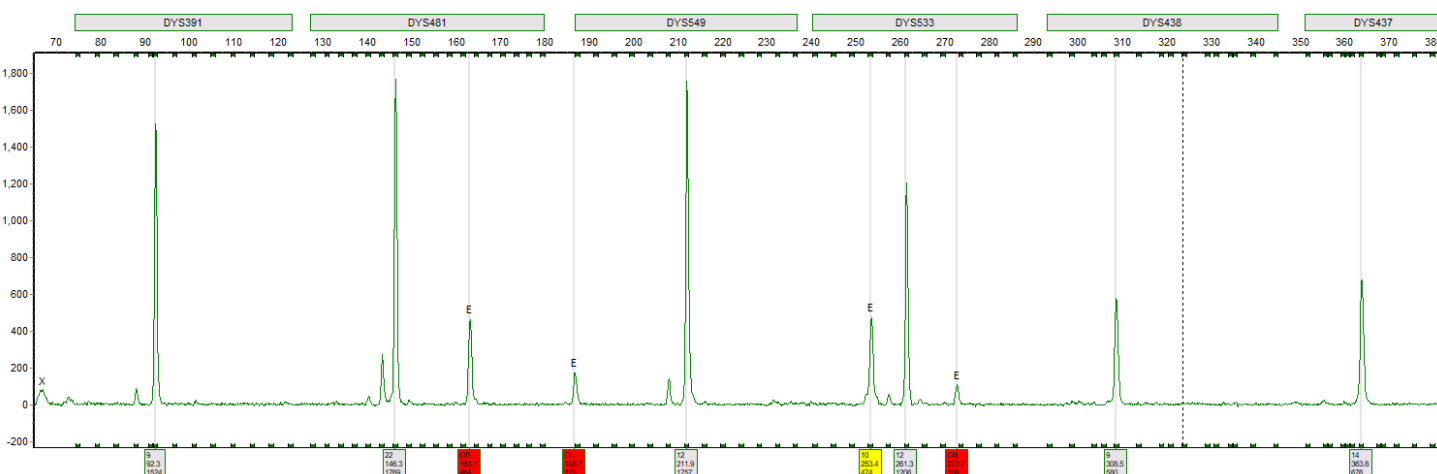
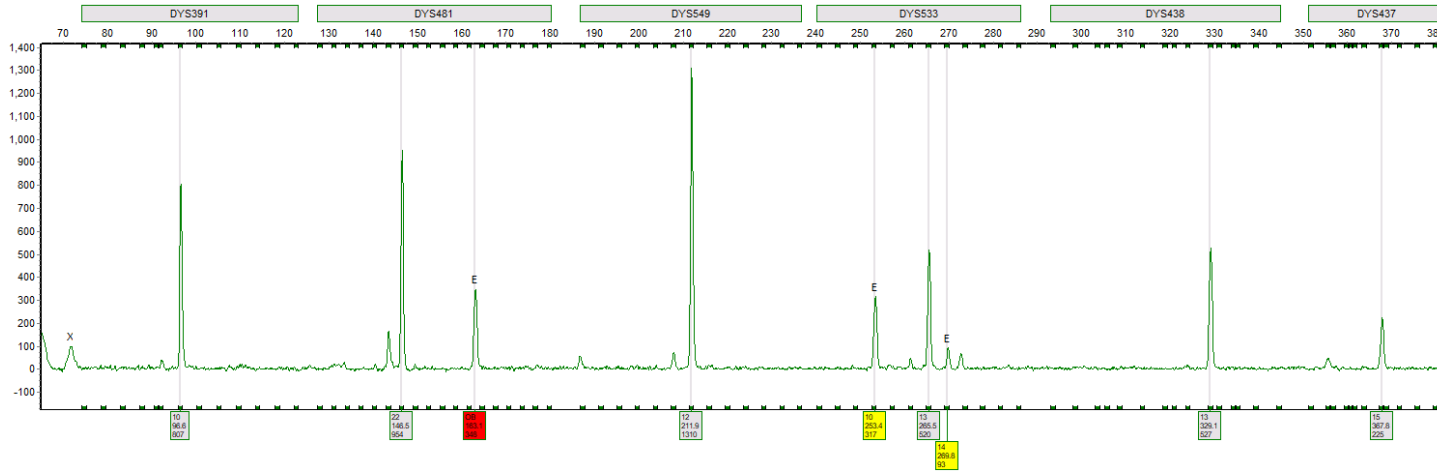
### 2.5.1 250pg male DNA in 1:5000 M:F ratio (Pavlov) / 100pg male DNA in 1:20,000 M:F ratio (Avogadro)



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## 2.5.2 100pg male DNA in 1:20,000 M:F ratio (Carmody) / 250pg male DNA in 1:20,000 M:F ratio (Pavlov)



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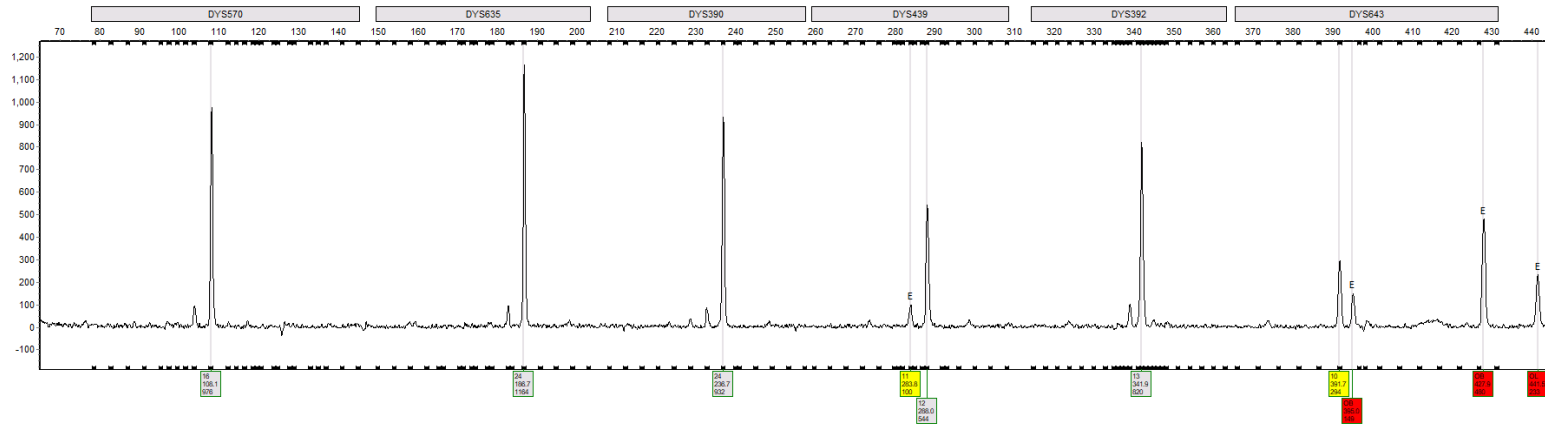
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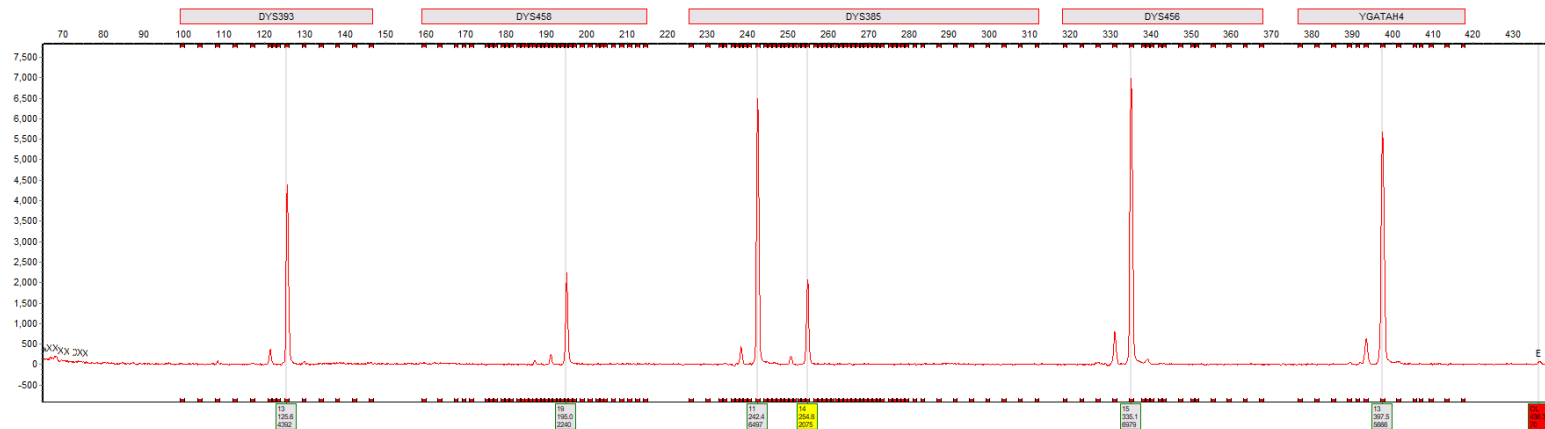
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### 2.5.3 100pg male DNA in 1:20,000 M:F ratio (Carmody)



### 2.5.4 100g male DNA in 1:5,000 M:F ratio (Carmody)



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### 3 Locus-specific stutter filter (non-additive) for each locus in GeneMarker® software.

#### BACK STUTTER

Locus	DYS576	DYS389 I	DYS448	DYS389 II	DYS19	DYS391	DYS481	DYS549	DYS533	DYS438	DYS437	DYS570	DYS635	DYS390	DYS439	DYS392	DYS643	DYS393	DYS458	DYS385	DYS456	YGATAH4
count	59	36	43	54	50	61	56	53	55	44	46	58	52	57	34	34	35	57	57	89	55	52
average	9.8%	5.6%	2.2%	11.4%	6.9%	6.6%	15.7%	5.7%	6.2%	2.6%	4.5%	8.5%	7.4%	7.9%	5.7%	9.6%	2.3%	9.2%	9.8%	8.5%	11.5%	7.9%
std.dev.	2.3%	0.7%	0.8%	2.0%	2.0%	1.7%	1.9%	1.1%	0.8%	1.0%	1.4%	2.7%	1.8%	1.1%	0.9%	1.9%	0.5%	1.7%	2.1%	1.7%	1.6%	1.6%
<b>Avg + 3STD FINAL FILTER</b>	16.83%	7.55%	4.74%	17.30%	12.97%	11.55%	21.44%	9.06%	8.50%	5.76%	8.84%	16.49%	12.75%	11.32%	8.35%	15.40%	3.86%	14.27%	16.16%	13.58%	16.17%	12.58%

#### FORWARD STUTTER

Locus	DYS576	DYS389 I	DYS448	DYS389 II	DYS19	DYS391	DYS481	DYS549	DYS533	DYS438	DYS437	DYS570	DYS635	DYS390	DYS439	DYS392	DYS643	DYS393	DYS458	DYS385	DYS456	YGATAH4
count	34			23	23	15	47	35	35		20	33	20	10	23	34		36	18	26	38	28
average	1.5%			1.5%	1.1%	0.3%	2.9%	1.1%	1.2%		0.7%	1.1%	0.8%	1.0%	1.1%	6.2%		2.0%	1.4%	1.2%	3.2%	2.0%
std.dev.	1.4%			0.6%	0.4%	0.2%	1.0%	0.4%	0.6%		0.3%	0.4%	0.4%	0.8%	0.3%	1.3%		0.3%	0.5%	0.3%	1.2%	0.7%
<b>Avg + 3STD FINAL FILTER</b>	5.82%			3.20%	2.27%	0.86%	5.74%	2.38%	3.08%		1.59%	2.48%	2.10%	3.35%	2.05%	9.95%		2.97%	2.95%	2.04%	6.76%	4.00%

#### DOUBLE BACK STUTTER

Locus	DYS576	DYS389 I	DYS448	DYS389 II	DYS19	DYS391	DYS481	DYS549	DYS533	DYS438	DYS437	DYS570	DYS635	DYS390	DYS439	DYS392	DYS643	DYS393	DYS458	DYS385	DYS456	YGATAH4
count	20			23		20	42	31	13			23		17		13		22	18	21	26	12
average	0.7%			1.1%		0.4%	2.9%	0.7%	0.4%			0.7%		0.7%		0.9%		0.8%	0.8%	0.8%	1.1%	0.7%
std.dev.	0.2%			0.3%		0.1%	0.8%	0.2%	0.1%			0.3%		0.1%		0.2%		0.2%	0.4%	0.3%	0.2%	0.3%
<b>Avg + 3STD FINAL FILTER</b>	1.13%			2.01%		0.74%	5.32%	1.37%	0.67%			1.72%		1.06%		1.52%		1.39%	2.09%	1.62%	1.69%	1.56%

#### HALF STUTTER (DYS19 ONLY)

DYS19	count	average	std.dev.	Avg + 3STD FINAL FILTER
N - 2	55	8.1%	1.2%	11.54%
N + 2	38	3.2%	0.7%	5.21%



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### 4 a) % of alleles called at each locus with varying amounts of DNA on 3500xL genetic analyzer (Pavlov)

DNA (pg)	DYS576	DYS389 I	DYS448	DYS389 II	DYS19	DYS391	DYS481	DYS549	DYS533	DYS438	DYS437	DYS570	DYS635	DYS390	DYS439	DYS392	DYS643	DYS393	DYS458	DYS385 (a)	DYS385 (b)	DYS456	YGATAH4	Average
15.6	100%	56%	67%	33%	44%	100%	89%	100%	67%	100%	44%	78%	100%	78%	67%	33%	56%	56%	89%	44%	33%	56%	44%	67%
31.25	89%	100%	89%	67%	78%	100%	100%	100%	100%	100%	89%	89%	89%	78%	89%	89%	89%	100%	89%	89%	78%	89%	89%	90%
62.5	100%	100%	100%	100%	100%	100%	100%	100%	100%	89%	100%	100%	100%	100%	100%	89%	100%	100%	100%	100%	100%	100%	100%	99%
125	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
250	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
500	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
800	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
1000	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%

### b) % of alleles called at each locus with varying amounts of DNA on 3500xL genetic analyzer (Carmody)

DNA (pg)	DYS576	DYS389 I	DYS448	DYS389 II	DYS19	DYS391	DYS481	DYS549	DYS533	DYS438	DYS437	DYS570	DYS635	DYS390	DYS439	DYS392	DYS643	DYS393	DYS458	DYS385 (a)	DYS385 (b)	DYS456	YGATAH4	Average
15.6	56%	67%	67%	11%	44%	100%	78%	89%	33%	89%	44%	78%	89%	78%	56%	11%	56%	44%	56%	44%	33%	44%	44%	57%
31.25	89%	78%	78%	56%	67%	100%	89%	78%	89%	100%	67%	89%	89%	100%	89%	78%	78%	100%	78%	100%	78%	78%	89%	84%
62.5	100%	100%	100%	89%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	89%	100%	89%	100%	100%	100%	100%	89%	98%
125	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
250	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
500	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
800	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
1000	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%



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### 5 a) Average peak height (RFU) at each locus with varying amounts of DNA on 3500xL genetic analyzer (Pavlov)

DNA (pg)	DYS576	DYS389 I	DYS448	DYS389 II	DYS19	DYS391	DYS481	DYS549	DYS533	DYS438	DYS437	DYS570	DYS635	DYS390	DYS439	DYS392	DYS643	DYS393	DYS458	DYS385 (a)	DYS385 (b)	DYS456	YGATAH4	Average
15.6	212	78	192	32	54	361	198	237	108	238	55	318	230	223	157	53	132	195	210	142	102	163	151	167
31.25	345	171	239	115	140	562	233	303	299	360	203	507	336	388	279	251	222	530	403	333	293	364	293	312
62.5	658	432	725	310	480	1375	859	904	750	422	470	919	765	1126	703	483	558	963	999	600	633	851	752	728
125	1095	941	1260	748	806	2531	1562	1250	1544	1338	918	1848	1791	1838	1171	1258	1368	2151	2075	1622	1911	1556	1657	1489
250	2799	1653	2262	1222	1694	4104	2844	2958	2699	2450	2211	4387	2867	3166	2530	2560	2353	3706	3922	2814	2579	2479	2790	2741
500	6338	3785	5619	3119	3805	10758	6873	7173	6275	6370	4525	9126	7160	7707	6666	6018	5409	8685	9594	8401	7784	5989	6536	6683
800	7177	4350	6227	3568	4341	12553	8014	9077	7962	6999	5822	12856	10371	10794	9472	9171	7310	11094	12675	10882	9931	8050	10772	8672
1000	10615	6763	9802	5267	7398	17886	11397	13397	11824	11807	8787	16304	13354	13852	10972	11087	9624	16528	16961	13775	13000	11717	14103	12010

### b) Average peak height (RFU) at each locus with varying amounts of DNA on 3500xL genetic analyzer (Carmody)

DNA (pg)	DYS576	DYS389 I	DYS448	DYS389 II	DYS19	DYS391	DYS481	DYS549	DYS533	DYS438	DYS437	DYS570	DYS635	DYS390	DYS439	DYS392	DYS643	DYS393	DYS458	DYS385 (a)	DYS385 (b)	DYS456	YGATAH4	Average
15.6	218	88	220	17	66	368	174	148	83	199	71	324	226	236	128	12	163	173	177	113	145	185	155	160
31.25	383	158	268	98	148	584	184	306	243	337	206	562	402	428	286	249	247	565	460	380	337	365	249	324
62.5	691	443	645	264	444	1462	877	864	685	359	418	976	849	1252	745	475	601	1094	1073	670	742	825	764	748
125	1214	1069	1329	737	878	2873	1791	1353	1577	1339	824	2031	2261	2025	1268	1288	1358	2449	2353	1808	2141	1603	1618	1617
250	3348	3345	3406	3441	3548	3633	3541	3555	3559	3587	3641	3727	3565	3555	3510	3576	3656	3771	3545	3361	3232	3186	3442	3348
500	7930	4692	6454	3535	4371	13638	8381	8275	7037	7314	4918	11720	8920	9042	7548	6489	6021	11035	10809	9805	9068	6530	7131	7855
800	9012	5346	7683	4257	5331	15840	9706	11151	9487	8664	7295	16440	12811	13117	11116	10887	9087	13824	14471	13489	11999	9617	13101	10597
1000	13261	8600	12254	6629	9641	22317	14040	16759	14891	15366	11453	21221	17760	18244	13999	14523	13314	20343	20913	18342	17334	15137	18625	15433

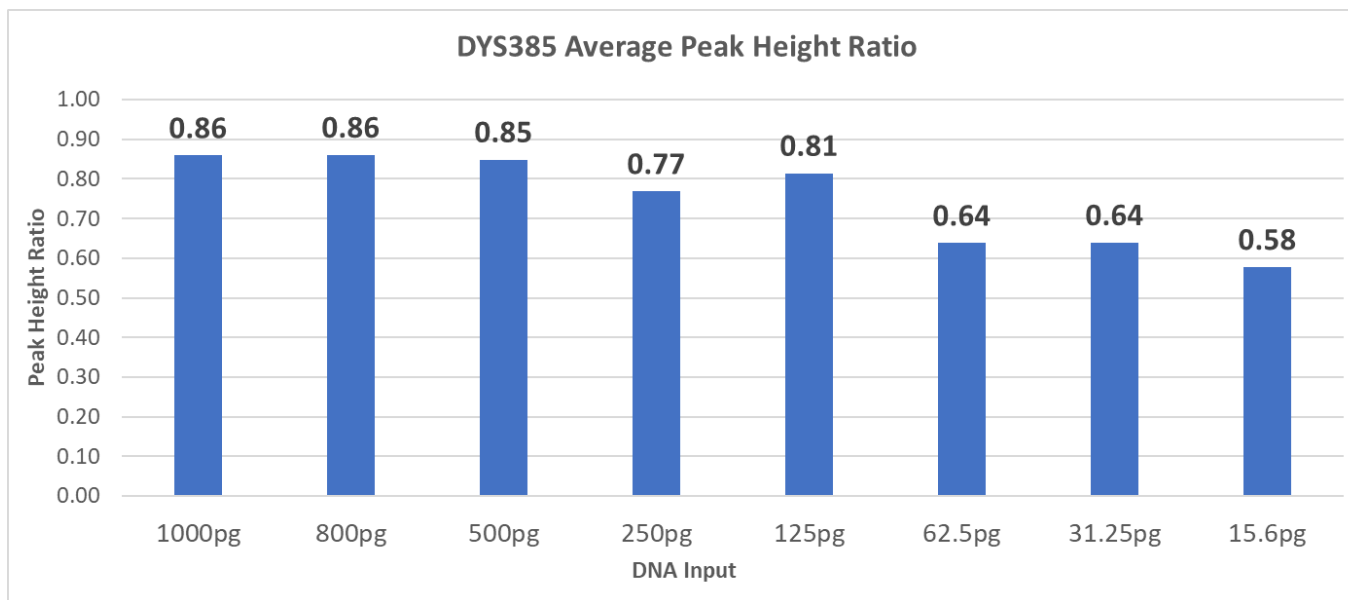
**LEGEND:** 0 RFU  10,000 ~ 22,000 RFU

## FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

### Manual Appendix for PowerPlex Y23 on 3500xL

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#### 6 Peak height ratio for single source samples with two true alleles present at DYS385a/b



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## 7 PPY23 locus-specific information from Promega Technical Manual (4)

STR Locus	Label	Size Range of Allelic Ladder Components <sup>1,2</sup> (bases)	Repeat Numbers of Allelic Ladder Components <sup>3</sup>	STR Locus	Label	Chromosomal Location <sup>1</sup>	Repeat Sequence <sup>2</sup> 5' → 3'
DYS576	Fluorescein	97–145	11–23	DYS576	Fluorescein	Y	AAAG
DYS389I	Fluorescein	147–179	9–17	DYS389I/II	Fluorescein	Y	(TCTG) (TCTA)
DYS448 <sup>4</sup>	Fluorescein	196–256	14–24	DYS448	Fluorescein	Y	AGAGAT
DYS389II	Fluorescein	259–303	24–35	DYS19	Fluorescein	Y	TAGA
DYS19	Fluorescein	312–352	9–19	DYS391	JOE	Y	TCTA
DYS391	JOE	86–130	5–16	DYS481	JOE	Y	CTT
DYS481	JOE	139–184	17–32	DYS549	JOE	Y	GATA
DYS549	JOE	198–238	7–17	DYS533	JOE	Y	ATCT
DYS533	JOE	245–285	7–17	DYS438	JOE	Y	TTTTTC
DYS438	JOE	293–343	6–16	DYS437	JOE	Y	TCTA
DYS437	JOE	344–380	11–18	DYS570	TMR-ET	Y	TTTC
DYS570	TMR-ET	90–150	10–25	DYS635	TMR-ET	Y	TSTA compound
DYS635	TMR-ET	150–202	15–28	DYS390	TMR-ET	Y	(TCTA) (TCTG)
DYS390	TMR-ET	207–255	17–29	DYS439	TMR-ET	Y	AGAT
DYS439	TMR-ET	263–307	6–17	DYS392	TMR-ET	Y	TAT
DYS392	TMR-ET	314–362	4–20	DYS643	TMR-ET	Y	CTTTT
DYS643	TMR-ET	368–423	6–17	DYS393	CXR-ET	Y	AGAT
DYS393	CXR-ET	101–145	7–18	DYS458	CXR-ET	Y	GAAA
DYS458	CXR-ET	159–215	10–24	DYS385a/b	CXR-ET	Y	GAAA
DYS385a/b	CXR-ET	223–307	7–28	DYS456	CXR-ET	Y	AGAT
DYS456	CXR-ET	316–364	11–23	Y-GATA-H4	CXR-ET	Y	TAGA
Y-GATA-H4	CXR-ET	374–414	8–18				

<sup>1</sup>The length of each allele in the allelic ladder has been confirmed by sequence analysis.

<sup>2</sup>When using an internal lane standard, such as the WEN Internal Lane Standard 500 Y23, the calculated sizes of allelic ladder components may differ from those listed. This occurs because different sequences in allelic ladder and ILS components may cause differences in migration. The dye label also affects migration of alleles.

<sup>3</sup>For a current list of microvariants, see the Variant Allele Report published at the U.S. National Institute of Standards and Technology (NIST) web site at: [https://strbase.nist.gov/var\\_tab.htm](https://strbase.nist.gov/var_tab.htm) and the Y Chromosome Haplotype Reference Database at: [www.yhrd.org](http://www.yhrd.org)

<sup>4</sup>A deletion has been reported at the DYS448 locus (35). Samples with this deletion will show two peaks (i.e., duplication) in DYS576 and a null allele in DYS448.

<sup>1</sup>Information about most of these loci can be found at: <https://strbase.nist.gov//chrom.htm>

<sup>2</sup>The August 1997 report (33,34) of the DNA Commission of the International Society for Forensic Haemogenetics (ISFH) states, “1) for STR loci within coding genes, the coding strand shall be used and the repeat sequence motif defined using the first possible 5’ nucleotide of a repeat motif; and 2) for STR loci not associated with a coding gene, the first database entry or original literature description shall be used”.

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Locus	Number of Repeats	
DYS576	tetranucleotide	4
DYS389I	tetranucleotide	4
DYS448	hexanucleotide	6
DYS389II	tetranucleotide	4
DYS19	tetranucleotide	4
DYS391	tetranucleotide	4
DYS481	trinucleotide	3
DYS549	tetranucleotide	4
DYS533	tetranucleotide	4
DYS438	pentanucleotide	5
DYS437	tetranucleotide	4
DYS570	tetranucleotide	4
DYS635	tetranucleotide	4
DYS390	tetranucleotide	4
DYS439	tetranucleotide	4
DYS392	trinucleotide	3
DYS643	pentanucleotide	5
DYS393	tetranucleotide	4
DYS458	tetranucleotide	4
DYS385a/b	tetranucleotide	4
DYS456	tetranucleotide	4
Y-GATA-H4	tetranucleotide	4

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- 8.4 Promega Technical Manual (DC2305 and DC2320).
- 8.5 Butler et al. Chromosomal Duplications Along the Y-Chromosome and Their Potential Impact on Y-STR Interpretation\*. *J Forensic Sci*, July 2005, Vol. 50, No. 4