

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

Manual Appendix for PowerPlex Fusion on 3500xL		
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1 PowerPlex® Fusion 5C Loci and Approximate Size Range (from the PowerPlex® Fusion System Technical Manual TMDO39- Promega Corporation)

STR Locus	Label	Size Range of Allelic Ladder Components ^{1,2} (bases)	Repeat Numbers of Allelic Ladder Components ³
Amelogenin ⁴	Fluorescein	89, 95	X, Y
D3S1358	Fluorescein	103–147	9–20
D1S1656	Fluorescein	161–208	9–14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18, 18.3, 19, 19.3, 20.3
D2S441	Fluorescein	214–250	8–11, 11.3, 12–17
D10S1248	Fluorescein	256–280	8–19
D13S317	Fluorescein	302–350	5–17
Penta E	Fluorescein	371–466	5–24
D16S539	JOE	84–132	4–16
D18S51	JOE	134–214	7–10, 10.2, 11–13, 13.2, 14–27
D2S1338	JOE	224–296	10, 12, 14–28
CSF1PO	JOE	318–362	5–16
Penta D	JOE	377–450	2.2, 3.2, 5–17
TH01	TMR-ET	72–115	3–9, 9.3, 10–11, 13.3
vWA	TMR-ET	127–183	10–24
D21S11	TMR-ET	203–259	24, 24.2, 25, 25.2, 26–28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36–38
D7S820	TMR-ET	269–313	5–16
D5S818	TMR-ET	321–369	6–18
TPOX	TMR-ET	393–441	4–16
DYS391	TMR-ET	442–486	5–16
D8S1179	CXR-ET	76–124	7–19
D12S391	CXR-ET	133–185	14–17, 17.3, 18, 18.3, 19–27
D19S433	CXR-ET	193–245	5.2, 6.2, 8–12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18, 18.2
FGA	CXR-ET	265–411	14–18, 18.2, 19, 19.2, 20, 20.2, 21, 21.2, 22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26–30, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 48.2, 50.2
D22S1045	CXR-ET	425–464	7–20

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

²When using an internal lane standard, such as the WEN Internal Lane Standard 500, 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 and linker also affect migration of alleles.

³For 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: www.cstl.nist.gov/div831/strbase/

⁴Amelogenin is not an STR.

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2 Average Peak Heights Obtained in the PowerPlex® Fusion Validation on 3500xLs:

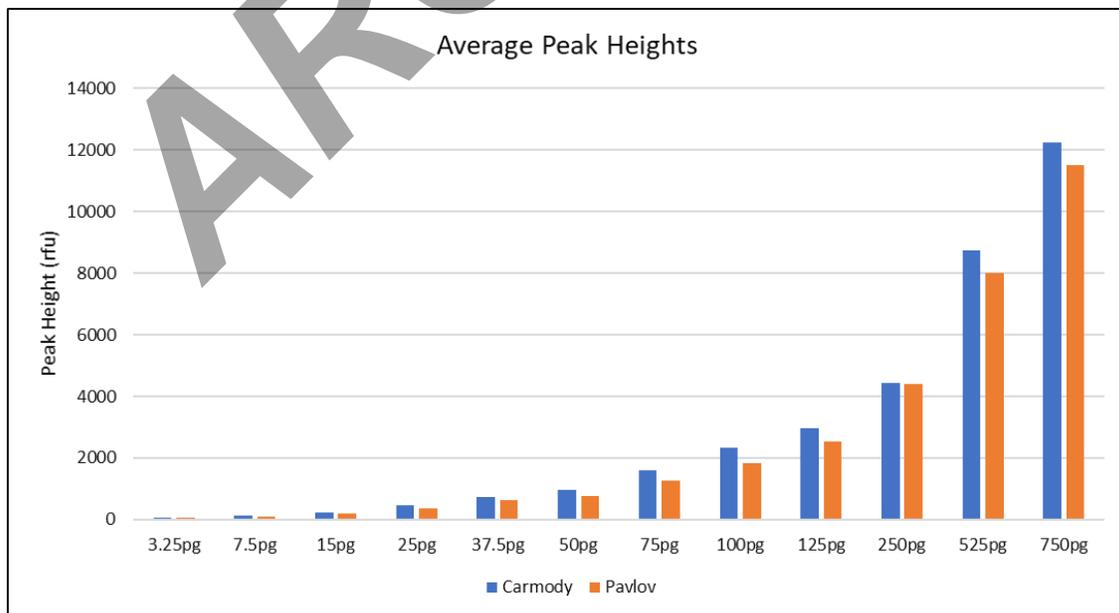
2.1 Average peak heights for six genomic DNA samples amplified using various template amounts (A) and average peak heights overall (B). Samples were amplified using PowerPlex® Fusion for 29 cycles on the Applied Biosystems® 9700. Samples were run on the Applied Biosystems® 3500xL (Pavlov and Carmody) using a 1.2kV, 24 second injection.

A.

		3.25pg	7.5pg	15pg	25pg	37.5pg	50pg	75pg	100pg	125pg	250pg	525pg	750pg
14F	Carmody	95	99	222	558	568	795	1279	1681	2333	3918	7680	11560
	Pavlov	76	106	165	384	545	463	1031	1505	2310	3656	7479	10270
34F	Carmody	25	139	*	*	*	944	1440	2018	3143	4480	7331	14315
	Pavlov	55	124	*	*	*	732	1127	1700	2210	3919	5883	11330
35F	Carmody	127	95	330	506	583	816	1547	2245	2679	4721	7869	9493
	Pavlov	99	91	283	406	606	536	1226	1744	2538	5198	7563	9805
12M	Carmody	48	130	177	374	1223	1521	2881	4207	4927	4104	10049	12987
	Pavlov	57	82	178	243	941	1402	2320	3079	4284	4083	8362	12257
21M	Carmody	21	90	258	471	755	1055	1209	1625	2207	5022	10146	12702
	Pavlov	23	88	213	468	602	722	803	1202	1735	5164	9543	12739
25M	Carmody	53	167	209	435	586	702	1255	2215	2463	4338	9307	12447
	Pavlov	58	147	204	341	436	650	1024	1687	2124	4420	9138	12635

* Values not included due to a pipetting error

B.



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3 Average Peak Height Ratios Observed during the PowerPlex® Fusion Validation on 3500xLs:

3.1 Average peak height ratios for six genomic DNA samples amplified using various template amounts. Samples were amplified using PowerPlex® Fusion for 29 cycles on the Applied Biosystems® 9700. Samples were run on the Applied Biosystems® 3500xL (Pavlov and Carmody) using a 1.2kV, 24 second injection. The three-color heat map was used to represent the lowest peak height ratios (red), the average peak height ratios (yellow), and the highest peak height ratios (dark green). Gray cells represent locations not included in analysis and white cells represent amplified amounts with complete dropout across all replicates. Note: Under 30pg shows a higher variability due to increased stochastic effects at these DNA amounts.

Average Peak Height Ratios (Pavlov)												
	3.25pg	7.5pg	15pg	25pg	37.5pg	50pg	75pg	100pg	125pg	250pg	525pg	750pg
AMEL	-	39%	51%	61%	66%	71%	76%	73%	76%	79%	87%	86%
D3S1358	83%	58%	57%	59%	70%	67%	72%	80%	75%	85%	86%	86%
D1S1656	-	80%	76%	68%	68%	68%	62%	74%	77%	83%	90%	90%
D2S441	40%	49%	68%	53%	46%	74%	61%	75%	70%	82%	84%	79%
D10S1248	-	-	36%	61%	68%	58%	79%	63%	65%	80%	68%	91%
D13S317	86%	-	75%	59%	54%	63%	77%	73%	63%	87%	82%	87%
Penta E	89%	74%	54%	71%	53%	72%	71%	67%	78%	74%	78%	80%
D16S539	-	87%	57%	64%	62%	56%	66%	76%	78%	84%	82%	84%
D18S51	47%	66%	65%	79%	59%	66%	67%	74%	69%	83%	83%	85%
D2S1338	56%	64%	61%	67%	64%	60%	65%	76%	64%	81%	88%	93%
CSF1PO	69%	-	50%	66%	63%	64%	73%	74%	68%	83%	83%	91%
Penta D	92%	40%	56%	69%	60%	57%	64%	65%	74%	83%	85%	89%
TH01	35%	72%	49%	55%	67%	84%	75%	71%	79%	80%	81%	89%
vWA	90%	50%	63%	62%	69%	63%	68%	68%	76%	82%	89%	87%
D21S11	-	-	44%	68%	66%	70%	76%	71%	77%	85%	89%	89%
D7S820	85%	53%	70%	66%	57%	59%	72%	72%	75%	79%	76%	86%
D5S818	91%	72%	50%	55%	57%	65%	62%	68%	77%	78%	85%	87%
TPOX	-	-	63%	49%	72%	63%	59%	77%	76%	76%	81%	83%
DYS391												
D8S1179	48%	48%	54%	59%	72%	68%	67%	79%	76%	87%	88%	92%
D12S391	84%	-	93%	64%	42%	64%	71%	75%	72%	78%	86%	85%
D19S433	52%	63%	42%	64%	65%	64%	72%	69%	72%	79%	81%	85%
FGA	-	-	70%	81%	56%	70%	61%	71%	83%	90%	74%	89%
D22S1045	89%	65%	100%	72%	58%	67%	61%	80%	72%	78%	88%	81%

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	Average Peak Height Ratios (Carmody)											
	3.25pg	7.5pg	15pg	25pg	37.5pg	50pg	75pg	100pg	125pg	250pg	525pg	750pg
AMEL	-	41%	52%	61%	65%	71%	75%	73%	75%	79%	88%	85%
D3S1358	90%	60%	63%	54%	71%	64%	64%	80%	74%	85%	86%	85%
D1S1656	59%	79%	60%	63%	68%	68%	65%	75%	76%	83%	89%	89%
D2S441	39%	49%	68%	52%	47%	74%	55%	75%	69%	81%	83%	77%
D10S1248	-	-	27%	60%	49%	58%	78%	65%	64%	81%	80%	89%
D13S317	84%	-	73%	57%	54%	61%	79%	74%	61%	86%	84%	86%
Penta E	82%	76%	52%	70%	53%	70%	77%	67%	77%	74%	78%	79%
D16S539	-	88%	54%	64%	61%	56%	66%	76%	77%	84%	83%	84%
D18S51	48%	63%	59%	79%	60%	65%	66%	73%	69%	82%	83%	84%
D2S1338	-	63%	45%	67%	63%	57%	62%	76%	63%	80%	88%	92%
CSF1PO	69%	56%	49%	58%	60%	66%	76%	73%	66%	82%	78%	89%
Penta D	89%	41%	61%	69%	60%	55%	70%	65%	72%	82%	87%	89%
TH01	33%	68%	49%	55%	66%	81%	70%	71%	78%	80%	83%	90%
vWA	93%	50%	63%	55%	70%	63%	77%	67%	76%	82%	89%	88%
D21S11	70%	-	45%	64%	66%	68%	79%	71%	76%	84%	84%	89%
D7S820	83%	51%	67%	63%	55%	58%	76%	72%	75%	78%	78%	86%
D5S818	93%	76%	51%	59%	57%	68%	59%	68%	74%	78%	83%	85%
TPOX	-	-	58%	49%	71%	64%	61%	78%	77%	75%	79%	83%
DYS391												
D8S1179	43%	49%	53%	59%	72%	67%	71%	80%	76%	87%	89%	91%
D12S391	92%	79%	79%	64%	43%	57%	72%	74%	72%	78%	89%	86%
D19S433	51%	48%	42%	55%	64%	65%	72%	65%	72%	78%	82%	83%
FGA	-	68%	70%	75%	50%	72%	56%	69%	83%	88%	76%	88%
D22S1045	-	69%	97%	70%	57%	69%	60%	80%	71%	78%	85%	80%

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4 Percent Profile Obtained during the PowerPlex® Fusion Validation on 3500xLs:

4.1 Percent profile obtained for samples amplified with PowerPlex® Fusion on an Applied Biosystems® 9700 for 29 cycles. Samples were run on the Applied Biosystems® 3500xL (Pavlov and Carmody) using a 1.2kV, 24 second injection. Samples were analyzed with a dye-specific analytical threshold. The three-color heat map was used to represent the lowest percent profile (red), the average percent profile (yellow), and the highest percent profile (dark green). Gray cells represent samples not included in analysis.

		Carmody % Profile of Sensitivity Series					
		14F	34F	35F	12M	21M	25M
3.25pg		57%	8%	51%	24%	9%	23%
		14%	24%	56%	29%	18%	31%
7.5pg		59%	54%	33%	50%	36%	54%
		39%	49%	38%	50%	36%	56%
15pg		77%		82%	66%	69%	74%
		52%		89%	63%	67%	69%
25pg		95%		89%	92%	89%	97%
		91%		96%	87%	91%	85%
37.5pg		98%		100%	100%	96%	97%
		98%		100%	100%	96%	100%
50pg		98%	100%	100%	100%	100%	100%
		98%	97%	100%	100%	98%	100%
75pg		100%	100%	100%	100%	100%	100%
		100%	100%	100%	100%	100%	100%
100pg		100%	100%	100%	100%	100%	100%
		100%	100%	100%	100%	100%	100%
125pg		100%	100%	100%	100%	100%	100%
		100%	100%	100%	100%	100%	100%
250pg		100%	100%	100%	100%	100%	100%
		100%	100%	100%	100%	100%	100%
525pg		100%	100%	100%	100%	100%	100%
		100%	100%	100%	100%	100%	100%
750pg		100%	100%	100%	100%	100%	100%
		100%	100%	100%	100%	100%	100%

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Pavlov % Profile of Sensitivity Series						
	14F	34F	35F	12M	21M	25M
3.25pg	52%	30%	47%	29%	18%	36%
	14%	27%	44%	34%	11%	28%
7.5pg	55%	49%	33%	45%	44%	51%
	45%	49%	38%	26%	33%	51%
15pg	70%		82%	63%	64%	77%
	52%		84%	74%	64%	67%
25pg	84%		89%	87%	91%	97%
	91%		96%	82%	89%	79%
37.5pg	95%		100%	100%	93%	97%
	98%		100%	100%	93%	97%
50pg	93%	100%	100%	100%	100%	100%
	91%	97%	98%	100%	96%	100%
75pg	100%	100%	100%	100%	100%	100%
	100%	100%	100%	100%	100%	100%
100pg	100%	100%	100%	100%	100%	100%
	100%	100%	100%	100%	100%	100%
125pg	100%	100%	100%	100%	100%	100%
	100%	100%	100%	100%	100%	100%
250pg	100%	100%	100%	100%	100%	100%
	100%	100%	100%	100%	100%	100%
525pg	100%	100%	100%	100%	100%	100%
	100%	100%		100%	100%	100%
750pg	100%	100%	100%	100%	100%	100%
	100%	100%	100%	100%	100%	100%

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5 Additional Possible Artifacts in PowerPlex® Fusion (from the PowerPlex® Fusion System Technical Manual TMDO39-Promega Corporation)

- 5.1 In addition to stutter peaks, other artifact peaks can be observed at some of the PowerPlex® Fusion System loci.
- 5.2 Low-level products can be seen in the -2bp and +2bp positions at some loci such as D1S1656, D13S317, D18S51, D21S11, D7S820, D5S818, D12S391 and D19S433.
- 5.3 -1bp peaks are sometimes present at Amelogenin and D2S441.
- 5.4 -3bp peaks are sometimes present at D12S391.
- 5.5 An artifact peak is sometimes present in anal and perianal samples at Penta E between 483 and 485bp.
- 5.6 Artifacts may also be seen in the following dye channels within the listed base pair ranges:
 - 5.6.1 Fluorescein: 62-65 bp*, 63-68 bp, 83-86 bp*
 - 5.6.2 JOE: 68-71 bp*, 79-80 bp, 214 bp, 247 bp
 - 5.6.3 TMR: 58-61 bp, 64-67 bp, 69-72 bp

*artifacts are independent of amplification and may occur in no-template samples