

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

Manual Appendix for PowerPlex Fusion		
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1 PowerPlex® Fusion 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 during the Powerplex® Fusion validation at NYC OCME:

2.1 Average peak heights from both Athena and Newton at 3kV 5s for the sensitivity series from 1ng down to 0pg using 29 cycles. The three color heat map was used to represent the lowest peak height (red), the average (yellow) and the highest peak height (dark green). The white cells represent loci that had complete dropout in all replicates

Average Peak Heights														
	1ng	500pg	250pg	150pg	125pg	100pg	75pg	50pg	30pg	25pg	15pg	7.5pg	3.25pg	0pg
AMEL	3049	2502	1038	787	469	433	428	229	133	117	83	25	18	-
D3S1358	3329	2110	825	533	429	353	294	205	96	98	39	56	19	-
D1S1656	2949	1835	805	506	428	284	279	150	72	98	42	21	-	-
D2S441	2846	1618	735	539	293	270	232	99	94	67	34	17	-	-
D10S1248	2488	1768	861	542	327	246	284	172	79	77	44	21	9	-
D13S317	2461	1595	723	530	333	234	267	170	89	72	32	24	9	-
Penta E	2062	1516	760	516	250	144	235	119	57	62	19	11	-	-
D16S539	3998	2415	1051	568	511	339	338	207	147	88	44	28	12	-
D18S51	3475	2135	996	548	454	286	312	168	93	92	40	11	8	-
D2S1338	2670	1952	835	607	406	276	284	208	141	64	24	21	15	-
CSF1PO	2460	1477	626	468	253	193	223	110	81	89	31	14	10	-
Penta D	2418	1787	802	464	305	226	260	143	68	72	41	22	-	-
TH01	3796	2094	988	659	440	369	332	206	130	71	70	45	10	-
vWA	3254	1835	865	526	387	360	286	181	92	74	23	20	11	-
D21S11	3096	1869	858	489	443	305	290	185	88	64	44	30	9	-
D7S820	3596	2012	971	520	392	365	293	176	91	94	18	22	18	9
D5S818	3595	1998	917	573	369	284	252	140	121	70	45	28	9	-
TPOX	2859	1662	822	559	363	298	263	143	72	88	38	25	12	-
DYS391	2264	1733	684	426	285	200	238	127	56	41	37	39	21	-
D8S1179	3160	2525	1215	722	496	479	383	233	170	109	86	25	19	-
D12S391	3632	2037	773	362	424	179	195	117	72	50	28	19	-	-
D19S433	2461	1419	613	394	254	237	215	103	105	39	17	16	8	-
FGA	2972	1864	740	423	325	285	261	169	91	77	31	13	13	-
D22S1045	1907	1212	520	307	204	199	168	78	65	65	31	13	9	-

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3 Average Peak Height Ratios observed during the Powerplex® Fusion validation at NYC OCME:

3.1 The average peak height ratios for both Athena (least sensitive instrument) and Newton (most sensitive instrument) at 3kV 5s for the sensitivity series from 1ng down to 0pg single source samples using 29 cycles are illustrated below. The three color heat map was used to represent the lowest peak height ratio (red), the average (yellow) and the highest peak height ratio (dark green)

Average Peak Height Ratios														
	1ng	500pg	250pg	150pg	125pg	100pg	75pg	50pg	30pg	25pg	15pg	7.5pg	3.25pg	0pg
AMEL	89%	86%	92%	92%	86%	72%	69%	56%	84%	79%	46%	-	-	-
D3S1358	95%	92%	82%	78%	88%	66%	68%	70%	82%	61%	71%	34%	82%	-
D1S1656	86%	80%	87%	75%	88%	82%	80%	77%	84%	82%	78%	-	-	-
D2S441	90%	83%	70%	75%	63%	73%	79%	66%	77%	75%	62%	-	-	-
D10S1248	87%	85%	70%	75%	70%	74%	67%	62%	77%	57%	69%	-	-	-
D13S317	85%	81%	84%	91%	84%	83%	67%	63%	63%	68%	92%	98%	-	-
Penta E	89%	81%	81%	79%	72%	64%	71%	72%	79%	72%	-	-	-	-
D16S539	95%	79%	86%	69%	84%	86%	83%	76%	79%	59%	27%	42%	-	-
D18S51	89%	87%	75%	71%	66%	69%	72%	74%	69%	58%	67%	-	-	-
D2S1338	93%	90%	81%	69%	85%	76%	61%	58%	62%	91%	98%	43%	-	-
CSF1PO	86%	87%	84%	61%	59%	74%	53%	66%	85%	74%	78%	-	-	-
Penta D	90%	87%	82%	81%	82%	82%	73%	54%	60%	81%	67%	95%	-	-
TH01	88%	85%	80%	74%	75%	76%	82%	72%	58%	60%	50%	76%	-	-
vWA	90%	83%	88%	85%	78%	86%	59%	67%	66%	82%	60%	-	-	-
D21S11	88%	84%	81%	80%	78%	63%	60%	67%	63%	55%	54%	91%	-	-
D7S820	88%	79%	75%	77%	78%	67%	68%	57%	84%	41%	85%	86%	-	-
D5S818	94%	83%	87%	76%	69%	81%	74%	67%	54%	66%	76%	40%	-	-
TPOX	85%	80%	84%	76%	68%	66%	77%	66%	71%	67%	-	-	-	-
DYS391														
D8S1179	89%	91%	84%	88%	76%	68%	85%	62%	77%	70%	82%	59%	80%	-
D12S391	91%	88%	86%	74%	74%	77%	76%	74%	64%	67%	-	-	-	-
D19S433	86%	81%	88%	83%	68%	79%	69%	84%	66%	47%	79%	-	-	-
FGA	92%	87%	93%	71%	75%	75%	82%	73%	86%	68%	64%	-	-	-
D22S1045	87%	78%	70%	78%	77%	57%	61%	61%	82%	51%	93%	-	-	-

Note: Under 30pg shows a higher variability due to increased stochastic effects at these DNA amounts.

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4 Percent Profile Obtained during the Powerplex® Fusion validation at NYC OCME:

4.1 Percent profile obtained for each sensitivity series using a 50 RFU threshold with a 3% minimum heterozygote filter and 3% global max filter at a 3kV 5 sec injection parameter. The three color heat map was used to represent the lowest peak height (red), the average (yellow) and the highest peak height (dark green).

	DNA 1		DNA 2	
	Athena	Newton	Athena	Newton
1ng	100%	100%	100%	100%
	100%	100%	100%	100%
	100%	100%	100%	100%
500pg	100%	100%	100%	100%
	100%	100%	100%	100%
	100%	100%	100%	100%
250pg	100%	100%	100%	100%
	100%	100%	100%	100%
	100%	100%	100%	*
150pg	100%	*	100%	100%
	100%	100%	100%	100%
	100%	100%	100%	100%
125pg	100%	100%	100%	100%
	100%	100%	100%	100%
	47%	56%	100%	100%
100pg	100%	100%	100%	100%
	98%	100%	100%	100%
	100%	100%	98%	100%
75pg	98%	98%	98%	100%
	98%	100%	100%	100%
	100%	100%	100%	100%
50pg	93%	96%	93%	98%
	89%	93%	91%	98%
	100%	100%	91%	98%
30pg	76%	84%	87%	93%
	71%	91%	60%	80%
	69%	82%	76%	93%
25pg	64%	82%	58%	67%
	67%	76%	62%	76%
	80%	91%	64%	69%
15pg	27%	51%	20%	56%
	36%	56%	36%	44%
	22%	51%	22%	51%
7.5pg	7%	29%	16%	27%
	27%	38%	13%	40%
	9%	40%	13%	22%
3.25pg	2%	20%	0%	9%
	2%	7%	2%	18%
	2%	7%	2%	9%
0pg	0%	0%	0%	0%
	0%	0%	0%	0%
	0%	0%	0%	2%
	* failed size standard			

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5 Powerplex® Fusion stutter filters

5.1 The table below lists the Stutter Filter ratios for the PowerPlex® Fusion System. The stutter filter is set as the mean stutter ratio plus three standard deviations. These are the stutter filters used in GeneMarker analysis at NYC OCME.

Minus Stutter Filter				
Locus	Count	Mean Stutter Ratio	Std Dev	Mean +3 Std Dev
AMEL	--	--	--	--
D3S1358	122	0.081	0.013	0.119
D1S1656	151	0.085	0.019	0.142
D2S441	110	0.048	0.015	0.092
D10S1248	123	0.079	0.015	0.124
D13S317	106	0.054	0.015	0.098
Penta E	47	0.039	0.012	0.076
D16S539	132	0.059	0.014	0.102
D18S51	164	0.082	0.021	0.146
D2S1338	177	0.083	0.019	0.139
CSF1PO	116	0.058	0.012	0.095
Penta D	11	0.029	0.013	0.068
TH01	80	0.023	0.008	0.046
vWA	108	0.062	0.017	0.112
D21S11	148	0.079	0.012	0.116
D7S820	118	0.052	0.019	0.110
D5S818	111	0.052	0.014	0.095
TPOX	82	0.027	0.009	0.055
DYS391	101	0.062	0.008	0.087
D8S1179	143	0.069	0.013	0.109
D12S391	157	0.087	0.024	0.158
D19S433	127	0.064	0.015	0.110
FGA	143	0.068	0.017	0.121
D22S1045	123	0.081	0.028	0.164
Plus Stutter	Count	Average	Std Dev	Mean +3 Std Dev
D22S1045	103	0.049	0.013	0.086
n-2				
D1S1656	56	0.025	0.004	0.036

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

- 6.1 In addition to stutter peaks, other artifact peaks can be observed at some of the PowerPlex® Fusion System loci.
- 6.2 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.
- 6.3 -1bp peaks are sometimes present at Amelogenin and D2S441.
- 6.4 -3bp peaks are sometimes present at D12S391.
- 6.5 An artifact peak is sometimes present in anal and perianal samples at Penta E between 483 and 485bp.
- 6.6 Artifacts may also be seen in the following dye channels within the listed base pair ranges:
 - 6.6.1 Fluorescein: 62-65 bp*, 63-68 bp, 83-86 bp*
 - 6.6.2 JOE: 68-71 bp*, 79-80 bp, 214 bp, 247 bp
 - 6.6.3 TMR: 58-61 bp, 64-67 bp, 69-72 bp

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

7 File Organization

- 7.1 Following completion of a run, .fsa files will collect in bulk in one folder. If there is one analysis set, all files should go within the main run folder on the network. If there are multiple analysis sets in one run (i.e., A, B, C, etc.), then multiple subfolders should be created to hold the run files and PDFs. Subfolders should be made for .fsa and PDF files within the analysis set folder.