



Office of Chief Medical Examiner – New York City
PowerPlex® Fusion System Amplification Kit on the Applied Biosystems 3130xl Genetic
Analyzer using GeneMarker HID v. 2.8.2 Analysis Software
Validation Report

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Contents

Office of Chief Medical Examiner – New York City	1
2. FBI Quality Assurance Standard and SWGDAM Recommended Studies	3
3. Materials and Methods	3
3.A.1 DNA Amplification	3
3.A.2 Data Analysis	3
3.A.3 Sensitivity Study	3
3.A.4 Analytical Threshold, and Stochastic Threshold.....	4
3.A.6 Precision.....	4
3.A.7 Reproducibility.....	4
3.A.8 Mixture Study	5
3.A.9 Contamination Assessment	5
3.A.10 Thermocycler Performance Check.....	5
4. Results	6
4.A.1 Analytical Threshold, and Stochastic Threshold.....	6
4.A.2 Sensitivity	10
4.A.3 Known Non Probative and Mock Casework Samples	22
4.A.4 Precision	27
4.A.5 Reproducibility and Repeatability	28
4.A.6 Mixture Study	32
4.A.7 Contamination Assessment	37
4.A.8 Thermocycler Performance Check.....	38
5. Conclusion	39
6. References	40
7. Appendix A – Lot Numbers	41
8. Appendix B – Analysis Methods	43
9. Appendix C – Stochastic Threshold Tables	47
10. Appendix D – Mixture Results	51



1. Introduction

The PowerPlex® Fusion system is a 24 locus system that includes all CODIS and ESS loci. The loci included are: Amelogenin, D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433, D22S1045, DYS391, and FGA.

Before an established method or procedure may be employed in a forensic laboratory, an internal validation must be completed to show that the method performs as expected. This validation summary outlines a set of experiments that shows conformance with the FBI Quality Assurance Standards for DNA Casework Laboratories (https://www.fbi.gov/about-us/lab/biometric-analysis/codis/qas_testlabs) and the validation guidelines outlined by the Scientific Working Group on DNA Analysis Methods (SWGDM) (http://swgdam.org/SWGDAM_Validation_Guidelines_APPROVED_Dec_2012.pdf). These experiments were designed to demonstrate the sensitivity and reliability of the PowerPlex® Fusion system. The studies completed were: sensitivity and stochastic studies, mixtures studies, precision, reproducibility, repeatability, known, non-probative evidence samples and mock evidence samples and contamination assessment.

2. FBI Quality Assurance Standard and SWGDAM Recommended Studies

Standard 8.3.1: Internal validation studies conducted after the date of this revision shall include as applicable: known and non-probative evidence samples or mock evidence samples, reproducibility and precision, sensitivity and stochastic studies, mixture studies, and contamination assessment. Internal validation studies shall be documented and summarized. The technical leader shall approve the internal validation studies.

3. Materials and Methods

3.A.1 DNA Amplification

DNA amplification was performed on an Applied Biosystems GeneAmp® PCR 9700 following the cycling parameters described in the PowerPlex® Fusion System technical manual using 29 cycles (TMD039). The protocol is 96°C for 1 minute, 29 cycles of 94°C for 10 seconds, 59°C for 1 minute, 72°C for 30 seconds, followed by 60°C for 10 minutes and a 4°C soak. A 12.5µl reaction was 2.5µl of 5X PowerPlex® Fusion master mix, 2.5µl of 5X PowerPlex® Fusion primer mix, and 7.5µl of DNA/water was added for all of the reactions. Negative controls contained 7.5µl water and the reaction mix. Please refer to Appendix A for the lot numbers of the reagents that were used during the validation. Applied Biosystems GeneAmp® PCR 9700 806F TC1 (serial number 805S6101009), Applied Biosystems GeneAmp® PCR 9700 806F-TC2 (serial number 805S7022692) and Applied Biosystems GeneAmp® PCR 9700 806F-TC3 (serial number 805S7022760) were used for the validation study.

3.A.2 Data Analysis

Spectral resolution was established using the PowerPlex® Fusion 5C matrix standard (TMD049). One microliter of amplified or allelic ladder was combined with 9.5µl of Hi-Di formamide and 0.5µl of the WEN Internal Lane Standard 500. The samples were heat denatured for 3 minutes at 95°C followed by an immediate chill for 3 minutes at 4°C. The samples were run on two 3130xl (Athena serial number 1594-010 and Newton 18231-006) genetic analyzers. Injection condition tested was 3kV for 5 second injection using a 2000 second run time. The data was analyzed using GeneMarkerHID v 2.8.2. Upon completion of the sensitivity study, the analytical threshold was calculated. As per the OCME-NYC, a 50 RFU threshold with a 3% minimum heterozygote filter and 3% global max filter in addition to the disabling of the pull-up correction was used for the rest of the validation.

3.A.3 Sensitivity Study

A sensitivity study was performed using the two extracted DNA samples, DNA1 and DNA 2. The final DNA templates tested were 4ng, 2ng, 1ng, 500pg, 250pg, 125pg, 100pg, 75pg, 50pg, 30pg, 25pg, 15pg, 7.5pg and 3.25pg. These samples were used to establish the analytical threshold and stochastic threshold as described in section 3.A.4. This study was also used to determine at which concentration allelic drop out first appears.



3.A.4 Analytical Threshold, and Stochastic Threshold

The analytical threshold is the point above which a peak can reliably be distinguished from baseline noise. The peak is either allelic or attributable to some known artifact such as pull-up or stutter. If a peak is not above the analytical threshold, it cannot be reliably considered to be a part of any developed DNA profile. If a true allelic peak is below the analytical threshold, that peak is considered to have “dropped out” of the profile. Any peak that is present above an analytical threshold that isn’t diagnosable artifact or due to a true allele from a known contributor would be considered to have ‘dropped-in’ to the profile.

The analytical threshold was calculated by Promega using two different methods of determination. Process #1 used the SWGDAM Interpretation Guidelines for Autosomal STR Typing recommendation. This method uses two times the intensity of the difference between the highest peak and lowest peak within the noise data. For this study, the sensitivity series, DNA 1 and DNA 2 from data generated on both Athena and Newton were reviewed. Process #2 targeted the limit of detection, a point at which the signal in the form of data peaks can be reliably distinguished from noise (LOD) and the limit of quantitation, a point in which signal can be reliably quantified (LOQ). 1ng and 500pg samples from sensitivity series, DNA 1 and DNA 2 that were injected on both Athena and Newton, were analyzed looking for the highest background noise in RFUs per dye lane. The average of the highest noise peak plus three times the standard deviation resulted in the LOD per dye lane. The average of the highest noise peaks plus ten times the standard deviation resulted in the LOQ per dye lane.

A stochastic threshold is the RFU level above which a single peak representing an allele at a locus can be determined to be a homozygote. If a single peak is observed below the stochastic threshold, then it must be assumed that a paired peak has potentially “dropped out” of the DNA profile. The stochastic threshold was calculated using the sensitivity series. The profiles were examined to determine the heterozygous loci in which one of the sister alleles had dropped below the analytical threshold. The average peak height of the surviving sister alleles was determined along with the standard deviation. Three times the standard deviation was added to the average to determine the stochastic threshold.

3.A.5 Known and Non-Probative Evidence Samples/ Mock Casework Samples

Amplification of known samples and non-probative evidence samples allows the laboratory to provide evidence of concordance using the new amplification system. For this study, ten known samples and twenty-four mock casework samples were amplified. Mock samples were generated from various touched items. In addition, all known and mock samples were amplified by two separate operators.

3.A.6 Precision

Sizing precision is critical for accurate genotyping. The migration of each allele in the ladder was evaluated. Thirteen ladders were run on each CE instrument (Athena and Newton). The average base pair size, standard deviation, minimum size, maximum size and range of base pair size was calculated for each allele present in the allelic ladder.

3.A.7 Reproducibility

The 2800M DNA sample supplied in the PowerPlex® Fusion amplification kit (0.25ng/μL) and the NIST SRM 2391c samples were used for the reproducibility study. The 2800M was amplified on all plates during the validation project; NIST SRM 2391c samples were amplified alongside known, mock casework and mixture samples. A combined total of 30 positive controls were injected throughout the validation on the CE instrument with the extracted DNA samples. In addition, 2800M and NIST SRM 2391c samples were amplified by two operators to show the reproducibility of the kit on different thermal cyclers and different operators.



3.A.8 Mixture Study

Mixture samples are commonly encountered in forensic casework samples. This study demonstrates the ability to detect major and minor profiles in samples, as well as the ability of the amplification kit to show mixtures at different ratios of contributors. For this study, two mixture series were generated using the following ratios: two person (20:1, 15:1, 10:1, 4:1, 2:1, 1:1, 1:2, 1:4, 1:10, 1:15, and 1:20), three person (5:1:1, 5:5:1, 1:1:1, 1:2:1, 3:2:1, and 5:2:1), four person (1:1:1:1, 1:3:3:1, and 1:3:5:1). Mixtures were amplified at four different target amounts: C1=750pg, C2=500pg, C3=250pg, and C4=100pg. Two person mixtures were evaluated for % profile obtained of the minor contributor using only unshared alleles. Three and four person mixtures were evaluated for the total number of alleles obtained. The three and four person mixtures will be evaluated during the OCME-NYC validation of STRmix® software program.

3.A.9 Contamination Assessment

Negative controls were run to assess contamination. A combined total of 35 negative controls were injected throughout the validation on both CE instruments. Each negative control was analyzed using the 50 RFU analytical threshold with a 3% minimum heterozygote filter and 3% global max filter.

3.A.10 Thermocycler Performance Check

Performance checks were conducted on ten thermocyclers. Seven replicates of 2800M were amplified using 750pg of input DNA. Alongside the seven replicates of 2800M, a negative control was run on each thermocycler. The results were evaluated using peak height and peak height ratios. Applied Biosystems GeneAmp® PCR 9700 705A TC11 (serial number 805S6101085, Applied Biosystems GeneAmp® PCR 9700 705A-TC12 (serial number 805S6101963), Applied Biosystems GeneAmp® PCR 9700 705A-TC13 (serial number 805S6101955), Applied Biosystems GeneAmp® PCR 9700 714C-TC6 (serial number 805S3110458), Applied Biosystems GeneAmp® PCR 9700 714C-TC8 (serial number 805S6101028), Applied Biosystems GeneAmp® PCR 9700 714C-TC9 (serial number 805S6101954), Applied Biosystems GeneAmp® PCR 9700 714C-TC11 (serial number 805S6101023), Applied Biosystems GeneAmp® PCR 9700 806C-TC6 (serial number 805S3120439), Applied Biosystems GeneAmp® PCR 9700 806F-TC1 (serial number 805S6101009), Applied Biosystems GeneAmp® PCR 9700 806F-TC2 (serial number 805S7022692), Applied Biosystems GeneAmp® PCR 9700 806F-TC3 (serial number 805S7022760), and Applied Biosystems GeneAmp® PCR 9700 610J-TC1 (serial number 805S3120445) were used for the performance check study.



4. Results

4.A.1 Analytical Threshold, and Stochastic Threshold

Following SWGDAM guidelines, process #1 used by Promega reviewed data generated from 2ng, 1ng, 500pg, 250pg, 125pg, 100pg, 75pg, 50pg, 30pg, 25pg, 15pg, 7.5pg, 3.25pg and 0pg on both Newton and Athena (Table 1). Process #2 used by Promega determined the LOD and LOQ of both the 1ng and 500pg samples that were injected on both Newton and Athena. (Table 2). In addition the analytical threshold was calculated by the OCME. As per the OCME, "Analysis of all the negative samples that were run for the Mock Evidence/R&R and Mixture studies was performed at 1 RFU, deleting any peaks that were obviously not noise, and exported the heights of the noise peaks that were called. 167 total data points were determined on Newton and 34 data points were determined on Athena. The average heights of all the noise peaks called was calculated and added to three times the standard deviation to arrive at the analytical threshold. Newton's average analytical threshold was 38.06 RFU, and Athena's was 27.24 RFU. The average for both instruments was 32.65 RFU. Considering the data above and in preparation for the STRmix validation project, 500pg samples were analyzed at varying analytical thresholds ranging from 1rfu to 80rfus. When considering the results of analysis at 40 RFU and 50 RFU, significantly fewer artifacts were present with the 50 RFU analytical threshold." The analytical threshold calculation described above by the OCME-NYC are shown in Table 3. As per the OCME-NYC, a 50 RFU threshold with a 3% minimum heterozygote filter and 3% global max filter was used for the rest of the validation. The analysis methods used to analyze the remainder of the validation data can be found in Appendix C. A stochastic threshold of 300 RFU can be used on both Athena and Newton (Table 4). The stochastic threshold tables can be found in Appendix C.

Due to the large number of artifacts that were observed using a 50 RFU analytical threshold, the OCME performed a global filter evaluation. As per the OCME, "A large number of artifacts were observed across all runs and between instruments. Troubleshooting steps of lowering input amount to 500pg and diluting the instrument matrix to make it less saturated were not successful in removing these artifacts. A global filter was evaluated to determine if artifacts could be reduced without excessive loss of data. Analysis of a portion of the samples run on both Athena and Newton was performed. A Global Max filter was applied during the run wizard, as well as a Min Heterozygote Imbalance (%) filter within the panel settings across all the locations. These samples were analyzed with a 10% filter, 8% filter, 5% filter and 4% filter set in both locations. After evaluation of a 4% filter it was noticed that almost half of the lost alleles could be recovered by going down to a 3% filter, while still greatly reducing the number of artifacts. For all samples, evidence and exemplars, it was decided a filter of 3% would be applied for the Global Max filter during the run wizard and across all locations within the panel for the Min Heterozygote Imbalance (%) filter."



Table 1: Analytical threshold calculated by Promega using Process #1 using sensitivity series and a calculation of 2* (maximum noise peak height – minimum noise peak height)

	Athena DNA1				Athena DNA 2				Newton DNA 1				Newton DNA 2			
	MIN	MAX	Difference	2X's	MIN	MAX	Difference	2X's	MIN	MAX	Difference	2X's	MIN	MAX	Difference	2X's
2ng	1	60	59	118	1	54	53	106	1	60	59	118	1	83	82	164
1ng	1	31	30	60	1	33	32	64	1	31	30	60	1	46	45	90
500pg	1	21	20	40	1	20	19	38	1	21	20	40	1	28	27	54
250pg	1	15	14	28	1	14	13	26	1	15	14	28	1	23	22	44
150pg	1	11	10	20	1	11	10	20	1	11	10	20	1	16	15	30
125pg	1	10	9	18	1	11	10	20	1	10	9	18	1	16	15	30
100pg	1	10	9	18	1	11	10	20	1	10	9	18	1	20	19	38
75pg	1	10	9	18	1	11	10	20	1	10	9	18	1	15	14	28
50pg	1	9	8	16	1	10	9	18	1	9	8	16	1	15	14	28
30pg	1	8	7	14	1	9	8	16	1	8	7	14	1	13	12	24
25pg	1	8	7	14	1	9	8	16	1	8	7	14	1	13	12	24
15pg	1	8	7	14	1	8	7	14	1	8	7	14	1	16	15	30
7.5pg	1	8	7	14	1	8	7	14	1	8	7	14	1	12	11	22
3.25pg	1	8	7	14	1	8	7	14	1	8	7	14	1	14	13	26
0pg	1	8	7	14	1	8	7	14	1	8	7	14	1	12	11	22



Table 2: Analytical threshold calculated by Promega using Process #2 using sensitivity samples near the optimal target.

Athena DNA1			Athena DNA2		
DNA Template	Background rfu (per dye channel)	Average Background (per template)	DNA Template	Background rfu (per dye channel)	Average Background (per template)
1ng	33	37	1ng	32	29
	29			26	
	36			36	
	48			22	
500pg	12	26	500pg	12	24
	28			23	
	31			31	
	31			31	
Newton DNA1			Newton DNA2		
DNA Template	Background rfu (per dye channel)	Average Background (per template)	DNA Template	Background rfu (per dye channel)	Average Background (per template)
1ng	55	56	1ng	57	54
	56			40	
	58			68	
	53			49	
500pg	21	31	500pg	39	36
	32			39	
	39			42	
	33			22	
	Average	STDEV	Limit of Detection (LOD) $AVG + 3 \times STDEV$	Limit of Quantitation (LOQ) $AVG + 10 \times STDEV$	
1ng and 500pg					
FL	33	16	81	195	
JOE	34	10	64	134	
TMR	43	12	79	165	
CXR	36	11	71	151	
OVERALLL =	36	13	76	170	
	Average	STDEV	Limit of Detection (LOD) $AVG + 3 \times STDEV$	Limit of Quantitation (LOQ) $AVG + 10 \times STDEV$	
500pg only					
FL	21	13	59	148	
JOE	31	7	51	98	
TMR	36	6	53	92	
CXR	29	5	44	78	
OVERALLL =	29	9	56	120	



Table 3: Analytical threshold calculated by OCME-NYC for casework at 29 cycles

Amplification Negative Noise Peaks at 1 RFU		
	Newton	Athena
Total Data Points	178	34
Average	15.64	13.82
Standard Deviation	9.61	11.09
Avg + 3x Std Dev	44.47	47.10
Average between Newton and Athena	45.79	

Table 4: Stochastic threshold for casework at 29 cycles for 3kV 5 sec.

		Average Height of Surviving Sister Allele	Standard Deviation	Maximum Peak Height of a Surviving Sister Allele	STOCHASTIC THRESHOLD
Athena	DNA 1	87	37	252	199
	DNA 2	94	44	251	227
Newton	DNA 1	103	53	320	262
	DNA 2	106	61	381	288



4.A.2 Sensitivity

The sensitivity data was analyzed using a 50 RFU threshold with a 3% minimum heterozygote filter and 3% global max filter. The samples that were used in the calculation were the 1ng, 500pg, 250pg, 125pg, 100pg, 75pg, 50pg, 30pg, 25pg, 15pg, 7.5pg, 3.25pg and 0pg for DNA1 and DNA2.

Full profiles in at least one replicate were obtained down to 50pg for DNA 1 on both Athena and Newton with 29 cycles; 75pg for DNA 2 (Table 5). Drop out in at least one replicate was first observed at 125pg for DNA 1 on both Athena and Newton; 100pg for DNA 2 on Athena and 50pg on Newton. As per the OCME a target optimal amount of 750pg would be appropriate for extracted DNA. The peak heights decreased with a decrease in template amount (Table 6-10). The decrease in template amount also decreased the peak height ratios for each of the DNA samples (Table 11-15). There were two instances of drop-in on Newton. Both were seen using a 50pg target amount; DNA 1 replicate A at 54 RFU and DNA 2 replicate C at 53 RFU (Figure 1).

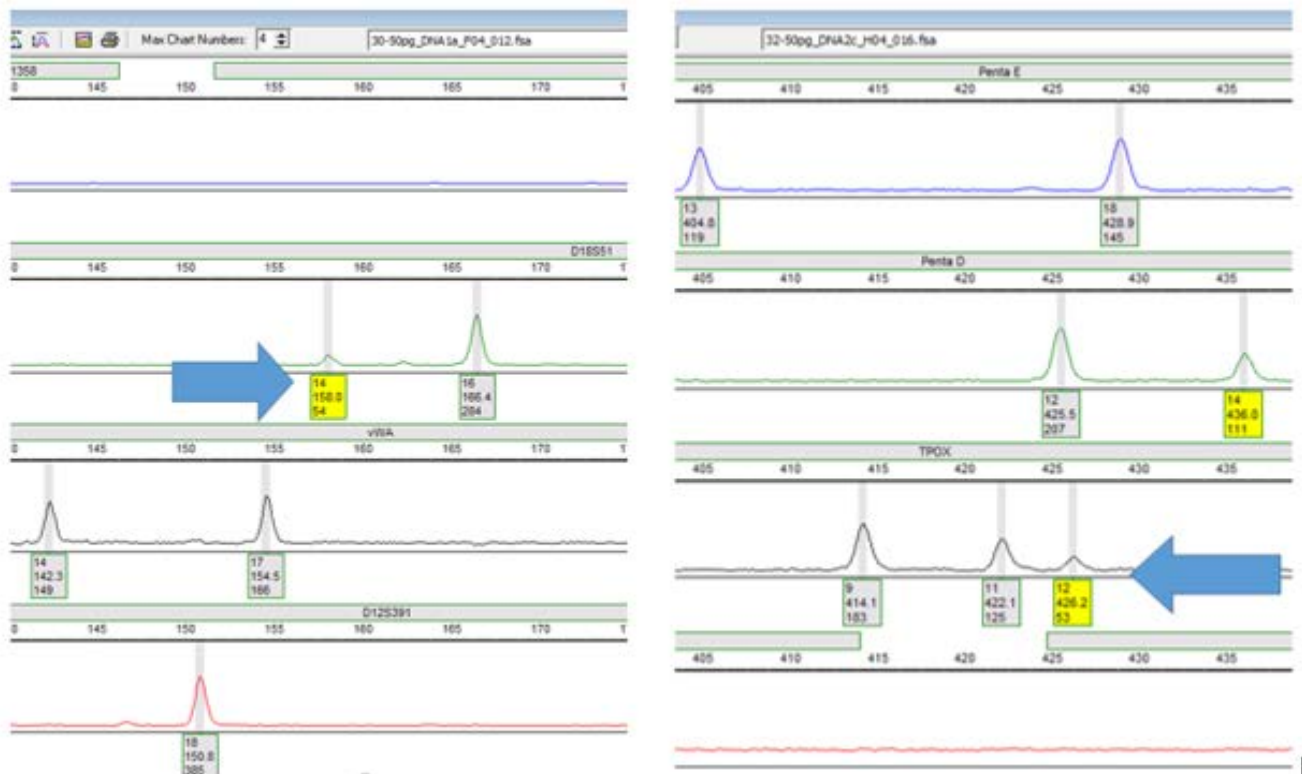


Figure 1: Instances of drop-in observed on Newton. Left side is DNA 1 replicate A with drop-in at D18S51 with an allelic assignment of 14 at 54 RFU. Right side is DNA 2 replicate C with an allelic assignment of 12 at 53 RFU.



Table 5: 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). The heat map allows for a quick visualization of the percent profile observed.

	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			



Table 6: 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 heat map allows for a quick visualization of the range of peak heights. 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	-



Table 7: Average peak heights for DNA 1 from Athena 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 heat map allows for a quick visualization of the range of peak heights. The white cells represent loci that had complete dropout in all replicates.

Athena DNA 1 Average Peak Heights														
	1ng	500pg	250pg	150pg	125pg	100pg	75pg	50pg	30pg	25pg	15pg	7.5pg	3.25pg	0pg
AMEL	2610	2097	905	683	316	413	372	189	101	110	43	-	9	-
D3S1358	2751	1726	703	451	313	298	181	145	67	68	32	48	-	-
D1S1656	2258	1498	601	419	273	242	224	138	62	74	42	12	-	-
D2S441	2205	1241	690	420	215	188	196	72	48	68	22	11	-	-
D10S1248	1952	1408	700	462	205	193	224	110	45	71	11	9	-	-
D13S317	1802	1148	497	393	218	165	236	135	46	50	17	-	-	-
Penta E	1551	1141	611	391	124	87	161	78	40	49	-	9	-	-
D16S539	3621	2102	999	554	470	317	300	180	101	60	19	11	9	-
D18S51	2943	1851	750	519	316	253	275	146	46	70	24	-	-	-
D2S1338	2259	1703	770	558	217	223	216	156	117	61	11	17	13	-
CSF1PO	2021	1136	510	452	147	146	201	94	56	99	-	-	-	-
Penta D	2083	1551	554	459	167	194	196	124	25	55	-	14	-	-
TH01	3281	1936	849	624	334	357	287	191	101	61	25	21	-	-
vWA	2768	1476	785	439	322	326	261	149	80	70	11	-	-	-
D21S11	2633	1770	712	472	356	263	230	142	41	50	48	23	-	-
D7S820	2930	1689	836	401	216	367	221	136	64	75	9	10	-	-
D5S818	2860	1785	833	501	234	260	189	106	108	81	36	9	-	-
TPOX	2294	1406	671	501	247	261	226	124	40	62	-	9	-	-
DYS391	1834	1482	585	469	218	163	192	106	45	37	18	-	-	-
D8S1179	2691	2313	1157	674	410	418	341	203	156	78	93	20	-	-
D12S391	3128	1924	610	362	390	155	173	92	47	44	22	-	-	-
D19S433	2039	1154	508	339	178	228	174	116	59	32	8	-	-	-
FGA	2326	1515	612	354	182	202	203	123	75	71	9	-	-	-
D22S1045	1384	888	378	230	104	166	101	55	50	51	-	-	-	-



Table 8: Average peak heights for DNA 2 from Athena 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 heat map allows for a quick visualization of the range of peak heights. The white cells represent loci that had complete dropout in all replicates.

Athena DNA 2 Average Peak Heights														
	1ng	500pg	250pg	150pg	125pg	100pg	75pg	50pg	30pg	25pg	15pg	7.5pg	3.25pg	0pg
AMEL	2095	1635	726	511	365	269	240	161	88	61	75	18	-	-
D3S1358	2326	1417	607	356	305	252	231	161	58	69	16	-	-	-
D1S1656	2200	1204	643	324	341	200	168	92	32	72	9	-	-	-
D2S441	2205	1233	549	420	220	236	134	71	83	23	-	13	-	-
D10S1248	1822	1231	643	363	268	186	182	141	60	37	22	10	-	-
D13S317	1977	1272	618	401	274	189	151	118	77	48	-	12	-	-
Penta E	1691	1204	573	427	252	128	188	91	44	41	-	-	-	-
D16S539	3351	2016	860	408	372	284	251	164	137	90	51	26	9	-
D18S51	2935	1707	948	429	428	246	225	129	93	81	32	9	-	-
D2S1338	2207	1585	708	446	453	251	244	175	110	37	22	-	9	-
CSF1PO	2200	1384	555	354	274	179	162	82	64	55	22	-	-	-
Penta D	2108	1490	778	364	341	203	227	104	78	68	32	20	-	-
TH01	2937	1548	823	463	368	269	242	138	92	52	69	49	-	-
vWA	2493	1543	663	411	287	281	192	147	59	49	9	15	-	-
D21S11	2373	1348	736	360	341	247	233	158	80	54	13	22	-	-
D7S820	2949	1660	804	455	409	253	244	149	74	75	10	-	-	-
D5S818	3098	1590	747	432	362	220	219	118	67	30	20	22	-	-
TPOX	2429	1404	751	447	339	241	201	97	60	83	31	17	-	-
DYS391														
D8S1179	2325	1730	821	466	349	372	244	159	101	89	36	13	-	-
D12S391	2737	1362	637	221	246	138	125	87	41	29	-	-	-	-
D19S433	1861	1152	505	289	204	162	161	39	96	18	-	-	-	-
FGA	2418	1487	596	317	313	264	199	135	54	44	29	-	-	-
D22S1045	1687	1064	437	267	209	154	160	56	38	44	-	-	-	-



Table 9: Average peak heights for DNA 1 from 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 heat map allows for a quick visualization of the range of peak heights. The white cells represent loci that had complete dropout in all replicates.

Newton DNA 1 Average Peak Heights														
	1ng	500pg	250pg	150pg	125pg	100pg	75pg	50pg	30pg	25pg	15pg	7.5pg	3.25pg	0pg
AMEL	4131	3476	1293	1071	586	635	669	291	184	192	94	9	27	-
D3S1358	4391	2844	1000	686	575	476	340	234	148	127	51	112	-	-
D1S1656	3653	2522	887	696	515	371	415	206	113	124	70	38	-	-
D2S441	3443	1956	955	599	380	288	347	119	96	120	70	15	-	-
D10S1248	3177	2345	1017	704	381	288	404	193	105	130	57	41	-	-
D13S317	2893	1838	720	638	393	272	420	215	101	103	27	29	-	-
Penta E	2419	1747	851	543	226	144	274	132	68	95	22	17	-	-
D16S539	4573	2759	1183	734	679	397	442	240	150	82	28	26	14	-
D18S51	4002	2548	950	615	472	327	412	202	88	108	55	10	8	-
D2S1338	3124	2292	990	768	328	277	319	219	175	93	33	33	28	-
CSF1PO	2725	1493	647	573	220	193	296	131	89	132	24	18	-	-
Penta D	2759	2031	711	528	253	243	292	173	56	79	-	24	-	-
TH01	4614	2664	1082	851	511	480	437	266	164	99	65	43	10	-
vWA	4044	2059	1022	619	510	446	399	210	126	110	27	-	-	-
D21S11	3851	2435	918	595	570	357	349	198	90	73	76	36	9	-
D7S820	4260	2315	1075	550	345	496	338	191	108	107	42	35	-	-
D5S818	4098	2383	1074	713	367	354	282	151	170	131	95	24	-	-
TPOX	3350	1896	864	632	386	341	338	193	81	96	24	37	-	-
DYS391	2694	1984	783	383	352	237	283	148	67	46	56	39	21	-
D8S1179	3958	3350	1550	1001	676	586	545	303	255	128	143	42	29	-
D12S391	4337	2738	807	506	665	218	278	137	90	73	34	28	-	-
D19S433	3002	1628	677	482	307	310	272	172	110	69	24	19	8	-
FGA	3464	2186	829	522	322	278	324	190	124	114	35	8	13	-
D22S1045	2084	1288	538	311	176	239	162	87	78	101	27	13	9	-



Table 10: Average peak heights for DNA 2 from 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 heat map allows for a quick visualization of the range of peak heights. The white cells represent loci that had complete dropout in all replicates.

Newton DNA 2 Average Peak Heights														
	1ng	500pg	250pg	150pg	125pg	100pg	75pg	50pg	30pg	25pg	15pg	7.5pg	3.25pg	0pg
AMEL	3362	2799	1228	884	609	415	431	275	157	106	122	47	19	-
D3S1358	3846	2455	990	641	524	385	423	280	110	126	57	10	19	-
D1S1656	3687	2114	1089	585	584	323	308	163	81	124	49	12	-	-
D2S441	3530	2042	745	716	359	369	251	132	149	58	9	30	-	-
D10S1248	3003	2086	1086	639	452	318	327	246	106	71	88	24	9	-
D13S317	3171	2122	1057	687	448	313	263	213	134	87	51	30	9	-
Penta E	2589	1974	1003	701	398	217	318	176	76	62	15	8	-	-
D16S539	4448	2783	1162	575	525	359	361	242	202	120	79	51	14	-
D18S51	4022	2434	1338	629	600	319	337	195	144	109	48	14	-	-
D2S1338	3089	2227	872	656	628	353	356	281	164	68	30	12	12	-
CSF1PO	2894	1894	792	494	372	253	234	134	114	71	49	10	10	-
Penta D	2721	2075	1167	507	460	264	325	172	112	89	50	32	-	-
TH01	4354	2230	1197	698	547	371	362	228	162	73	119	69	10	-
vWA	3710	2260	991	637	427	387	293	217	103	68	44	25	11	-
D21S11	3527	1923	1067	529	506	354	346	243	141	79	40	41	-	-
D7S820	4243	2386	1170	674	599	345	371	230	118	116	14	21	18	9
D5S818	4326	2232	1013	646	514	305	317	185	138	41	30	59	9	-
TPOX	3364	1943	1004	655	479	350	289	160	108	113	60	35	12	-
DYS391														
D8S1179	3667	2707	1334	748	550	541	403	268	169	141	70	23	10	-
D12S391	4327	2126	1040	361	398	203	205	152	111	56	-	11	-	-
D19S433	2941	1741	761	465	325	248	252	85	157	37	19	12	-	-
FGA	3679	2266	925	499	486	396	318	227	113	78	50	18	-	-
D22S1045	2471	1607	728	420	326	237	248	113	94	63	35	-	-	-



Table 11: Average peak height ratios for 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 ratio (red), the average (yellow) and the highest peak height ratio (dark green). The heat map allows for a quick visualization of the range of peak heights ratios. The gray cells represent the Y-STR locus with no heterozygous calculation. The white cells represent loci in with complete dropout in all replicates.

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%	-	-	-



Table 12: Average peak height ratios for DNA 1 from Athena 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 ratio (red), the average (yellow) and the highest peak height ratio (dark green). The heat map allows for a quick visualization of the range of peak heights ratios. The gray cells represent the Y-STR locus with no heterozygous calculation. The white cells represent loci in with complete dropout in all replicates.

Athena DNA 1 Average Peak Height Ratios														
	1ng	500pg	250pg	150pg	125pg	100pg	75pg	50pg	30pg	25pg	15pg	7.5pg	3.25pg	0pg
AMEL	90%	86%	92%	93%	90%	71%	69%	56%	82%	78%	-	-	-	-
D3S1358	97%	90%	92%	69%	92%	65%	61%	59%	91%	50%	77%	-	-	-
D1S1656														
D2S441	95%	80%	71%	67%	65%	84%	77%	66%	61%	78%	-	-	-	-
D10S1248	84%	82%	70%	78%	64%	76%	73%	72%	88%	69%	-	-	-	-
D13S317	84%	84%	78%	87%	95%	74%	67%	46%	-	42%	-	-	-	-
Penta E	92%	77%	88%	77%	70%	75%	79%	73%	-	62%	-	-	-	-
D16S539	97%	81%	87%	66%	80%	87%	80%	70%	86%	58%	-	-	-	-
D18S51	92%	80%	75%	80%	60%	86%	81%	64%	-	53%	87%	-	-	-
D2S1338	96%	89%	86%	72%	87%	85%	57%	63%	73%	88%	-	-	-	-
CSF1PO	86%	89%	84%	42%	62%	82%	52%	74%	91%	77%	-	-	-	-
Penta D	92%	93%	75%	80%	84%	75%	63%	65%	-	68%	-	-	-	-
TH01	90%	87%	83%	69%	75%	85%	90%	73%	69%	33%	-	-	-	-
vWA	87%	88%	89%	81%	74%	87%	58%	67%	62%	77%	-	-	-	-
D21S11	84%	92%	79%	87%	72%	52%	62%	77%	68%	52%	54%	93%	-	-
D7S820	91%	79%	73%	72%	76%	50%	58%	44%	85%	27%	-	-	-	-
D5S818	95%	79%	82%	77%	62%	77%	68%	56%	57%	70%	-	-	-	-
TPOX	85%	89%	81%	69%	71%	75%	98%	66%	75%	37%	-	-	-	-
DYS391														
D8S1179	92%	91%	85%	90%	77%	66%	84%	57%	63%	85%	74%	-	-	-
D12S391														
D19S433	84%	73%	79%	84%	74%	83%	73%	92%	77%	38%	-	-	-	-
FGA	93%	88%	90%	73%	59%	73%	76%	65%	81%	62%	-	-	-	-
D22S1045	85%	76%	66%	83%	77%	43%	60%	48%	78%	-	-	-	-	-



Table 13: Average peak height ratios for DNA 2 from Athena 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 ratio (red), the average (yellow) and the highest peak height ratio (dark green). The heat map allows for a quick visualization of the range of peak heights ratios. The gray cells represent the Y-STR locus with no heterozygous calculation. The white cells represent loci in with complete dropout in all replicates.

Athena DNA 2 Average Peak Height Ratios														
	1ng	500pg	250pg	150pg	125pg	100pg	75pg	50pg	30pg	25pg	15pg	7.5pg	3.25pg	0pg
AMEL														
D3S1358	93%	93%	72%	90%	84%	67%	74%	82%	89%	75%	-	-	-	-
D1S1656	86%	80%	81%	74%	88%	82%	81%	77%	82%	81%	-	-	-	-
D2S441	84%	85%	66%	90%	60%	60%	92%	70%	95%	-	-	-	-	-
D10S1248	89%	89%	73%	71%	73%	71%	61%	58%	68%	-	-	-	-	-
D13S317	86%	76%	87%	91%	73%	90%	68%	82%	76%	87%	-	-	-	-
Penta E	89%	84%	71%	78%	73%	68%	62%	86%	81%	80%	-	-	-	-
D16S539	94%	76%	86%	71%	88%	86%	85%	81%	72%	59%	-	-	-	-
D18S51	86%	95%	76%	59%	72%	52%	63%	83%	71%	68%	43%	-	-	-
D2S1338	89%	91%	79%	65%	83%	64%	66%	52%	50%	89%	-	-	-	-
CSF1PO	87%	86%	83%	76%	56%	66%	54%	55%	76%	70%	78%	-	-	-
Penta D	90%	82%	90%	81%	79%	87%	81%	47%	62%	100%	-	98%	-	-
TH01	85%	82%	73%	84%	74%	65%	73%	84%	47%	77%	46%	88%	-	-
vWA	93%	77%	87%	89%	84%	84%	59%	67%	74%	91%	-	-	-	-
D21S11	92%	77%	81%	75%	84%	74%	57%	58%	70%	57%	-	86%	-	-
D7S820	83%	79%	77%	84%	78%	84%	76%	68%	90%	56%	-	-	-	-
D5S818	94%	87%	89%	72%	78%	85%	81%	81%	42%	-	90%	-	-	-
TPOX	84%	71%	86%	76%	64%	56%	71%	73%	58%	87%	-	-	-	-
DYS391														
D8S1179	87%	92%	83%	84%	74%	70%	84%	68%	89%	62%	90%	-	-	-
D12S391	91%	87%	88%	73%	75%	78%	76%	74%	58%	-	-	-	-	-
D19S433	87%	89%	99%	76%	65%	74%	65%	77%	55%	-	-	-	-	-
FGA	90%	86%	95%	63%	85%	79%	87%	78%	92%	-	-	-	-	-
D22S1045	88%	79%	77%	69%	77%	71%	59%	72%	87%	54%	-	-	-	-



Table 14: Average peak height ratios for DNA 1 from 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 ratio (red), the average (yellow) and the highest peak height ratio (dark green). The heat map allows for a quick visualization of the range of peak heights ratios. The gray cells represent the Y-STR locus with no heterozygous calculation. The white cells represent loci in with complete dropout in all replicates.

Newton DNA 1 Average Peak Height Ratios														
	1ng	500pg	250pg	150pg	125pg	100pg	75pg	50pg	30pg	25pg	15pg	7.5pg	3.25pg	0pg
AMEL	88%	86%	93%	91%	83%	72%	69%	56%	85%	79%	46%	-	-	-
D3S1358	95%	89%	92%	63%	92%	65%	62%	60%	59%	47%	72%	34%	-	-
D1S1656														
D2S441	95%	81%	72%	53%	66%	84%	77%	66%	59%	71%	62%	-	-	-
D10S1248	84%	82%	70%	79%	66%	77%	72%	61%	81%	66%	75%	-	-	-
D13S317	84%	85%	78%	93%	95%	76%	68%	45%	38%	58%	-	98%	-	-
Penta E	90%	78%	89%	82%	70%	58%	78%	60%	-	61%	-	-	-	-
D16S539	97%	82%	88%	67%	80%	87%	79%	72%	85%	62%	-	-	-	-
D18S51	92%	80%	76%	84%	60%	83%	81%	63%	66%	38%	92%	-	-	-
D2S1338	97%	89%	87%	74%	86%	87%	56%	64%	73%	90%	98%	43%	-	-
CSF1PO	86%	88%	82%	51%	63%	82%	52%	75%	91%	77%	-	-	-	-
Penta D	91%	92%	75%	85%	83%	76%	64%	63%	58%	59%	-	-	-	-
TH01	90%	87%	85%	61%	75%	86%	92%	72%	68%	52%	56%	50%	-	-
vWA	87%	89%	89%	80%	71%	88%	59%	66%	61%	66%	-	-	-	-
D21S11	84%	90%	78%	83%	71%	52%	62%	73%	63%	51%	54%	95%	-	-
D7S820	93%	81%	72%	68%	77%	51%	59%	45%	70%	31%	85%	-	-	-
D5S818	97%	79%	82%	83%	60%	78%	68%	53%	53%	63%	61%	-	-	-
TPOX	86%	89%	84%	84%	72%	76%	68%	50%	74%	55%	-	-	-	-
DYS391														
D8S1179	92%	91%	83%	94%	78%	64%	84%	58%	64%	79%	74%	59%	80%	-
D12S391														
D19S433	84%	73%	79%	95%	64%	84%	74%	90%	75%	55%	79%	-	-	-
FGA	94%	87%	90%	86%	70%	74%	76%	64%	85%	64%	64%	-	-	-
D22S1045	86%	78%	67%	90%	76%	44%	63%	49%	75%	49%	-	-	-	-



Table 15: Average peak height ratios for DNA 2 from 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 ratio (red), the average (yellow) and the highest peak height ratio (dark green). The heat map allows for a quick visualization of the range of peak heights ratios. The gray cells represent the Y-STR locus with no heterozygous calculation. The white cells represent loci in with complete dropout in all replicates.

Newton DNA 2 Average Peak Height Ratios														
	1ng	500pg	250pg	150pg	125pg	100pg	75pg	50pg	30pg	25pg	15pg	7.5pg	3.25pg	0pg
AMEL														
D3S1358	93%	94%	74%	90%	84%	68%	74%	82%	88%	71%	64%	-	82%	-
D1S1656	86%	80%	92%	75%	89%	82%	80%	78%	86%	82%	78%	-	-	-
D2S441	85%	85%	71%	91%	63%	63%	68%	64%	94%	-	-	-	-	-
D10S1248	89%	88%	66%	71%	75%	73%	62%	58%	70%	37%	63%	-	-	-
D13S317	87%	77%	91%	93%	72%	92%	68%	82%	75%	86%	92%	-	-	-
Penta E	86%	84%	77%	79%	73%	57%	64%	69%	77%	83%	-	-	-	-
D16S539	92%	76%	84%	71%	87%	86%	86%	81%	72%	60%	27%	42%	-	-
D18S51	86%	95%	73%	59%	72%	53%	62%	87%	70%	71%	46%	-	-	-
D2S1338	89%	91%	73%	66%	82%	67%	66%	53%	50%	95%	-	-	-	-
CSF1PO	86%	85%	89%	76%	56%	66%	55%	60%	84%	72%	79%	-	-	-
Penta D	89%	82%	87%	80%	81%	88%	83%	41%	62%	99%	67%	92%	-	-
TH01	87%	83%	80%	82%	75%	68%	73%	61%	47%	80%	47%	92%	-	-
vWA	93%	78%	87%	89%	83%	85%	59%	68%	67%	91%	60%	-	-	-
D21S11	92%	77%	85%	74%	84%	73%	59%	58%	50%	59%	-	91%	-	-
D7S820	84%	79%	75%	85%	79%	82%	78%	69%	92%	50%	-	86%	-	-
D5S818	93%	88%	97%	72%	75%	86%	81%	79%	63%	-	76%	40%	-	-
TPOX	84%	70%	84%	76%	66%	57%	71%	75%	76%	88%	-	-	-	-
DYS391														
D8S1179	87%	91%	86%	84%	75%	70%	87%	67%	91%	54%	90%	-	-	-
D12S391	91%	88%	85%	75%	74%	77%	76%	74%	71%	67%	-	-	-	-
D19S433	88%	89%	97%	76%	68%	74%	65%	75%	55%	-	-	-	-	-
FGA	90%	88%	99%	64%	85%	76%	87%	83%	86%	78%	-	-	-	-
D22S1045	88%	80%	70%	70%	77%	71%	61%	76%	87%	51%	93%	-	-	-



4.A.3 Known Non Probative and Mock Casework Samples

All known non-probative samples were amplified using a 750pg target amount; mock casework samples were amplified targeting 750pg or max input volume. Known, non-probative and mock casework samples were amplified by two separate operators and injected for 3kV 5sec. Samples were analyzed using a 50 RFU threshold with a 3% minimum heterozygote filter and 3% global max filter. All ten known samples and mock casework samples were reviewed for concordance. The known, non-probative and mock casework samples had consistent genotypes with those provided. All known non-probative samples generated full profiles. Some mock casework displayed inconsistent results when compared to the provided results. These inconsistencies are most likely due to the nature of touched samples and the variations in overall target amount leading to drop out. In addition, multiple alleles not assigned to the original donor were called using PowerPlex® Fusion System (Tables 16-21).

Table 16: Mock casework key for profiles generated during the PowerPlex® Fusion internal validation.

	Touched Item	SA pg/ul
Mock 1	bottle	63.2
Mock 2	bottle	2155.4
Mock 3	bottle	276.3
Mock 4	bottle	463.7
Mock 5	bottle	173.0
Mock 6	bottle	53.3
Mock 7	bottle	474.9
Mock 8	bottle	1344.9
Mock 9	bottle	1803.5
Mock 10	Rubber Piece	24.9
Mock 11	Altoid Tin	5.8
Mock 12	Scissors	2.9
Mock 13	Fork Handle	516.8
Mock 14	Glasses Piece	62.1
Mock 15	Altoid Tin	3.6
Mock 16	Hair Drier	7.6
Mock 17	Timer	16.5
Mock 18	Ice Cream Scoop	190.6
Mock 19	Spoon Handle	6.7
Mock 20	Brown Sunglasses	25.2
Mock 21	Stress Toy	53.6
Mock 22	Ear Buds	9.8
Mock 23	Aviator Sunglasses	7.0
Mock 24	Cup	10.9



Table 17: Known profile success for operator 1. The bolded profiles were provided by the OCME-NYC and the non-bolded samples below are the observed profile during the validation.

Known Samples Newton Operator 1																								
	AMEL	D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E	D16S539	D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818	TPOX	DYS391	D8S1179	D12S391	D19S433	FGA	D22S1045
Known 1	XY	16 17	15 16	10 14	16	8 9	12 18	9 11	13 15	17 25	11 12	11 12	7 9	16 18	29 31.2	8 10	11	11	10	11 14	19 21	13	22 23	16 17
Known_1	XY	16 17	15 16	10 14	16	8 9	12 18	9 11	13 15	17 25	11 12	11 12	7 9	16 18	29 31.2	8 10	11	11	10	11 14	19 21	13	22 23	16 17
Known 2	XY	14 16	16.3 17.3	11 14	13 16	9	14 19	12 13	14 17	16 19	10	9 10	6 8	15 18	29 32.2	9 10	10 11	8 9	9	13	17 22	13 14	18 22	15
Known_2	XY	14 16	16.3 17.3	11 14	13 16	9	14 19	12 13	14 17	16 19	10	9 10	6 8	15 18	29 32.2	9 10	10 11	8 9	9	13	17 22	13 14	18 22	15
Known 3	XY	15 16	14 16	10 12	15 16	11	5 7	10 11	16 21	18 19	12	10 11	6 9	14 16	27 31	8 11	12 13	8 11	10	12 13	18 20	12	23 24	17
Known_3	XY	15 16	14 16	10 12	15 16	11	5 7	10 11	16 21	18 19	12	10 11	6 9	14 16	27 31	8 11	12 13	8 11	10	12 13	18 20	12	23 24	17
Known 4	XY	16	12	10 14	14 17	11 13	12 13	12	14 18	20 22	10 11	9 11	8 9.3	14 17	29 31.2	8 12	11 12	9 11	10	12 13	18 21	12 14	23	11 15
Known_4	XY	16	12	10 14	14 17	11 13	12 13	12	14 18	20 22	10 11	9 11	8 9.3	14 17	29 31.2	8 12	11 12	9 11	10	12 13	18 21	12 14	23	11 15
Known 5	XY	16	12 16	12 14	14 17	10 14	5 12	11	13 18	19 25	11 12	11 12	6 7	16 18	27 29	10	12 13	8	10	12 14	20 22	13 14.2	21 22	15 17
Known_5	XY	16	12 16	12 14	14 17	10 14	5 12	11	13 18	19 25	11 12	11 12	6 7	16 18	27 29	10	12 13	8	10	12 14	20 22	13 14.2	21 22	15 17
Known 6	XY	15 16	16 18.3	12 14	13 14	8 10	16 20	10 12	14	19 22	10 11	9 13	7 9.3	14 17	29	8	11	8 11	9	10 15	19 21	12.2 14.2	18 21	16 17
Known_6	XY	15 16	16 18.3	12 14	13 14	8 10	16 20	10 12	14	19 22	10 11	9 13	7 9.3	14 17	29	8	11	8 11	9	10 15	19 21	12.2 14.2	18 21	16 17
Known 7	X	16 17	13 15	11 14	15	12 13	5 8	9 11	16 18	16 24	10	8 13	7 8	15 19	29 32.2	9 11	12 13	9	**	14 15	15 17	14 14.2	22	15 16
Known_7	X	16 17	13 15	11 14	15	12 13	5 8	9 11	16 18	16 24	10	8 13	7 8	15 19	29 32.2	9 11	12 13	9	-	14 15	15 17	14 14.2	22	15 16
Known 8	X	15 16	15 17.3	11	14	10 11	12 22	10 12	12 14	20	12	8 12	7 8	16 19	31 32.2	11 12	7 9	8 11	**	16	18 20	13 15.2	19 23	16
Known_8	X	15 16	15 17.3	11	14	10 11	12 22	10 12	12 14	20	12	8 12	7 8	16 19	31 32.2	11 12	7 9	8 11	-	16	18 20	13 15.2	19 23	16
Known 9	X	15 16	15 16	11	13 14	8 12	5 12	11 12	14 19	17 23	10 12	8 12	7 9	14	30 31	9.1 10	10 12	8 10	**	10 14	22	13 14	24 26	15
Known_9	X	15 16	15 16	11	13 14	8 12	5 12	11 12	14 19	17 23	10 12	8 12	7 9	14	30 31	9.1 10	10 12	8 10	-	10 14	22	13 14	24 26	15
Known 10	X	17	11 15	10 14	13	12 13	7 12	9 10	15 18	19 23	10 12	9 10	6 7	14 18	29 30	10 11	11 12	8	**	13	17 18	12.2 15.2	20 26	15 16
Known_10	X	17	11 15	10 14	13	12 13	7 12	9 10	15 18	19 23	10 12	9 10	6 7	14 18	29 30	10 11	11 12	8	-	13	17 18	12.2 15.2	20 26	15 16

** and – represent loci where no alleles were present.



Table 18: Known profile success for operator 2. The top bolded profiles were provided by the OCME-NYC and the non-bolded samples below are the observed profile during the validation.

Known Samples Newton Operator 2																								
	AMEL	D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E	D16S539	D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818	TPOX	DYS391	D8S1179	D12S391	D19S433	FGA	D22S1045
Known 1	XY	16 17	15 16	10 14	16	8 9	12 18	9 11	13 15	17 25	11 12	11 12	7 9	16 18	29 31.2	8 10	11	11	10	11 14	19 21	13	22 23	16 17
Known_1	XY	16 17	15 16	10 14	16	8 9	12 18	9 11	13 15	17 25	11 12	11 12	7 9	16 18	29 31.2	8 10	11	11	10	11 14	19 21	13	22 23	16 17
Known 2	XY	14 16	16.3 17.3	11 14	13 16	9	14 19	12 13	14 17	16 19	10	9 10	6 8	15 18	29 32.2	9 10	10 11	8 9	9	13	17 22	13 14	18 22	15
Known_2	XY	14 16	16.3 17.3	11 14	13 16	9	14 19	12 13	14 17	16 19	10	9 10	6 8	15 18	29 32.2	9 10	10 11	8 9	9	13	17 22	13 14	18 22	15
Known 3	XY	15 16	14 16	10 12	15 16	11	5 7	10 11	16 21	18 19	12	10 11	6 9	14 16	27 31	8 11	12 13	8 11	10	12 13	18 20	12	23 24	17
Known_3	XY	15 16	14 16	10 12	15 16	11	5 7	10 11	16 21	18 19	12	10 11	6 9	14 16	27 31	8 11	12 13	8 11	10	12 13	18 20	12	23 24	17
Known 4	XY	16	12	10 14	14 17	11 13	12 13	12	14 18	20 22	10 11	9 11	8 9.3	14 17	29 31.2	8 12	11 12	9 11	10	12 13	18 21	12 14	23	11 15
Known_4	XY	16	12	10 14	14 17	11 13	12 13	12	14 18	20 22	10 11	9 11	8 9.3	14 17	29 31.2	8 12	11 12	9 11	10	12 13	18 21	12 14	23	11 15
Known 5	XY	16	12 16	12 14	14 17	10 14	5 12	11	13 18	19 25	11 12	11 12	6 7	16 18	27 29	10	12 13	8	10	12 14	20 22	13 14.2	21 22	15 17
Known_5	XY	16	12 16	12 14	14 17	10 14	5 12	11	13 18	19 25	11 12	11 12	6 7	16 18	27 29	10	12 13	8	10	12 14	20 22	13 14.2	21 22	15 17
Known 6	XY	15 16	16 18.3	12 14	13 14	8 10	16 20	10 12	14	19 22	10 11	9 13	7 9.3	14 17	29	8	11	8 11	9	10 15	19 21	12.2 14.2	18 21	16 17
Known_6	XY	15 16	16 18.3	12 14	13 14	8 10	16 20	10 12	14	19 22	10 11	9 13	7 9.3	14 17	29	8	11	8 11	9	10 15	19 21	12.2 14.2	18 21	16 17
Known 7	X	16 17	13 15	11 14	15	12 13	5 8	9 11	16 18	16 24	10	8 13	7 8	15 19	29 32.2	9 11	12 13	9	**	14 15	15 17	14 14.2	22	15 16
Known_7	X	16 17	13 15	11 14	15	12 13	5 8	9 11	16 18	16 24	10	8 13	7 8	15 19	29 32.2	9 11	12 13	9	-	14 15	15 17	14 14.2	22	15 16
Known 8	X	15 16	15 17.3	11	14	10 11	12 22	10 12	12 14	20	12	8 12	7 8	16 19	31 32.2	11 12	7 9	8 11	**	16	18 20	13 15.2	19 23	16
Known_8	X	15 16	15 17.3	11	14	10 11	12 22	10 12	12 14	20	12	8 12	7 8	16 19	31 32.2	11 12	7 9	8 11	-	16	18 20	13 15.2	19 23	16
Known 9	X	15 16	15 16	11	13 14	8 12	5 12	11 12	14 19	17 23	10 12	8 12	7 9	14	30 31	9.1 10	10 12	8 10	**	10 14	22	13 14	24 26	15
Known_9	X	15 16	15 16	11	13 14	8 12	5 12	11 12	14 19	17 23	10 12	8 12	7 9	14	30 31	9.1 10	10 12	8 10	-	10 14	22	13 14	24 26	15
Known 10	X	17	11 15	10 14	13	12 13	7 12	9 10	15 18	19 23	10 12	9 10	6 7	14 18	29 30	10 11	11 12	8	**	13	17 18	12.2 15.2	20 26	15 16
Known_10	X	17	11 15	10 14	13	12 13	7 12	9 10	15 18	19 23	10 12	9 10	6 7	14 18	29 30	10 11	11 12	8	-	13	17 18	12.2 15.2	20 26	15 16

** and – represent loci where no alleles were present.



4.A.4 Precision

The precision assessment was done using 13 allelic ladders that were run in conjunction with the sensitivity samples at 3kV 5s on Athena, and 13 allelic ladders at 3kV 5s on Newton. The size of the alleles (in bp) was evaluated. The highest standard deviation observed on Athena was 0.1 (Figure 2). The highest standard deviation observed on Newton was 0.1 (Figure 3). Three times the standard deviation is less 0.5bp.

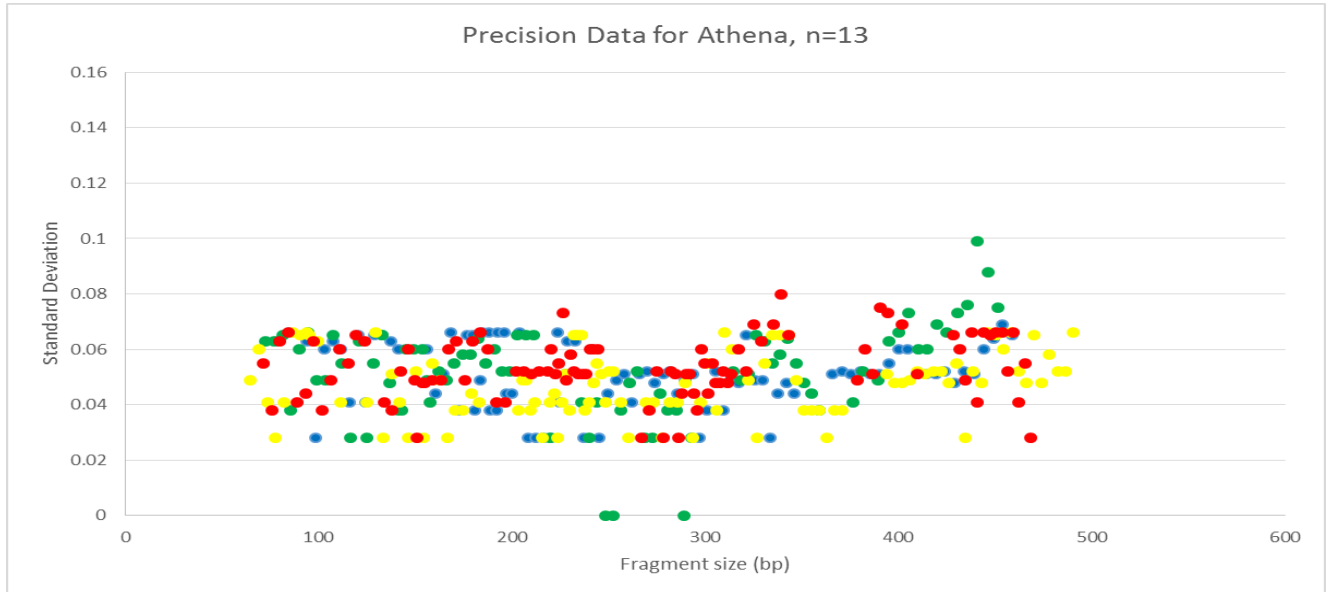


Figure 2: Precision of the PowerPlex® Fusion system on a 3130xl Genetic Analyzer. Thirteen ladders were run on the Applied Biosystems® 3130xl using a 3kV, 5 second injection on Athena. For each allele, the average fragment size (in bases) was plotted against the standard deviation of the mean size observed. Generally, a standard deviation of 0.16 ($3 * \text{st.dev} < 0.5$) is an acceptable precision for the system.

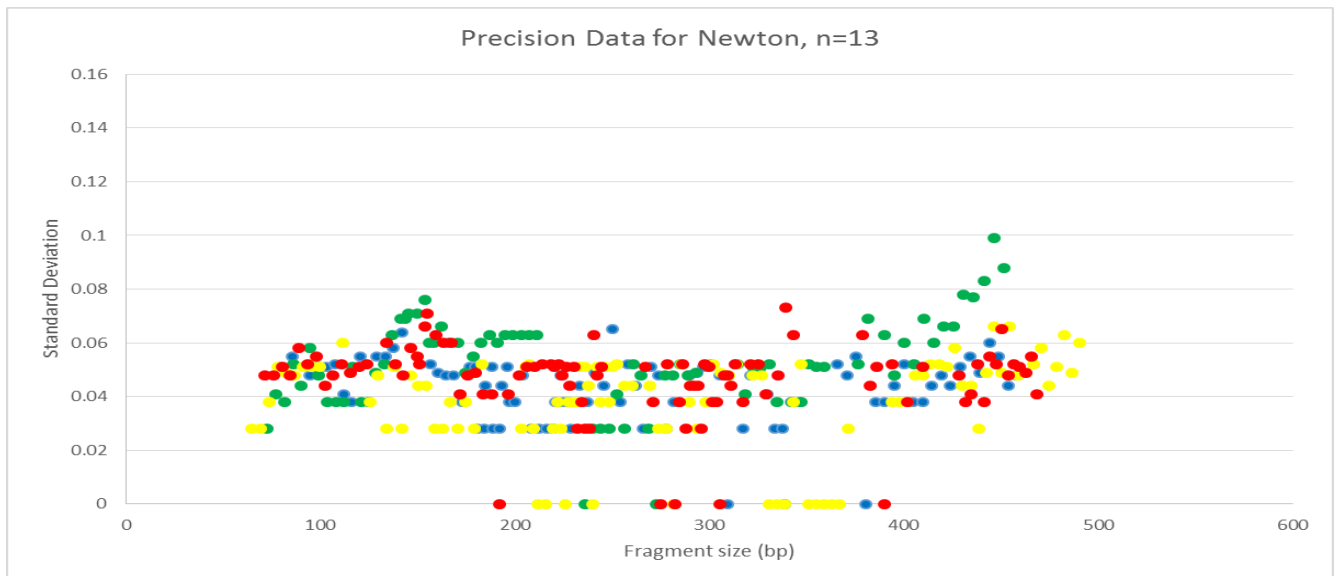


Figure 3: Precision of the PowerPlex® Fusion system on a 3130xl Genetic Analyzer. Thirteen ladders were run on the Applied Biosystems® 3130xl using a 3kV, 5 second injection on Newton. For each allele, the average fragment size (in bases) was plotted against the standard deviation of the mean size observed. Generally, a standard deviation of 0.16 ($3 * \text{st.dev} < 0.5$) is an acceptable precision for the system.



4.A.5 Reproducibility and Repeatability

Positive controls were amplified on each amplification plate. Every positive control (2800M) resulted in the same profile which is concordant with the profile provided in TMD045 Appendix 9.A in the Technical Manual (Table 22). Replicates of 2800M were also amplified by two different operators and injected on both Athena and Newton. All profiles yielded the correct genotype. (Table 23) NIST SRM 2391c samples were also amplified by two different operators. One replicate of NIST C amplified by operator 1 initially resulted in an OB at DYS391; the sample was re-injected. The subsequent re-injection results in the correct allelic assignment of 11 at DYS391. All results were concordant with the NIST 2391c certificate with the exception of NIST D at D12S391 allele 19. Allele 19 not called due to resolution between the 18.3 and 19 allele. (Table 24) (Figure 4). The resolution of 18.3 and 19 at D12S391 is concordant with the results obtained during the PowerPlex® Fusion developmental validation.

Table 22: Genotype results of all positive controls amplified during the validation.

		OCME Repeatability of 2800M																								
		AMEL	D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E	D16S539	D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818	TPOX	DYS391	D8S1179	D12S391	D19S433	FGA	D22S1045	
Athena	2800M	XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
	02-Amplification_Positive	XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
	24-Amplification_Positive	XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
	24-Amplification_Positive	XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
	24-Amplification_Positive																									
	26-Amplification_Positive	XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
	27-Amplification_Positive	XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
	28-Amplification_Positive	XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
	52-Amp_Pos_a	XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
	52-Amp_Pos_a	XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
	53-Amp_Pos_b	XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
	53-Amp_Pos_b	XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
	54-Amp_Pos_c	XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
	54-Amp_Pos_c	XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
	09-Amp_Pos_1	XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
	10-Amp_Pos_2	XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
	11-Amp_Pos_3	XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
	12-Amp_Pos_4	XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
	Newton	02-Amplification_Positive	XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16
		24-Amplification_Positive	XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16
		26-Amplification_Positive	XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16
		27-Amplification_Positive	XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16
		28-Amplification_Positive	XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16
		52-Amp_Pos_a	XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16
52-Amp_Pos_a		XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
53-Amp_Pos_b		XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
53-Amp_Pos_b		XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
54-Amp_Pos_c		XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
54-Amp_Pos_c		XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
09-Amp_Pos_1		XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
09-Amp_Pos_1		XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
10-Amp_Pos_2		XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
10-Amp_Pos_2		XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
11-Amp_Pos_3		XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
11-Amp_Pos_3		XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
12-Amp_Pos_4		XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
12-Amp_Pos_4		XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
09-Amp_Pos_1		XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
10-Amp_Pos_2		XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
11-Amp_Pos_3		XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	
12-Amp_Pos_4		XY	17 18	12 13	10 14	13 15	9 11	7 14	9 13	16 18	22 25	12	12 13	6 9.3	16 19	29 31.2	8 11	12	11	10	14 15	18 23	13 14	20 23	16	



Table 23: Results of 2800M amplified by two different operators and injected on both Newton and Athena.

		OCME Reproducibility of 2800M																								
		AMEL	D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E	D16S539	D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818	TPOX	DYS391	D8S1179	D12S391	D19S433	FGA	D22S1045	
2800M		XY	1718	1213	1014	1315	911	714	913	1618	2225	12	1213	69.3	1619	2931.2	811	12	11	10	1415	1823	1314	2023	16	
Newton	Operator 1	09-Amp_Pos_1	XY	1718	1213	1014	1315	911	714	913	1618	2225	12	1213	69.3	1619	2931.2	811	12	11	10	1415	1823	1314	2023	16
		09-Amp_Pos_1	XY	1718	1213	1014	1315	911	714	913	1618	2225	12	1213	69.3	1619	2931.2	811	12	11	10	1415	1823	1314	2023	16
		10-Amp_Pos_2	XY	1718	1213	1014	1315	911	714	913	1618	2225	12	1213	69.3	1619	2931.2	811	12	11	10	1415	1823	1314	2023	16
		10-Amp_Pos_2	XY	1718	1213	1014	1315	911	714	913	1618	2225	12	1213	69.3	1619	2931.2	811	12	11	10	1415	1823	1314	2023	16
	11-Amp_Pos_3	XY	1718	1213	1014	1315	911	714	913	1618	2225	12	1213	69.3	1619	2931.2	811	12	11	10	1415	1823	1314	2023	16	
	11-Amp_Pos_3	XY	1718	1213	1014	1315	911	714	913	1618	2225	12	1213	69.3	1619	2931.2	811	12	11	10	1415	1823	1314	2023	16	
	12-Amp_Pos_4	XY	1718	1213	1014	1315	911	714	913	1618	2225	12	1213	69.3	1619	2931.2	811	12	11	10	1415	1823	1314	2023	16	
	12-Amp_Pos_4	XY	1718	1213	1014	1315	911	714	913	1618	2225	12	1213	69.3	1619	2931.2	811	12	11	10	1415	1823	1314	2023	16	
Athena	Operator 2	09-Amp_Pos_1	XY	1718	1213	1014	1315	911	714	913	1618	2225	12	1213	69.3	1619	2931.2	811	12	11	10	1415	1823	1314	2023	16
		10-Amp_Pos_2	XY	1718	1213	1014	1315	911	714	913	1618	2225	12	1213	69.3	1619	2931.2	811	12	11	10	1415	1823	1314	2023	16
		11-Amp_Pos_3	XY	1718	1213	1014	1315	911	714	913	1618	2225	12	1213	69.3	1619	2931.2	811	12	11	10	1415	1823	1314	2023	16
		12-Amp_Pos_4	XY	1718	1213	1014	1315	911	714	913	1618	2225	12	1213	69.3	1619	2931.2	811	12	11	10	1415	1823	1314	2023	16



Table 24: Results of NIST SRM 2391c sample concordance.

		OCME Reproducibility of 2391c NIST																							
		AMEL	D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E	D16S539	D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818	TPOX	DYS391	D8S1179	D12S391	D19S433	FGA	D22S1045
NIST A		X	15 16	17.3	10	15 16	8	5 10	10 11	12 15	18 23	10	9 13	89.3	18 19	28 32.2	11	11 12	8	-	13 14	18.3 22	13 14	21 23	15
Operator 1	NISTA1	X	15 16	17.3	10	15 16	8	5 10	10 11	12 15	18 23	10	9 13	89.3	18 19	28 32.2	11	11 12	8	-	13 14	18.3 22	13 14	21 23	15
	NISTA1	X	15 16	17.3	10	15 16	8	5 10	10 11	12 15	18 23	10	9 13	89.3	18 19	28 32.2	11	11 12	8	-	13 14	18.3 22	13 14	21 23	15
	NISTA2	X	15 16	17.3	10	15 16	8	5 10	10 11	12 15	18 23	10	9 13	89.3	18 19	28 32.2	11	11 12	8	-	13 14	18.3 22	13 14	21 23	15
	NISTA2	X	15 16	17.3	10	15 16	8	5 10	10 11	12 15	18 23	10	9 13	89.3	18 19	28 32.2	11	11 12	8	-	13 14	18.3 22	13 14	21 23	15
Operator 2	NISTA1	X	15 16	17.3	10	15 16	8	5 10	10 11	12 15	18 23	10	9 13	89.3	18 19	28 32.2	11	11 12	8	-	13 14	18.3 22	13 14	21 23	15
	NISTA2	X	15 16	17.3	10	15 16	8	5 10	10 11	12 15	18 23	10	9 13	89.3	18 19	28 32.2	11	11 12	8	-	13 14	18.3 22	13 14	21 23	15
NIST B		XY	15 19	11 14	10 14	13	9 12	7 15	10 13	13 16	17	10 11	8 12	69.3	17 18	32 32.2	10	12 13	8 11	10	10 13	19 24	16 16.2	20 23	15 17
Operator 1	NISTB1	XY	15 19	11 14	10 14	13	9 12	7 15	10 13	13 16	17	10 11	8 12	69.3	17 18	32 32.2	10	12 13	8 11	10	10 13	19 24	16 16.2	20 23	15 17
	NISTB1	XY	15 19	11 14	10 14	13	9 12	7 15	10 13	13 16	17	10 11	8 12	69.3	17 18	32 32.2	10	12 13	8 11	10	10 13	19 24	16 16.2	20 23	15 17
	NISTB2	XY	15 19	11 14	10 14	13	9 12	7 15	10 13	13 16	17	10 11	8 12	69.3	17 18	32 32.2	10	12 13	8 11	10	10 13	19 24	16 16.2	20 23	15 17
	NISTB2	XY	15 19	11 14	10 14	13	9 12	7 15	10 13	13 16	17	10 11	8 12	69.3	17 18	32 32.2	10	12 13	8 11	10	10 13	19 24	16 16.2	20 23	15 17
Operator 2	NISTB1	XY	15 19	11 14	10 14	13	9 12	7 15	10 13	13 16	17	10 11	8 12	69.3	17 18	32 32.2	10	12 13	8 11	10	10 13	19 24	16 16.2	20 23	15 17
	NISTB2	XY	15 19	11 14	10 14	13	9 12	7 15	10 13	13 16	17	10 11	8 12	69.3	17 18	32 32.2	10	12 13	8 11	10	10 13	19 24	16 16.2	20 23	15 17
NIST C		XY	16 18	11 15	10	12 16	11	12 13	10	16 19	19	10 12	10 11	6 8	16 18	29 30	10 12	10 11	11	11	10 17	19 23	13.2 15.2	24 26	16
Operator 1	NISTC1	XY	16 18	11 15	10	12 16	11	12 13	10	16 19	19	10 12	10 11	6 8	16 18	29 30	10 12	10 11	11	11	10 17	19 23	13.2 15.2	24 26	16
	NISTC1	XY	16 18	11 15	10	12 16	11	12 13	10	16 19	19	10 12	10 11	6 8	16 18	29 30	10 12	10 11	11	11	10 17	19 23	13.2 15.2	24 26	16
	NISTC2	XY	16 18	11 15	10	12 16	11	12 13	10	16 19	19	10 12	10 11	6 8	16 18	29 30	10 12	10 11	11	11	10 17	19 23	13.2 15.2	24 26	16
	NISTC2**	XY	16 18	11 15	10	12 16	11	12 13	10	16 19	19	10 12	10 11	6 8	16 18	29 30	10 12	10 11	11	OB	10 17	19 23	13.2 15.2	24 26	16
	NISTC2	XY	16 18	11 15	10	12 16	11	12 13	10	16 19	19	10 12	10 11	6 8	16 18	29 30	10 12	10 11	11	11	10 17	19 23	13.2 15.2	24 26	16
Operator 2	NISTC1	XY	16 18	11 15	10	12 16	11	12 13	10	16 19	19	10 12	10 11	6 8	16 18	29 30	10 12	10 11	11	11	10 17	19 23	13.2 15.2	24 26	16
	NISTC2	XY	16 18	11 15	10	12 16	11	12 13	10	16 19	19	10 12	10 11	6 8	16 18	29 30	10 12	10 11	11	11	10 17	19 23	13.2 15.2	24 26	16
NIST D		XY	15 16 18	11 15 17.3	10	12 15 16	8 11	5 10 12 13	10 11	12 15 16 19	18 19 23	10 12	9 10 11 13	6 8 9.3	16 18 19	28 29 30 32.2	10 11 12	10 11 12	8 11	11	10 13 14 17	18.3 19 22 23	13 13.2 14 15.2	21 23 24 26	15 16
Operator 1	NISTD1	XY	15 16 18	11 15 17.3	10	12 15 16	8 11	5 10 12 13	10 11	12 15 16 19	18 19 23	10 12	9 10 11 13	6 8 9.3	16 18 19	28 29 30 32.2	10 11 12	10 11 12	8 11	11	10 13 14 17	18.3 22 23	13 13.2 14 15.2	21 23 24 26	15 16
	NISTD1	XY	15 16 18	11 15 17.3	10	12 15 16	8 11	5 10 12 13	10 11	12 15 16 19	18 19 23	10 12	9 10 11 13	6 8 9.3	16 18 19	28 29 30 32.2	10 11 12	10 11 12	8 11	11	10 13 14 17	18.3 22 23	13 13.2 14 15.2	21 23 24 26	15 16
	NISTD2	XY	15 16 18	11 15 17.3	10	12 15 16	8 11	5 10 12 13	10 11	12 15 16 19	18 19 23	10 12	9 10 11 13	6 8 9.3	16 18 19	28 29 30 32.2	10 11 12	10 11 12	8 11	11	10 13 14 17	18.3 22 23	13 13.2 14 15.2	21 23 24 26	15 16
	NISTD2	XY	15 16 18	11 15 17.3	10	12 15 16	8 11	5 10 12 13	10 11	12 15 16 19	18 19 23	10 12	9 10 11 13	6 8 9.3	16 18 19	28 29 30 32.2	10 11 12	10 11 12	8 11	11	10 13 14 17	18.3 22 23	13 13.2 14 15.2	21 23 24 26	15 16
Operator 2	NISTD1	XY	15 16 18	11 15 17.3	10	12 15 16	8 11	5 10 12 13	10 11	12 15 16 19	18 19 23	10 12	9 10 11 13	6 8 9.3	16 18 19	28 29 30 32.2	10 11 12	10 11 12	8 11	11	10 13 14 17	18.3 22 23	13 13.2 14 15.2	21 23 24 26	15 16
	NISTD2	XY	15 16 18	11 15 17.3	10	12 15 16	8 11	5 10 12 13	10 11	12 15 16 19	18 19 23	10 12	9 10 11 13	6 8 9.3	16 18 19	28 29 30 32.2	10 11 12	10 11 12	8 11	11	10 13 14 17	18.3 22 23	13 13.2 14 15.2	21 23 24 26	15 16

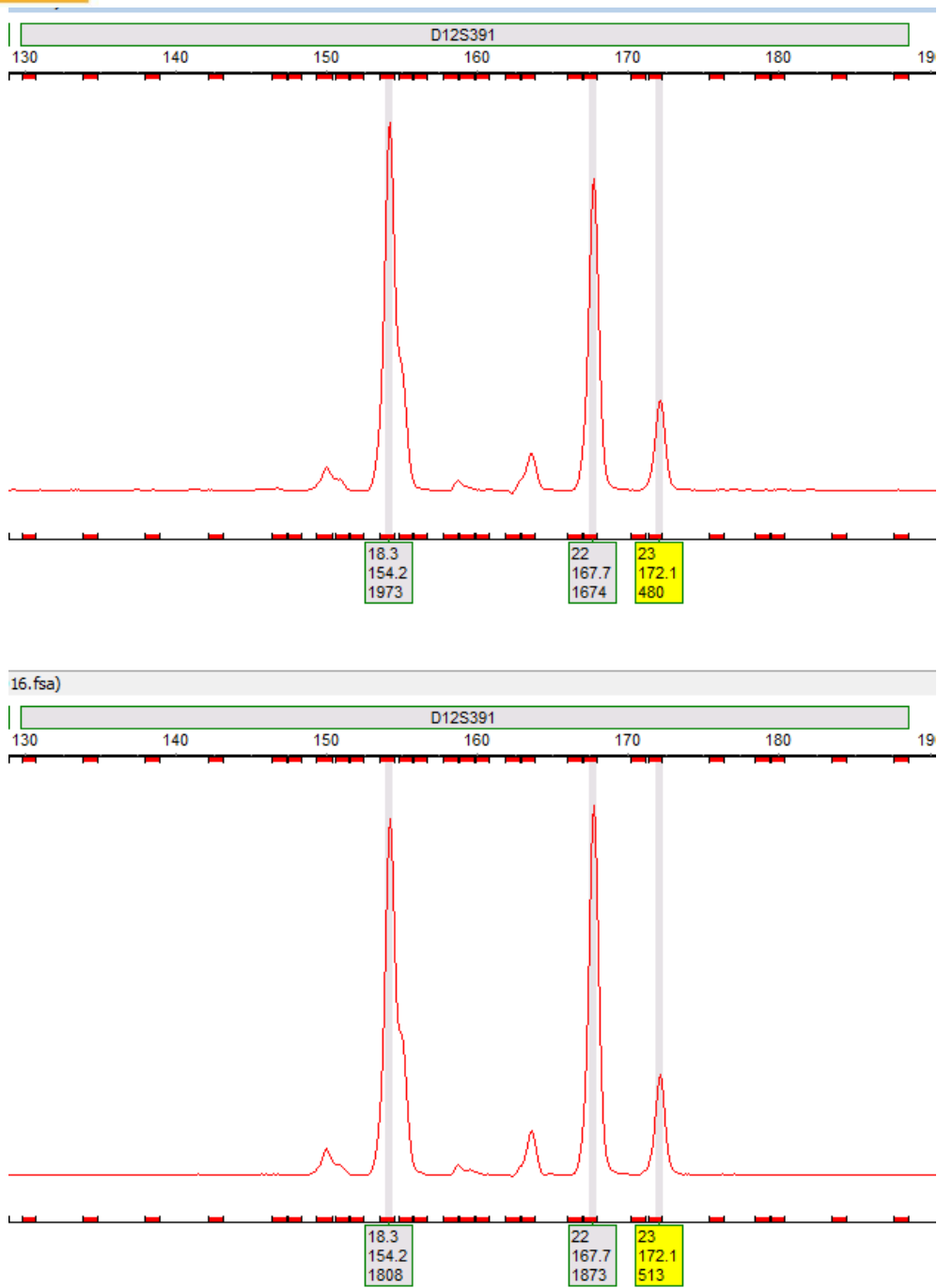


Figure 4: NIST SRM 2391c sample D Applied Biosystems® 3130xl injected on Athena using 3kv 5 sec injection parameter.



4.A.6 Mixture Study

Eighty-eight two person mixtures were amplified during this validation study. Two person mixtures were generated using samples 6M and 22F (mixture 1) and 15M and 5F (mixture 2). Profiles were compared and a composite profile was generated containing only unshared alleles in the minor contributor. The mixtures were then compared to determine what percentage of the unique minor alleles was detected in each mixture ratio. For two person mixtures injected on Athena, 100% of the unique alleles from the minor donor was obtained at 2-1 and 4-1 mixtures using a 750pg, 500pg, and 250pg for both mixture series. Mixture series #2 obtained a full profile at a 750pg 10-1 ratio (Tables 25). Two person mixtures injected on Newton obtained 100% of the minor alleles for 2-1 mixtures down to 100pg and 4-1 mixtures down to 250pg for both mixture series. Mixture series #2 obtained a full profile at a 750pg 10-1 ratio (Table 26).

Forty-eight three person mixtures were amplified during this study. Three person mixtures were generated using samples 12F, 13M, and 6M, (mixture 1), and 17M, 27F, and 9M (mixture 2). Twelve four person mixtures were generated using 17M, 27F, 9M, and 28F. Three and four person mixtures were evaluated for the total number of alleles obtained. Three person mixtures obtained full profiles for mixture #1 on Athena and Newton at a 5-1-1, 5-5-1, 1-1-1, 1-2-1, 3-2-1, and 5-2-1 at 750pg; 1-1-1, 1-2-1, 3-2-1, and 5-2-1 at 500pg; 5-1-1, 1-2-1, 3-2-1, and 5-2-1 at 250pg. Full profiles were obtained for mixture #2 on Athena and Newton at a 1-1-1, 1-2-1, 3-2-1, and 5-2-1 at 750pg; 1-1-1, 1-2-1, and 3-2-1 at 500pg. Four person mixtures obtained full profiles on both Athena and Newton using a 750pg target at 1-1-1-1 and 1-3-3-1. (Table 27 and 28). See Appendix E for individual mixture results.

There were numerous instances of pull-up noticed throughout both mixture series on both instruments. Mixtures amplified using 750pg target amount at times had a significant amount of pull up seen in the sample. As expected, when lowering the target amount of DNA amplified the instances of pull up decreased as well (Table 29 and 30).

Table 25: Percentage of minor profile obtained for two person mixtures injected at 3kV 5 sec on Athena

		Athena Two Person Mixtures									
		20-1	1-20	15-1	1-15	10-1	1-10	4-1	1-4	2-1	1-2
750pg	Mix 1	41%	61%	85%	93%	93%	89%	100%	100%	100%	100%
	Mix 2	75%	59%	92%	85%	100%	93%	100%	100%	100%	100%
500pg	Mix 1	59%	68%	63%	71%	89%	86%	100%	100%	100%	100%
	Mix 2	75%	52%	83%	56%	92%	81%	100%	100%	100%	100%
250pg	Mix 1	56%	43%	48%	46%	74%	75%	100%	100%	100%	100%
	Mix 2	42%	33%	38%	48%	67%	78%	100%	100%	100%	100%
100pg	Mix 1	26%	4%	15%	11%	37%	25%	52%	86%	78%	93%
	Mix 2	13%	11%	17%	4%	33%	33%	54%	74%	83%	81%

Table 26: Percentage of minor profile obtained for two person mixtures injected at 3kV 5 sec on Newton

		Newton Two Person Mixtures									
		20-1	1-20	15-1	1-15	10-1	1-10	4-1	1-4	2-1	1-2
750pg	Mix 1	37%	68%	81%	93%	93%	93%	100%	100%	96%	100%
	Mix 2	75%	67%	88%	89%	100%	89%	100%	100%	100%	100%
500pg	Mix 1	59%	71%	63%	79%	93%	89%	100%	100%	100%	100%
	Mix 2	79%	67%	83%	63%	96%	81%	100%	100%	100%	100%
250pg	Mix 1	67%	57%	59%	50%	78%	82%	100%	100%	100%	100%
	Mix 2	46%	44%	58%	56%	75%	81%	100%	100%	100%	100%
100pg	Mix 1	44%	14%	41%	29%	52%	57%	70%	71%	100%	100%
	Mix 2	25%	15%	38%	19%	42%	56%	75%	85%	92%	96%



Table 27: Percentage of total alleles obtained for three person mixtures injected at 3kV 5 sec

Allele Counts for 3 Person Mixtures					
		Athena		Newton	
		M1	M2	M1	M2
750pg	5-1-1	100%	99%	100%	98%
	5-5-1	100%	98%	100%	99%
	1-1-1	100%	100%	100%	100%
	1-2-1	100%	100%	100%	100%
	3-2-1	100%	100%	100%	100%
	5-2-1	100%	100%	100%	100%
500pg	5-1-1	100%	100%	99%	100%
	5-5-1		98%		
	1-1-1	100%	100%	100%	100%
	1-2-1	100%	100%	100%	100%
	3-2-1	100%	100%	100%	100%
	3-2-1		100%		
5-2-1	100%	99%	100%	100%	
250pg	5-1-1	100%	97%	100%	99%
	5-5-1	97%	92%	97%	98%
	1-1-1	97%	100%	99%	98%
	1-2-1	100%	99%	100%	100%
	3-2-1	100%	99%	100%	100%
	5-2-1	100%	95%	100%	99%
100pg	5-1-1	97%	68%	99%	97%
	5-1-1	79%	68%	90%	84%
	5-5-1	83%	77%	88%	87%
	1-1-1	85%	76%	96%	98%
	1-2-1	92%	82%	96%	87%
	3-2-1	90%	82%	96%	89%
	5-2-1	77%	81%	87%	86%



Table 28: Percentage of total alleles obtained for four person mixtures injected at 3kV 5 sec

Allele Counts for 4 Person Mixtures			
		Athena	Newton
750pg	1-1-1-1	100%	100%
	1-3-3-1	100%	100%
	1-3-5-1	96%	98%
500pg	1-1-1-1	81%	89%
	1-3-3-1	94%	94%
	1-3-5-1	94%	94%
250pg	1-1-1-1	90%	92%
	1-3-3-1	87%	88%
	1-3-5-1	94%	94%
100pg	1-1-1-1	91%	92%
	1-3-3-1	90%	93%
	1-3-5-1	66%	83%



Table 29: Instances of pull up documented when analyzing Mixture 1 using GeneMarkerHID v.2.8.2 using a 50 RFU threshold with a 3% minimum heterozygote filter and 3% global max filter.

Mixture 1			
		Athena	Newton
750pg	20-1	5	6
	15-1	3	7
	10-1	4	3
	4-1	2	4
	2-1	3	2
	1-1	2	5
	1-2	4	1
	1-4	13	7
	1-10	0	4
	1-15	2	1
	1-20	4	2
500pg	20-1	2	1
	15-1	0	1
	10-1	2	2
	4-1	0	1
	2-1	1	8
	1-1	1	9
	1-2	0	4
	1-4	0	4
	1-10	5	2
	1-15	0	5
	1-20	7	2
250pg	20-1	0	2
	15-1	2	1
	10-1	1	2
	4-1	0	0
	2-1	0	0
	1-1	0	2
	1-2	3	0
	1-4	1	3
	1-10	1	2
	1-15	0	2
	1-20	0	0
100pg	20-1	0	0
	15-1	0	0
	10-1	0	0
	4-1	0	0
	2-1	0	0
	1-1	0	0
	1-2	0	0
	1-4	0	0
	1-10	0	0
	1-15	0	0
	1-20	0	0



Table 30: Instances of pull up documented when analyzing Mixture 2 using GeneMarkerHID v.2.8.2 using a 50 RFU threshold with a 3% minimum heterozygote filter and 3% global max filter.

Mixture 2			
		Athena	Newton
750pg	20-1	2	4
	15-1	4	4
	10-1	1	1
	4-1	1	3
	2-1	5	2
	1-1	4	5
	1-2	3	0
	1-4	7	9
	1-10	3	6
	1-15	2	2
	1-20	4	1
500pg	20-1	1	1
	15-1	0	1
	10-1	0	1
	4-1	0	1
	2-1	3	0
	1-1	1	2
	1-1	1	1
	1-2	0	3
	1-4	0	6
	1-10	4	1
	1-15	1	3
	1-20	6	1
250pg	20-1	0	1
	15-1	0	0
	10-1	0	0
	4-1	0	1
	2-1	0	0
	1-1	0	0
	1-2	2	0
	1-4	0	4
	1-10	0	2
	1-15	0	0
	1-20	0	0
100pg	20-1	0	0
	15-1	0	0
	10-1	0	0
	4-1	0	0
	2-1	0	0
	1-1	0	0
	1-2	0	0
	1-4	0	0
	1-10	0	0
	1-15	0	0
	1-20	0	0



4.A.7 Contamination Assessment

Thirty-five negative controls were examined throughout the validation study. One negative control injected on Newton generated an allele call of 10 at D7S820. This result was not reproducible (Table 31).

Table 31: Results of contamination assessment

		OCME Contamination Assessment																										
		AMEL	D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E	D16S539	D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818	TPOX	DYS391	D8S1179	D12S391	D19S433	FGA	D22S1045			
Athena	03-Amplification_Negative																											
	13-Amp_Neg_1																											
	14-Amp_Neg_2																											
	15-Amp_Neg_3																											
	17-Amp_Neg_4																											
	29-Amplification_Negative																											
	30-Amplification_Negative																											
	31-Amplification_Negative																											
	49-Opg_a																											
	49-Opg_a																											
	50-Opg_b																											
	50-Opg_b																											
	51-Opg_c																											
	51-Opg_c																											
72-Amplification_Negative																												
Newton	03-Amplification_Negative																											
	13-Amp_Neg_1																											
	13-Amp_Neg_1																											
	14-Amp_Neg_2																											
	14-Amp_Neg_2																											
	15-Amp_Neg_3																											
	15-Amp_Neg_3																											
	17-Amp_Neg_4																											
	17-Amp_Neg_4																											
	17-Amp_Neg_4**																											
	29-Amplification_Negative																											
	30-Amplification_Negative																											
	31-Amplification_Negative																											
	72-Amplification_Negative																											
	49-Opg_a																											
	49-Opg_a																											
	50-Opg_b																											
50-Opg_b																												
51-Opg_c																												
51-Opg_c																												
**Sample was re injected and the run is shown in the row above																												



4.A.8 Thermocycler Performance Check

Ten thermocyclers were performance checked using 2800M. All positive controls yielded the correct genotype; all negative controls were negative. Three positive controls run on 806F-TC3, 610J-TC1, and 705A-TC12, had failed size standards. Thermocyclers were also compared by peak heights and peak height ratios (Table 32 and 33)

Table 32: Average peak heights generated during the performance check of 10 internal OCME-NYC thermocyclers

Performance Check Peak Heights										
	610J-TC1	705A-TC11	705A-TC12	705A-TC13	714C-TC6	714C-TC8	714C-TC9	714C-TC11	806C-TC6	806F-TC3
AMEL	1786	1840	2009	1961	1797	1828	1980	1997	1965	2149
D3S1358	2259	2026	2177	2080	1929	2373	2260	2087	2128	2587
D1S1656	2297	2149	2196	2009	2061	2353	2322	2238	2162	2651
D2S441	1727	1657	1861	1770	1718	1857	1786	1848	1817	1851
D10S1248	1669	1665	1769	1469	1589	1798	1712	1684	1732	1967
D13S317	1660	1591	1774	1576	1495	1709	1652	1685	1747	1770
Penta E	1416	1369	1543	1537	1513	1407	1461	1600	1545	1632
D16S539	3329	3425	3103	3092	3476	3692	3121	3419	3852	3316
D18S51	2537	2718	2540	2383	2441	2835	2557	2562	2686	2717
D2S1338	2405	2447	2494	2411	2398	2434	2419	2438	2514	2364
CSF1PO	2090	2005	2338	2017	1943	2124	2043	2058	2058	2147
Penta D	1461	1570	1936	1654	1583	1304	1607	1652	1677	1803
TH01	3464	3291	3392	3373	3544	3554	3286	3466	3851	3386
vWA	2343	2824	2311	2267	2140	2861	2174	2218	2682	2339
D21S11	2463	2236	2192	1876	1995	2301	2330	2011	2230	2610
D7S820	2300	2422	2473	2218	2174	2277	2245	2246	2515	2471
D5S818	2552	2331	2595	2444	2299	2390	2538	2384	2530	2518
TPOX	2279	2168	2357	2072	2047	2130	2137	2006	2283	2275
DYS391	2275	2182	2275	2171	2077	2086	2047	2105	2216	2219
D8S1179	1652	1688	1963	1987	1782	1749	2070	1961	1968	2592
D12S391	2364	2180	2272	2267	2176	2605	2332	2205	2482	2795
D19S433	1601	1548	1646	1559	1667	1562	1573	1644	1726	1710
FGA	1848	1870	2130	1959	1826	1865	1882	1971	2048	2025
D22S1045	1233	1461	1522	1447	1283	1335	1324	1388	1438	1446



Table 33: Average peak height ratios generated during the performance check of 10 internal OCME-NYC thermocyclers. The gray cells represent loci with no heterozygous calculations.

Performance Check Peak Height Ratios										
	610J-TC1	705A-TC11	705A-TC12	705A-TC13	714C-TC6	714C-TC8	714C-TC9	714C-TC11	806C-TC6	806F-TC3
AMEL	85%	93%	94%	91%	91%	92%	90%	84%	91%	96%
D3S1358	80%	90%	92%	92%	93%	90%	89%	90%	86%	91%
D1S1656	90%	95%	90%	90%	89%	88%	91%	90%	88%	94%
D2S441	91%	89%	90%	91%	88%	90%	87%	88%	89%	90%
D10S1248	92%	93%	91%	89%	88%	90%	92%	94%	90%	85%
D13S317	92%	86%	93%	90%	86%	94%	86%	85%	89%	89%
Penta E	88%	88%	91%	91%	85%	88%	89%	92%	87%	88%
D16S539	90%	90%	94%	88%	90%	93%	90%	92%	90%	91%
D18S51	87%	94%	89%	86%	93%	90%	91%	88%	89%	94%
D2S1338	91%	87%	91%	89%	90%	91%	87%	88%	93%	87%
CSF1PO										
Penta D	86%	88%	88%	87%	87%	83%	89%	87%	86%	96%
TH01	89%	92%	91%	89%	92%	89%	94%	94%	89%	88%
vWA	90%	89%	88%	94%	92%	92%	89%	90%	92%	91%
D21S11	93%	84%	90%	92%	88%	88%	93%	87%	90%	90%
D7S820	87%	94%	89%	94%	87%	93%	88%	89%	87%	85%
D5S818										
TPOX										
DYS391										
D8S1179	84%	88%	90%	83%	94%	87%	84%	92%	93%	87%
D12S391	83%	79%	91%	87%	85%	89%	83%	84%	87%	85%
D19S433	93%	87%	88%	87%	88%	91%	85%	90%	86%	80%
FGA	91%	89%	78%	85%	93%	92%	90%	92%	87%	85%
D22S1045										

5. Conclusion

The PowerPlex® Fusion system was evaluated on the Applied Biosystems® GeneAmp® PCR 9700 thermal cycler. Samples were amplified using the extracted DNA protocol at half reaction with 29 cycles. The amplified product was run using a 3kV 5 second injection with a 2000 second run time on two Applied Biosystems® 3130xl. As per the OCME-NYC, an analytical threshold of 50 RFU with a 3% minimum heterozygote filter and 3% global max filter was used for the validation with 29 cycles. A stochastic threshold of 300 RFU was established. The results showed that the system was capable of producing reliable and reproducible results. The precision of the system is within the recommended range. The studies performed in this validation meet the criteria for an internal validation and have shown that the PowerPlex® Fusion is suitable for use in a forensic laboratory.



6. References

Quality Assurance Standards for Forensic DNA Testing Laboratories (effective July 1, 2009)

SWGDM Validation Guidelines – Approved December 2012 (SWGDM.org)

Butler, J.M. (2010) Fundamentals of Forensic DNA Typing, San Diego; Elsevier Academic Press, Chapter 13 “Quality Assurance”, p. 300

Butler, J.M. (2012) Advanced Topics in Forensic DNA Typing: Methodology, San Diego: Elsevier Academic Press, Chapter 7 “Quality Assurance and Validation”, p. 183

PowerPlex® Fusion System Technical Manual TMD039, Revision Date 6/16 from Promega Corporation

PowerPlex® 5C Matrix Standard Technical Manual TMD049, Revision Date 10/15 from Promega Corporation

Internal Validation Guide of Autosomal STR Systems for Forensic Laboratories, Promega Reference Manual (2013)

Erica L.R Butts, M.C. Kline, D.L. Duewer, C. R. Hill, H.M. Butler, P. M Vallone. NIST validation studies on the 3500 Genetic Analyzer. Forensic Science International: Genetics Supplement Series 3. 2011 e184-185.



7. Appendix A – Lot Numbers

Pre-Amplification Product	Manufacturer	Catalog Number	Lot Number	Expiration Date
PowerPlex® 5C Matrix Standard	Promega	DG4850	183166	07/23/2017
PowerPlex® Fusion kit	Promega	DC2408	154236	10/20/2017
PowerPlex® Fusion 5X Master Mix	Promega	DP207A	140564	11/06/2017
PowerPlex® Fusion 5X Primer Pair Mix	Promega	DK236B	148691	01/04/2018
2800M Control DNA	Promega	DD710A	118893	10/06/2019
Water, Amplification Grade	Promega	DW099A	135976	10/27/2019
Post Amplification Product	Manufacturer	Catalog Number	Lot Number	Expiration Date
PowerPlex® Fusion Allelic Ladder Mix	Promega	DG381B	120080	10/20/2017
WEN ILS 500	Promega	DG500A	183317	10/20/2018
Array (16 capillary)	Applied Biosystems	4404687	See table below	
Polymer (POP 4) 960 samples	Applied Biosystems	4393710	10224026	05/07/2016



Array lot numbers used during the validation study

Run	Array Internal LIMS Lot Number
Athena031016 12-15	36cmCap1602014396
Athena031016 16-19	36cmCap1602014396
Athena032416 75-76	36cmCap1602014396
Athena040716_112-117	36cmCap1602014396
Athena040716_118-122	36cmCap1602014396
Athena040816 127-128	36cmCap1602014396
Athena040816 129-130	36cmCap1602014396
Athena041916 138	36cmCap1602014396
Athena042116 143-145	36cmCap1602014396
Athena042116 146-148	36cmCap1602014396
Newton031116 56-59	36cmCap1401271359
Newton031116 60-63	36cmCap1401271359
Newton031116 64	36cmCap1401271359
Newton032416_41-44	36cmCap1602014398
Newton032416_45-46	36cmCap1602014398
Newton032516 47-50	36cmCap1602014398
Newton032516 51	36cmCap1602014398
Newton032516_52	36cmCap1602014398
Newton040616 108-113	36cmCap1602014398
Newton040616 114-118	36cmCap1602014398
Newton041916 11	36cmCap1406051623




8. Appendix B – Analysis Methods

Run Wizard

Data Process - HID Analysis

Set data process options

Raw Data Analysis

Auto Range (frame) 

Start: End:

Smooth Enhanced Smooth

Baseline Subtraction:

Superior Classic Enhanced


Pull-up Correction Spike Removal

Saturation Repair

Allele Call

Auto Range (bps)

Start: End:

Peak Detection Threshold: 


Min Intensity: Max Intensity:

Percentage > Global Max

Note: Use Panel Editor to set Min Intensity and % Global Max for peaks within Markers

Size Call

Local Southern Cubic Spline

 Save



Run Wizard, Additional Settings

Run Wizard ✕

Additional Settings - HID Analysis

Set additional options related to the different analysis type

Allelic Ladder:

Positive Control Template:

Allele Evaluation

Peak Score:

Reject < Check < Pass

Mixture Evaluation

Valid Mixture Peak Percentage: %

Min Mixture Marker Number:

Auto Select Best Ladder

Auto Panel Adjustment



Panel Editor, Edit Marker

Edit Marker [X]

Marker Parameters

Marker Name:

Nucleotide Repeats (x):

Boundary: To

Min Homozygote Intensity:

< = Inconclusive <=

Min Heterozygote Intensity:

< = Inconclusive <=

Max Heterozygote Imbalance(%):

Min Heterozygote Imbalance(%):

Apply Homo/Hetero Settings to All Markers

Stutter Filter:

Use Marker-Specific Values

$N - 2x$ %

$N + x$ % $N - x$ %

$N+0.5x$ % $N-0.5x$ %

Use Allele-Specific Values(From Panel)

Apply Stutter Settings to All Markers

OK Cancel



Promega Stutter Percentages used in GeneMarker v.2.8.2

Promega Stutter Percentages			
AMEL	0%	N-x	Minus
D3S1358	11.9%	N-x	Minus
D1S1656	14.2%	N-x	Minus
	3.6%	N-0.5x	Minus
D2S441	9.2%	N-x	Minus
D10S1248	12.4%	N-x	Minus
D13S317	9.8%	N-x	Minus
Penta E	7.6%	N-x	Minus
D16S539	10.2%	N-x	Minus
D18S51	14.6%	N-x	Minus
D2S1338	13.9%	N-x	Minus
CSF1PO	9.5%	N-x	Minus
Penta D	6.8%	N-x	Minus
TH01	4.6%	N-x	Minus
vWA	11.2%	N-x	Minus
D21S11	11.6%	N-x	Minus
D7S820	11.0%	N-x	Minus
D5S818	9.5%	N-x	Minus
TPOX	5.5%	N-x	Minus
DYS391	8.7%	N-x	Minus
D8S1179	10.9%	N-x	Minus
D12S391	15.8%	N-x	Minus
D19S433	11.0%	N-x	Minus
FGA	12.1%	N-x	Minus
D22S1045	16.4%	N-x	Minus
	8.6%	N+x	Plus



Stochastic threshold for 29 cycles for DNA 2 Athena

TEMPLATE PROFILE	AMEL		D3S1358		D1S1656		D2S441		D10S1248		D13S317		Penta E		D16S539		D18S51		D2S1338		CSF1PO		Penta D		TH01		vWA		D21S11		D7S820		D5S818		TPOX		DYS391		D8S1179		D12S391		D19S433		FGA		D22D45	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2						
	X	X	16	17	16	17.3	11	14	14	15	8	11	13	18	10	11	15	17	17	24	13	14	12	14	7	9	15	19	30	32.2	10	12	9	13	9	11	11	11	11	13	15	21	15.2	16.2	21	22	15	16
07-1ng_DNA2a	4096	2319	2337	2297	2592	2198	2775	1932	1636	2375	2546	1997	1599	3370	3476	3289	2889	2422	2691	2593	2241	2206	2414	2671	2289	2402	2456	2057	2366	3225	2278	3576	3008	2077	2525	2185	1709	2413	2419	2082	1730	2155	2422	1674	1469			
09-1ng_DNA2a	3663	2103	1884	2032	1705	2000	1923	1580	1456	1381	1753	1509	1451	2801	2450	2326	2644	2018	1671	2022	1766	2008	1773	2779	3415	2642	2221	2267	2247	3023	2903	3176	3047	2925	2175	1924	2181	2910	2237	1659	1950	2473	2036	1665	1699			
10-1ng_DNA2b	4812	2783	2511	2121	2451	1888	2447	2045	2280	2038	1767	1873	1716	4076	3931	3562	2899	2166	2274	2430	2150	2246	2003	3414	3051	2585	2652	2782	2521	3453	2814	3391	3388	2378	2491	3049	2901	3272	3172	1797	1950	2720	2700	2023	1593			
11-50ng_DNA2c	2911	1361	1286	976	1158	1074	924	1438	1170	1246	995	938	884	1521	2198	1652	1554	1562	1376	1528	1317	1315	1237	1211	1557	1549	1116	1256	1446	1439	1671	1457	1668	1190	1660	1910	1816	1361	1251	1009	1094	1373	1388	965	1067			
12-50ng_DNA2a	3560	1365	1538	1088	1451	1574	1419	1241	1300	1685	1249	1437	1209	2827	1711	2109	2148	1864	1826	1513	1216	1654	1356	1763	1623	1910	1709	1266	1692	2097	1664	1621	2059	1765	1368	1503	1814	1402	1520	1594	1229	1789	1869	1364	865			
13-50ng_DNA2b	3336	1454	1495	1150	1400	1351	1055	1179	1057	1401	1055	1575	1178	1931	1906	1433	1344	1533	1347	1418	1309	1991	1389	1369	1766	1751	1224	1439	989	1278	1813	1333	1400	1485	954	1681	1654	1484	1151	977	1009	1522	983	1153	967			
14-25ng_DNA2c	1567	609	524	745	709	367	437	738	315	656	557	447	563	897	719	1139	635	665	461	480	428	1018	796	926	989	601	678	679	798	809	1120	643	614	596	695	802	1012	717	604	464	468	530	532	456	395			
15-25ng_DNA2a	1334	741	457	562	493	644	362	670	772	609	628	814	558	909	817	918	1035	688	523	601	670	750	720	574	856	654	777	665	773	723	567	739	812	794	641	750	840	725	631	481	490	634	638	641	345			
16-25ng_DNA2b	1452	535	773	842	506	939	547	640	723	704	555	646	412	851	968	894	1065	918	994	671	477	676	709	592	1003	596	670	631	871	721	884	928	748	927	851	838	685	547	596	568	561	579	662	373	410			
18-15ng_DNA2a	1125	363	289	199	368	382	429	223	336	298	363	361	452	577	423	581	287	550	242	260	294	456	417	379	450	401	448	365	328	523	525	254	414	433	440	432	229	196	267	199	188	294	281	174				
19-15ng_DNA2b	879	390	388	356	322	423	489	393	539	342	337	446	455	377	204	447	319	604	669	464	320	290	361	534	399	540	493	329	153	404	300	393	654	528	357	370	466	255	159	212	375	434	196	282	372			
20-15ng_DNA2c	1060	335	371	391	306	390	407	290	398	552	515	306	541	401	468	333	604	379	231	464	322	272	386	493	525	269	315	519	467	552	427	427	448	488	560	623	465	282	203	335	345	442	348	292	203			
21-12ng_DNA2a	631	329	339	318	352	322	264	371	420	267	273	207	334	332	264	713	446	517	443	317	189	338	446	339	320	210	205	288	315	409	402	349	246	438	281	268	171	360	252	239	145	297	300	202	196			
22-12ng_DNA2b	720	191	218	281	341	147	250	184	103	188	241	295	181	359	431	486	310	569	446	371	261	299	277	394	397	349	226	436	264	454	327	492	430	529	314	446	578	225	151	152	252	420	338	138	187			
23-12ng_DNA2c	836	455	300	393	359	96	242	303	228	229	347	242	254	423	422	320	291	406	339	364	140	404	280	174	583	387	345	373	370	336	523	371	281	279	194	281	347	226	261	251	185	299	222	329	201			
24-10ng_DNA2a	480	235	348	169	119	209	364	88	204	155	166	121	178	260	219	360	256	240	299	242	182	289	262	434	412	321	244	275	311	318	282	166	224	275	342	628	460	111	166	225	141	331	169	255	111			
25-10ng_DNA2b	465	293	344	278	281	218	227	286	228	242	263	123	183	264	317	274	109	217	338	216	133	174	144	248	119	257	285	216	311	234	177	265	303	334	196	241	293	149	123	125	141	323	282	124	123			
26-10ng_DNA2c	668	95	194	155	199	86	311	149	163	164	141	162		303	339	327	148	280	130	184	115	163	184	134	264	310	269	223	145	238	271	172	236	65	411	577	157	128	200	142	237	241	128	181				
27-75ng_DNA2a	404	199	162	188	178	229		206	132	104	160	67	230	294	251	174	115	235	329	94	186	248	397	296	262	107	318	165	380	275	125	218	120	233	194	264	279	170	81	142	249	188	224	214	239			
28-75ng_DNA2b	385	262	301	149	245	118	137	225	159	78	126	217	157	179	245	269	83	89	118	88	152	198	186	211	223	178	204	142	296	237	282	146	164	166	185	267	234	108	98	57	104	198	176	123	74			
29-75ng_DNA2c	648	159	304	115	130	162	159	120	249	250	189	250	208	264	271	339	369	461	230	158	291	154	177	123	335	220	125	183	233	271	272	328	336	121	304	248	172	138	154	225	188	216	192	241	69			
30-50ng_DNA2a	281	157	117	57	87	60	74	133	71	120	131	134	124	129	163	146	171	142	201	139	64	175		227		240	181	227	213	108	198	68		101	66	195	146	115	90	79	102	192	107	60	76			
31-50ng_DNA2b	336	142	152	95	84	96		78	129	135	165	125		182	227	139	122	150	275	55		170	72	188	213	169	100	179	90	134	94	86	60	102	120	169	58	80	64	51		67	77	58	89			
32-50ng_DNA2c	347	225	174	227	125	73	165	268	64	60	90	73	92	154	130	85	112	65	219	91	142	136	69	112	89	77	116	55	185	199	160	257	235	112	78	187	197	68	105		190	175	53					
33-30ng_DNA2a	244	99	97	78	64	61	66	75	61	64	70	80	65	127	286	69	118	111	74	90	80	126	61	74	128	105	55	62		166	98	231	50		122		60		76	121	81	76	90	78				
34-30ng_DNA2b	142			52		72		61	112	70	81	121		92	85	147		218	71			124		96		85		188		75	84	72		97		251			68	99		80	88	60				
35-30ng_DNA2c	144	67	84			148	151	52		114	66			101	129	121	100	123	64	129	82	133	82	69	186	54	52	94	134	56	62			135	78	109	122	69	119	157	52							
36-25ng_DNA2a	153	56		119	113	136		107		85	98	124		89	60	55						132		80		91		131		108				73	98	80	128	65		50								
37-25ng_DNA2b	93	78	51	56				117		69	55	69	55	55	87	121	51	63	71	70	69	103	103	87	73	83		85		75	135			97	98	140						59						
38-25ng_DNA2c	121	123	105	86	58					104				171	77	125	134	87		56	137	68		88	61	61	67	53	93	110		74	134	109	114	69	106		55				57					



Stochastic threshold for 29 cycles for DNA 2 Newton

TEMPLATE PROFILE	AMEL		D3S1338		D151656		D2S441		D10S1248		D13S317		Penta E		D16S539		D18S51		D2S1338		CSF1PO		Penta D		TH01		vWA		D21S11		D7S820		D5S818		TPOX		DYS391		D8S1179		D12S391		D19S433		FGA		D221045	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2		
	X	X	16	17	16	17.3	11	14	14	15	8	11	13	18	10	11	15	17	17	24	13	14	12	14	7	9	15	19	30	32.2	10	12	9	13	9	11	11	11	11	13	15	21	15.2	16.2	21	22	15	16
07-1ng_DNA2a	6784	3961	3918	3963	4394	3578	4504	3243	2729	3851	4169	3172	2493	4814	5130	4819	4308	3668	3989	3696	3142	3144	3487	4119	3606	3804	3879	3256	3741	5014	3508	4052	4578	3209	3869	3762	2962	4186	4262	3571	2954	3709	4101	2874	2495			
09-1ng_DNA2a	5914	3500	3099	3355	2827	3094	2996	2386	2198	1959	2447	1924	1826	3757	3305	3063	3509	2624	2084	2303	1967	2195	1939	4566	5613	4262	3590	3682	3600	4517	4348	4387	4209	3859	2815	3331	3795	4803	3768	2641	3123	3626	2995	2091	2168			
10-1ng_DNA2b	7475	4511	4087	3484	4097	3109	3901	3575	3887	3508	3092	3322	2797	4995	4687	4642	3790	3038	3130	3367	2871	2943	2616	4294	3926	3317	3406	3629	3256	4371	3699	4517	4213	3163	3267	4221	3931	4569	4373	2618	2741	3874	3770	2876	2322			
11-500pg_DNA2c	4595	2224	2084	1619	1919	1667	1445	2293	1864	1962	1574	1451	1351	1970	2844	2257	2091	2056	1854	2006	1720	1702	1613	1635	2075	2133	1560	1710	1970	1954	2272	1965	2206	1557	2201	2796	2617	1954	1820	1433	1539	1939	1961	1369	1506			
12-500pg_DNA2a	6133	2415	2694	1900	2576	2614	2351	2135	2219	2827	2092	2354	1977	3894	2344	2973	2996	2574	2492	2050	1606	2225	1840	2494	2328	2739	2462	1751	2340	2919	2397	2208	2833	2408	1813	2347	2864	2208	2352	2366	1835	2744	2792	1963	1267			
13-500pg_DNA2b	6066	2624	2691	2101	2569	2350	1827	2140	1863	2437	1842	2703	2008	2856	2790	2231	2056	2366	2018	2072	1911	2986	2083	2118	2728	2747	1919	2230	1539	1961	2812	2056	2126	2246	1490	2823	2792	2496	1927	1603	1668	2506	1654	1906	1629			
14-250pg_DNA2c	2685	1037	911	1326	1247	637	747	1391	598	1202	1042	876	1014	1223	968	1607	915	1024	726	743	652	1477	1154	1285	1371	864	976	994	1178	1159	1625	960	913	920	1058	1241	1547	1100	928	767	762	869	863	747	646			
15-250pg_DNA2b	2228	1263	790	938	845	1024	573	1112	1243	970	1014	1267	855	1297	1159	1329	1502	989	749	842	930	1039	996	849	1284	979	1145	963	1133	1059	836	1086	1093	1131	906	1214	1332	1153	979	734	779	994	974	983	534			
18-150pg_DNA1a	1865	625	498	358	640	629	701	385	573	499	588	575	710	787	568	809	418	764	350	353	389	606	553	550	670	588	660	534	468	748	771	369	595	465	622	659	666	369	315	408	308	283	441	419	266			
19-150pg_DNA2b	1558	729	723	667	597	754	871	708	981	611	615	762	773	553	306	682	500	920	1012	672	461	430	542	860	651	895	811	540	240	639	485	633	1042	836	554	641	812	442	283	358	642	742	349	479	622			
20-150pg_DNA2c	1082	610	663	695	552	656	682	503	682	933	878	508	879	568	668	468	896	557	333	648	440	370	539	686	768	407	459	736	655	772	626	594	640	682	771	982	726	432	325	525	549	650	530	432	302			
21-125pg_DNA2a	1029	538	558	519	589	492	419	624	684	584	425	316	517	449	358	971	609	591	421	248	451	248	481	588	481	459	301	280	396	447	572	570	477	323	594	390	381	263	554	395	349	232	451	449	301	282		
22-125pg_DNA2b	1214	342	387	507	590	263	420	326	185	316	406	474	288	495	601	663	424	798	595	489	340	379	359	580	581	506	332	623	396	640	468	696	570	718	441	682	891	354	241	234	387	611	489	201	265			
23-125pg_DNA2c	1411	794	522	676	620	160	397	505	389	378	578	391	403	621	623	491	450	611	513	530	205	579	406	267	911	600	545	588	588	526	820	577	443	431	300	483	589	382	459	420	329	519	394	562	345			
24-100pg_DNA2a	696	347	511	258	183	311	529	148	326	210	232	171	239	329	269	467	336	337	404	293	223	349	328	557	552	417	334	377	427	410	335	216	260	344	417	666	374	163	230	331	212	478	241	331	157			
25-100pg_DNA2b	707	444	528	437	426	319	324	403	324	335	356	148	209	335	389	329	137	265	401	230	140	171	145	396	199	379	435	306	464	309	251	340	378	424	242	382	459	226	178	175	207	449	374	135	139			
26-100pg_DNA2c	1085	166	313	277	355	170	561	342	365	385	357	122	413	394	438	445	201	472	236	393	240	272	320	182	341	404	350	334	214	347	418	343	290	508	166	568	798	233	188	326	236	404	431	268	389			
27-75pg_DNA2a	694	347	273	323	300	77	380	344	224	171	271	118	352	396	345	252	164	339	463	134	257	340	531	420	367	162	460	241	536	397	201	303	161	328	261	411	404	263	127	213	375	287	333	317	355			
28-75pg_DNA2b	765	519	580	297	497	233	270	446	325	156	242	399	297	281	387	428	133	141	186	140	237	313	296	342	368	287	331	235	471	383	446	240	261	262	282	466	436	198	179	92	179	343	302	201	131			
29-75pg_DNA2c	1128	286	531	198	230	268	275	206	419	418	317	401	338	380	379	498	546	681	324	220	417	225	247	193	482	332	186	267	328	404	394	464	472	172	427	411	287	217	246	348	303	342	301	377	105			
30-50pg_DNA2a	449	270	200	98	154	102	123	239	118	214	231	243	220	169	216	204	224	213	300	217	98	61	249	302	61	313	235	313	297	154	273	93	152	107	307	213	180	142	108	160	291	175	93	121				
31-50pg_DNA2b	641	273	294	186	171	176	89	153	247	263	318	243	87	295	368	230	218	260	473	60	88	283	123	301	361	284	172	310	157	229	167	149	109	181	210	311	106	155	124	98	131	138	113	168				
32-50pg_DNA2c	559	361	281	371	193	193	111	274	446	104	147	119	145	219	185	125	167	106	333	134	205	207	111	193	151	119	177	87	292	312	244	408	350	183	125	329	341	119	190	79	65	323	306	83	100			
33-30pg_DNA2a	429	186	176	138	120	106	117	131	111	102	122	142	110	191	423	115	185	172	109	127	120	183	127	213	163	89	58	97	262	154	353	83	89	223	110	58	119	205	148	123	154	133						
34-30pg_DNA2b	252	134	164	64	75	269	278	104	187	210	117	117	137	125	116	207	100	302	100	61	58	168	64	147	71	128	369	50	117	129	103	69	134	125	381	73	74	106	151	123	136	78	94					
35-30pg_DNA2c	262	62	103	207	192	222	171	13																																								



10. Appendix D – Mixture Results

Mixture Results generated for 2 person Mixture Series 1 on Athena

Mixture		AMEL	D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E	D16S539	D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818	TPOX	DYS391	D8S1179	D12S391	D19S433	FGA	D22S1045
1	6M	XY	15 17	15	10 12	13 15	8 9	11 17	9 11	15 16	19 23	12	9	9	14 19	30	9.1 11	11 12	10	10	14	19 20	12 13	23 25	11 16
	22F	X	15 17	15 16	11	14	8 11	11 19	11	13 19	17	11	11 15	6 9	17 18	27 31.2	8 10	9 11	8	**	14 16	21	13.2 15	21 24	11 15
750pg	20-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17	9 11	15 16 19	17 19 23	11 12	9	6 9	14 19	30	9.1 11	11 12	8 10	10	14	19 20 21	12 13	21 23 25 26	11 16
	15-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30	9.1 10 11	9 11 12	8 10	10	14	19 20 21	12 13 13.2 15	21 23 24 25	11 16
	10-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17	9 11	13 15 16 19	17 19 23	11 12	9 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	4-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	2-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 15 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-2	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 15 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-4	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 15 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-10	XY	15 17	15 16	11 12	14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-15	XY	15 17	15 16	10 11 12	13 14	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	20 21	12 13 13.2 15	21 23 24 25	11 15 16
1-20	X	15 17	15 16	11	14	8 9 11	11 19	11	13 15 16 19	17 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 10 11	9 11 12	8 10	10	14 16	19 21	12 13 13.2 15	21 24 25	11 15 16	
500pg	20-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17	9 11	13 15 16	17 19 23	11 12	9 11	9	14 18 19	27 30	8 9.1 11	9 11 12	10	10	14	19 20 21	12 13 15	21 23 24 25	11 16
	15-1	XY	15 17	15	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9	6 9	14 17 18 19	30	8 9.1 11	9 11 12	8 10	10	14 16	19 20 21	12 13	21 23 25	11 16
	10-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16	17 19 23	11 12	9 15	6 9	14 17 18 19	27 30 31.2	9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	4-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	2-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 15 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-2	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-4	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-10	XY	15 17	15 16	10 11 12	14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 21	13 13.2 15	21 23 24 25	11 15 16
	1-15	X	15 17	15 16	11 12	14	8 9 11	11 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	21	13 13.2 15	21 23 24 25	11 15 16
1-20	X	15 17	15 16	10 11 12	14	8 9 11	11 17 19	9 11	13 15 16 19	17	11 12	9 11 15	6 9	14 17 18	27 30 31.2	8 9.1 10 11	9 11 12	8	10	14 15 16	19 21	12 13 13.2 15	21 23 24 25	11 15	
250pg	20-1	XY	15 17	15	10 11 12	13 14 15	8 9 11	11 17	9 11	13 15 16	17 19 23	11 12	9 15	9	14 17 18 19	30	8 9.1 11	9 11 12	10	10	14	19 20 21	12 13	21 23 24 25	11 15 16
	15-1	XY	15 17	15 16	10 11 12	13 14 15	8 9	11 17	9 11	15 16 19	19 23	11 12	9	9	14 17 18 19	30	8 9.1 10 11	11 12	8 10	10	14	19 20 21	12 13	21 23 25	11 15 16
	10-1	XY	15 17	15 16	10 11 12	13 14 15	8 9	11 17	9 11	13 15 16 19	17 19 23	11 12	9	6 8 9	14 18 19	27 30	8 9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 15	21 23 24 25	11 16
	4-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	2-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-2	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2 35	8 9.1 10 11	9 11 12	8 10	10	14 15 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-4	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 15 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-10	XY	15 17	15 16	11 12	13 14 15	8 9 11	11 19	11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10	9 11 12	8 10	10	14 16	19 20 21	13.2 15	21 23 24 25	11 15
	1-15	X	15 17	15 16	10 11	14 15	8 11	11 19	9 11	13 19	17	11 12	9 11 15	6 9	14 17 18	27 31.2	8 9.1 10 11	9 11	8 10	10	14 15 16	21	12 13 13.2 15	21 24 25	11 15
1-20	X	15 17	15 16	11	14 15	8 11	11 19	11	13 15 16 19	17 19 23	11	9 11 15	6 9	14 17 18	27 31.2	8 10 11	9 11 12	8 10	10	14 16	19 21	12 13.2 15	21 24	11 15	
100pg	20-1	XY	15 17	15	10 12	13 15	8 9	11 17	9 11	15 16	17 19 23	11 12	9 11	9	14 18 19	30	9.1 11	11 12	8 10	10	14 16	19 20	12 13	23 24 25	11 16
	15-1	XY	15 17	15	10 12	13 15	8 9	11 17	9 11	13 15 16	19 23	11 12	9	6 9	14 19	30	8 9.1 11	11 12	10	10	14	19 20	12 13	23 25	11 16
	10-1	XY	15 17	15	10 11 12	13 14 15	8 9	11 17	9 11	15 16	19 23	11 12	9 11 15	6 9	14 19	27 30 31.2	9.1 11	11 12	8 10	10	14	19 20 21	12 13	23 24 25	11 15 16
	4-1	XY	15 17	15	10 11 12	13 14 15	8 9 11	11 17	9 11	13 15 16 19															



Mixture Results generated for 2 person Mixture Series 2 on Athena

		AMEL	D3S1358	D151656	D2S441	D10S1248	D13S317	Penta E	D16S539	D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818	TPOX	DYS391	D8S1179	D12S391	D19S433	FGA	D22S1045
Mixture	15 M	XY	17 18	12 14	11	13 14	9 12	5 12	11 13	15 20	17 25	12	11 14	89.3	17	29 30	9 10	10 12	11	10	12 13	19 21	14 14.2	18 24	15
2	5 F	X	14 16	14	11 14	14	9 13	5 7	12 13	12 16	16 18	12	11 14	7 9	17	31.2 32.2	8 10	10 13	8	**	9 14	21 23	13 15.2	20 23	12 16
750pg	20-1	XY	14 16 17 18	12 14	11	13 14	9 12 13	5 7 12	11 12 13	12 15 16 20	17 18 25	12	11 14	7 8 9 9.3	17	29 30 31.2	9 10	10 12 13	8 11	10	9 12 13 14	19 21 23	13 14 14.2 15.2	18 24	12 15
	15-1	XY	14 16 17 18	12 14	11 14	13 14	9 12 13	5 7 12	11 12 13	12 15 16 20 21	17 18 25	12	11 14	7 8 9 9.3	17	29 30 31.2 32.2	9 10	10 12 13	8 11	10	12 13 14	19 21 23	13 14 14.2 15.2	18 20 23 24	12 15 16
	10-1	XY	14 16 17 18	12 14	11 14	13 14	9 12 13	5 7 12	11 12 13	12 15 16 20	16 17 18 25	12	11 14	7 8 9 9.3	17	29 30 31.2 32.2	8 9 10	10 12 13	8 11	10	9 12 13 14	19 21 23	13 14 14.2 15.2	18 20 23 24	12 15 16
	4-1	XY	14 16 17 18	12 14	11 14	13 14	9 12 13	5 7 12	11 12 13	12 15 16 20	16 17 18 25	12	11 14	7 8 9 9.3	17	29 30 31.2 32.2	8 9 10	10 12 13	8 11	10	9 12 13 14	19 21 23	13 14 14.2 15.2	18 20 23 24	12 15 16
	2-1	XY	14 16 17 18	12 14	11 14	13 14	9 12 13	5 7 12	11 12 13	12 15 16 20	16 17 18 25	12	11 14	7 8 9 9.3	17	29 30 31.2 32.2	8 9 10	10 12 13	8 11	10	9 12 13 14	19 21 23	13 14 14.2 15.2	18 20 23 24	12 15 16
	1-1	XY	14 16 17 18	12 14	11 14	13 14	9 12 13	5 7 12	11 12 13	12 15 16 20	16 17 18 25	12	11 14	7 8 9 9.3	17	29 30 31.2 32.2	8 9 10	10 12 13	8 11	10	9 12 13 14	19 21 23	13 14 14.2 15.2	18 20 23 24	12 15 16
	1-2	XY	14 16 17 18	12 14	11 14	13 14	9 12 13	5 7 12	11 12 13	12 15 16 20	16 17 18 25	12	11 14	7 8 9 9.3	17	29 30 31.2 32.2	8 9 10	10 11 12 13	8 11	10	9 12 13 14	19 21 23	13 14 14.2 15.2	18 20 23 24	12 15 16
	1-4	XY	14 16 17 18	12 14	11 14	13 14	9 12 13	5 7 12	11 12 13 14	12 15 16 20	16 17 18 25	12	11 14	7 8 9 9.3	17	29 30 31.2 32.2	8 9 10	10 11 12 13	8 11	10	9 12 13 14	19 21 23	13 14 14.2 15.2	18 20 23 24	12 15 16
	1-10	XY	14 16 17 18	12 14	11 14	14	9 12 13	5 7 12	11 12 13	12 15 16 20	16 17 18	12	11 14	7 8 9 9.3	17	29 30 31.2 32.2	8 9 10	10 12 13	8 11	10	9 12 13 14	19 21 23	13 14 14.2 15.2	18 20 23 24	12 15 16
	1-15	XY	14 16 17 18	12 14	11 14	14	9 12 13	5 7 12	11 12 13	12 16 20	16 17 18 25	12	11 14	7 8 9 9.3	17	29 30 31.2 32.2	8 10	10 12 13	8 11	10	9 12 13 14	19 21 23	13 14 14.2 15.2	18 20 23 24	12 15 16
	1-20	X	14 16 17 18	12 14	11 14	14	9 13	5 7 12	11 12 13 14	12 16 20	16 18 25	12	11 14	7 8 9 9.3	17	29 30 31.2 32.2	8 10	10 12 13	8	10	9 12 14	19 21 23	13 14 15.2	20 23	12 15 16
	500pg	20-1	XY	14 16 17 18	12 14	11 14	13 14	9 12 13	5 7 12	11 12 13	12 15 20	17 18 25	12	11 14	7 8 9 9.3	17	29 30 31.2 32.2	9 10	10 12	8 11	10	9 12 13	19 21	13 14 14.2 15.2	18 24
15-1		XY	14 16 17 18	12 14	11 14	13 14	9 12 13	5 7 12	11 12 13	12 15 20 21	17 18 25	12	11 14	7 8 9 9.3	17	29 30 31.2	9 10	10 12 13	8 11	10	9 12 13 14	19 21 23	13 14 14.2	18 20 23 24	15 16
10-1		XY	14 16 17 18	12 14	11 14	13 14	9 12 13	5 7 12	11 12 13	12 15 16 20	17 25	12	11 14	7 8 9 9.3	17	29 30 31.2 32.2	8 9 10	10 12 13	8 11	10	9 12 13 14	19 21 23	13 14 14.2 15.2	18 20 23 24	15 16
4-1		XY	14 16 17 18	12 14	11 14	13 14	9 12 13	5 7 12	11 12 13	12 15 16 20	16 17 18 25	12	11 14	7 8 9 9.3	17	29 30 31.2 32.2	8 9 10	10 12 13	8 11	10	9 12 13 14	19 21 23	13 14 14.2 15.2	18 20 23 24	12 15 16
2-1		XY	14 16 17 18	12 14	11 14	13 14	9 12 13	5 7 12	11 12 13	12 15 16 20	16 17 18 25	12	11 14	7 8 9 9.3	17	29 30 31.2 32.2	8 9 10	10 12 13	8 11	10	9 12 13 14	19 21 23	13 14 14.2 15.2	18 20 23 24	12 15 16
1-1		XY	14 16 17 18	12 14	11 14	13 14	9 12 13	5 7 12	11 12 13	12 15 16 20	16 17 18 25	12	11 14	7 8 9 9.3	17	29 30 31.2 32.2	8 9 10	10 12 13	8 11	10	9 12 13 14	19 21 23	13 14 14.2 15.2	18 20 23 24	12 15 16
1-1		XY	14 16 17 18	12 14	11 14	13 14	9 12 13	5 7 12	11 12 13	12 15 16 20	17 18 25	12	11 14	7 8 9 9.3	17	29 30 31.2 32.2	8 9 10	10 12 13	8 11	10	9 12 13 14	19 21 23	13 14 14.2 15.2	18 20 23 24	15 16
1-2		XY	14 16 17 18	12 14	11 14	13 14	9 12 13	5 7 12	11 12 13	12 15 16 20	16 17 18 25	12	11 14	7 8 9 9.3	17	29 30 31.2 32.2	8 9 10	10 12 13	8 11	10	9 12 13 14	19 21 23	13 14 14.2 15.2	18 20 23 24	12 15 16
1-4		XY	14 16 17 18	12 14	11 14	13 14	9 12 13	5 7 12	11 12 13 14	12 15 16 20	16 17 18 25	12	11 14	7 8 9 9.3	17	29 30 31.2 32.2	8 9 10	10 12 13	8 11	10	9 12 13 14	19 21 23	13 14 14.2 15.2	18 20 23 24	12 15 16
1-10		XY	14 16 17 18	12 14	11 14	14	9 12 13	5 7 12	11 12 13	12 15 16 20	16 18 25	12	11 14	7 8 9 9.3	17	29 31.2 32.2	8 10	10 12 13	8 11	10	9 12 13 14	19 21 23	13 14 14.2 15.2	18 20 23 24	12 15 16
1-15		X	14 16 17 18	12 14	11 14	14	9 12 13	5 7 12	11 12 13	12 16 20	16 18 25	12	11 14	7 8 9 9.3	17	30 31.2 32.2	8 10	10 13	8	10	9 13 14	19 21 23	13 15.2	18 20 23	12 15 16
1-20		X	14 16 17	14	11 14	14	9 12 13	5 7 12	11 12 13 14	12 15 16	16 18 25	12	11 14	7 8 9 9.3	17	29 31.2 32.2	8 10	10 11 12 13	8 11	10	9 12 13 14	18.3 21 23	13 14 15.2	20 23	12 16
250pg	20-1	XY	16 17 18	12 14	11	13 14	9 12 13	5 12	11 12 13	15 20	17 25	12	11 14	7 8 9 9.3	17	29 30 31.2	9 10	10 12	11	10	9 12 13 14	19 21	13 14 14.2	18 24	15
	15-1	XY	14 16 17 18	12 14	11	13 14	9 12	5 12	11 12 13	12 15 16 20	17 25	12	11 14	8 9 9.3	17	29 30 31.2	9 10	10 12	11	10	9 12 13	19 21	14 14.2	18 23 24	15
	10-1	XY	14 17 18	12 14	11 14	13 14	9 12	5 12	11 12 13	12 15 20	16 17 25	12	11 14	8 9 9.3	17	29 30	9 10	10 12 13	8 11	10	9 12 13 14	19 21 23	14 14.2 15.2	18 20 23 24	12 15
	4-1	XY	14 16 17 18	12 14	11 14	13 14	9 12 13	5 7 12	11 12 13	12 15 16 20	16 17 18 25	12	11 14	7 8 9 9.3	17	29 30 31.2 32.2	8 9 10	10 12 13	8 11	10	9 12 13 14	19 21 23	13 14 14.2 15.2	18 20 23 24	12 15 16
	2-1	XY	14 16 17 18	12 14	11 14	13 14	9 12 13	5 7 12	11 12 13	12 15 16 20	16 17 18 25	12	11 14	7 8 9 9.3	17	29 30 31.2 32.2	8 9 10	10 12 13	8 11	10	9 12 13 14	19 21 23	13 14 14.2 15.2	18 20 23 24	12 15 16
	1-1	XY	14 16 17 18	12 14	11 14	13 14	9 12 13	5 7 12	11 12 13	12 15 16 20	16 17 18 25	12	11 14	7 8 9 9.3	17	29 30 31.2 32.2	8 9 10	10 12 13	8 11	10	9 12 13 14	19 21 23	13 14 14.2 15.2	18 20 23 24	12 15 16
	1-2	XY	14 16 17 18	12 14	11 14	13 14	9 12 13	5 7 12	11 12 13	12 15 16 20	16 17 18 25	12	11 14	7 8 9 9.3	17	29 30 31.2 32.2	8 9 10	10 11 12 13	8 11	10	9 12 13 14	19 21 23	13 14 14.2 15.2	18 20 23 24	12 15 16
	1-4	XY	14 16 17 18	12 14	11 14	13 14	9 12 13	5 7 12	11 12 13	12 15 16 20	16 17 18 25	12	11 14	7 8 9 9.3	17	29 30 31.2 32.2	8 9 10	10 12 13	8 11	10	9 12 13 14	19 21 23	13 14 14.2 15.2	18 20 23 24	12 15 16
	1-10	XY	14 16 17 18	12 14	11 14	14	9 12 13	5 7	11 12 13	12 15 16 20	16 17 18 25	12	11 14	7 8 9	17	29 30 31.2 32.2	8 9 10	10 12 13	8 11	10	9 12 13 14	19 21 23	13 14.2 15.2	18 20 23	12 15 16
	1-15	X	14 16 17 18	14	11 14	14	9 12 13	5 7	11 12 13	12 16	16 17 18	12	11 14	7 9 9.3	17	29 31.2 32.2	8 10	10 12 13	8	10	9 12 14	19 21 23	13 14 15.2	20 23 24	12 15 16
	1-20	XY	14 16	14	11 14	14	9 12 13	5 7	11 12 13	12 16	16 18	12	11 14	7 8 9	17	29 30 31.2 32.2	8 10	10 12 13	8	10	9 12 14	19 21 23	13 14.2 15.2	20 23	12 16
	100pg	20-1	XY	17 18	12 14	11	13 14	9 12	5 12	11 13	15 20	17 25	12	11 14	8 9.3	17	29 30 32.2	9 10	10 12	8 11	10	12 13	19 21	14 14.2	18 24
15-1		XY	17 18	12 14	11	13 14	9 12	5 12	11 13	15 20	17 25	12	11 14	7 8 9 9.3	17										



Mixture Results generated for 2 person Mixture Series 1 on Newton

		AMEL	D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E	D16S539	D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818	TPOX	DYS391	D8S1179	D12S391	D19S433	FGA	D22S1045
Mixture 1	6M	XY	15 17	15	10 12	13 15	8 9	11 17	9 11	15 16	19 23	12	9	9	14 19	30	9.1 11	11 12	10	10	14	19 20	12 13	23 25	11 16
	22F	X	15 17	15 16	11	14	8 11	11 19	11	13 19	17	11	11 15	6 9	17 18	27 31.2	8 10	9 11	8	**	14 16	21	13.2 15	21 24	11 15
750pg	20-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17	9 11	15 16 19	17 19 23	11 12	9	9	14 19	30	9.1 11	11 12	8 10	10	14	19 20 21	12 13	23 25	11 15 16
	15-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11	6 9	14 17 18 19	27 30	9.1 10 11	9 11 12	8 10	10	14	19 20 21	12 13 13.2 15	21 23 24 25	11 16
	10-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17	9 11	13 15 16 19	17 19 23	11 12	9 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	4-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	2-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 15 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-2	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 15 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-4	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 15 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-10	XY	15 17	15 16	11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 14 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-15	XY	15 17	15 16	10 11 12	13 14	8 9 11	11 17 19	9 11	13 15 16 19 20	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	20 21	12 13 13.2 15	21 23 24 25	11 15 16
1-20	X	15 17	15 16	11	14	8 9 11	11 17 19	11	13 15 16 19	17 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	6 8 10 11	9 11 12	8 10	10	14 16	19 21	12 13 13.2 15	21 24 25	11 15 16	
500pg	20-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17	9 11	13 15 16	17 19 23	11 12	9 11	9	14 18 19	27 30	8 9.1 11	9 11 12	10	10	14	19 20 21	12 13 13.2 15	21 23 24 25	11 16
	15-1	XY	15 17	15	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9	6 9	14 17 18 19	30	8 9.1 11	9 11 12	8 10	10	14 16	19 20 21	12 13 15	21 23 25	11 16
	10-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	9 11 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 25	11 15 16
	4-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	2-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 15 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-2	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-4	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-10	XY	15 17	15 16	10 11 12	14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 21	12 13 13.2 15	21 23 24 25	11 15
	1-15	X	15 17	15 16	11 12	14	8 9 11	11 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	21	12 13 13.2 15	21 23 24 25	11 15 16
1-20	X	15 17	15 16	10 11 12	14	8 9 11	11 17 19	9 11	13 15 16 19	17	11 12	9 11 15	6 9	14 17 18	27 30 31.2	8 9.1 10 11	9 11 12	8	10	14 15 16	19 21	12 13 13.2 15	21 23 24 25	11 15	
250pg	20-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16	17 19 23	11 12	9 15	9	14 17 18 19	30	8 9.1 10 11	9 11 12	10	10	14	19 20 21	12 13	21 23 24 25	11 15 16
	15-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	15 16 19	17 19 23	11 12	9	9	14 17 18 19	30	8 9.1 10 11	11 12	8 10	10	14	19 20 21	12 13 13.2	21 23 25	11 15 16
	10-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17	9 11	13 15 16 19	17 19 23	11 12	9	6 9	14 18 19	27 30	8 9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 24 25	11 16
	4-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	2-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-1	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 8 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-2	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 15 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-4	XY	15 17	15 16	10 11 12	13 14 15	8 9 11	11 17 19	9 11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 15 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15 16
	1-10	XY	15 17	15 16	11 12	13 14 15	8 9 11	11 19	11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18 19	27 30 31.2	8 9.1 10 11	9 11 12	8 10	10	14 16	19 20 21	12 13 13.2 15	21 23 24 25	11 15
	1-15	X	15 17	15 16	10 11	14 15	8 11	11 19	9 11	13 19	17	11 12	9 11 15	6 9	14 17 18	27 31.2	8 9.1 10 11	9 11	8 10	10	14 15 16	19 21	12 13 13.2 15	21 24 25	11 15
1-20	X	15 17	15 16	11	14 15	8 9 11	11 19	11	13 15 16 19	17 19 23	11 12	9 11 15	6 9	14 17 18	27 31.2	8 10 11	9 11 12	8 10	10	14 16	19 21	12 13 13.2 15	21 23 24	11 15	
100pg	20-1	XY	15 17	15	10 11 12	13 14 15	8 9	11 17	9 11	15 16	17 19 23	11 12	9 11	9	14 18 19	30	8 9.1 10 11	11 12	8 10	10	14 16	19 20	12 13	23 24 25	11 15 16
	15-1	XY	15 17	15	10 11 12	13 14 15	8 9	11 17	9 11	13 15 16	19 23	11 12	9 11	6 9	14 19	30 31.2	8 9.1 11	11 12	10	10	14	19 20 21	12 13 15	23 24 25	11 16
	10-1	XY	15 17	15	10 11 12	13 14 15	8 9	11 17	9 11	15 16	17 19 23	11 12	9 11 15	6 9	14 19	27 30 31.2	9 11 11	11 12	8 10	10	14	19 20 21	12 13 15	23 24 25	11 15 16
	4-1	XY	15 17	15 16	10 11 1																				



Mixture Results generated for 3 person Mixture Series 1 on Athena. Red numbers indicate artifacts potentially due to pull up or elevated stutter.

		AMEL	D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E	D16S539	D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818	TPOX	DYS391	D8S1179	D12S391	D19S433	FGA	D22S1045
Mixture 1	12F	X	16	14 17	10	13 15	9 10	12 15	11	14	19 22	10 11	12	6	16 17	30 31.2	11	11 13	8	**	12 13	17 19	13 15.2	24 25	16
	13M	XY	15 16	14 16	10	13 14	8	14 17	9 13	13 14	23 24	10	10 11	6 7	14 20	29	11 12	13	8	10	11 14	20	15.2 16	22	11 17
	6M	XY	15 17	15	10 12	13 15	8 9	11 17	9 11	15 16	19 23	12	9	9	14 19	30	9.1 11	11 12	10	10	14	19 20	12 13	23 25	11 16
750pg	5-1-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 18 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	5-5-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	1-1-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 12 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	1-2-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	3-2-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	5-2-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 18 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
500pg	5-1-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 13 16	14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	21 22 23 24 25	11 16 17
	5-5-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 13 16	13 14 15 16 18.2	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25 50.2	11 16 17
	1-1-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 12 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	1-2-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	3-2-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 13 16	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	5-2-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
250pg	5-1-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2	22 23 24 25	11 16 17
	5-5-1	XY	15 16 17	14 15 16 17	10	13 14 15	8 9 10	12 14 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	1-1-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 12 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	1-2-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 12 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	3-2-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	5-2-1	XY	15 16 17	14 15 16 17	10	13 14 15	8 9 10	11 12 14 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	13 15.2 16	22 23 24 25	11 16 17
100pg	5-1-1	XY	15 16	14 15 16 17	10	13 14 15	8 9 10	12 15 17	9 11 13	14 16	19 22 23 24	10 11	9 11 12	6 9	14 16 17 20	29 30 31.2	11	11 12 13	8 10	10	11 12 13 14	17 19 20	13 15.2 16	23 24 25	16
	5-5-1	XY	15 16 17	14 15 16 17	10	13 14 15	8 9	15 17	9 11 13	13 14	19 22 23 24	10 11 12	10 11 12	6 7 9	14 16 17 20	29 30 31.2	9.1 11 12	11 12 13	8	10	11 12 13 14	17 19 20	13 15.2 16	22 24 25	16 17
	1-1-1	XY	15 16 17	14 15 17	10 12	13 14 15	8 9	12 15 17	9 11 13	13 14 15 16	19 22 23 24	10 12	9 12	6 7 9	14 16 17 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 14	17 19 20	13 15.2 16	22 23 24 25	11 16
	1-2-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	14 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	15.2 16	22 23 24 25	11 16
	3-2-1	XY	15 16 17	14 15 16 17	10	13 15	8 9 10	14 17	9 11 13	13 14 15	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	13 15.2	22 23 24 25	11 16 17
	5-2-1	XY	15 16 17	14 16 17	10	13 14 15	8 9 10	12 15	9 11	13 14 16	19 22 23	10 11	9 11 12	6 7 9	14 16 17	29 30 31.2	11	11 12 13	8 10	10	11 12 13 14	17 19 20	13 15.2	22 24 25	11 16



Mixture Results generated for 3 person Mixture Series 2 on Athena. Red numbers indicate artifacts potentially due to pull up or elevated stutter.

		AMEL	D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E	D16S539	D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818	TPOX	DYS391	D8S1179	D12S391	D19S433	FGA	D22S1045
Mixture 2	17M	XY	14 16	11 17	10 14	14	8 12	12	11 12	13 14	19 23	10 13	9 13	6 8	14 17	30 31	11	13	8	10	12 14	18	12 16	20 22	16
	27F	X	12 15	12 15	11 13	14	11 14	7 14	11	18 20	20	12	9 10	99.3	16 19	28 29	10	8 12	6 10	**	14	15 18	14.2	21 28	15 16
	9M	XY	15 16	17	11 14	13 14	8	12 18	11	14 15	19 25	11 12	10 12	9	15 18	30	10 11	10 11	11	10	10 13	17 21	13.2 15.2	23 25	11 15
750pg	5-1-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12 13	7 9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	5-5-1	XY	9 12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20 21.2	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 11 12 13	6 8 10 11	10	10 12 13 14	15 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	1-1-1	XY	9 12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20 21.2	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	1-2-1	XY	9 12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	3-2-1	XY	9 12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20 21.2	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	5-2-1	XY	9 12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31 32	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
500pg	5-1-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20 21.2	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	5-5-1	XY	12 14 15 16	11 12 15 16 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31 32	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 16	20 21 22 23 28	11 15 16
	1-1-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	1-2-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	3-2-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20 21.2	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	5-2-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
250pg	5-1-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 20	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 14.2 15.2 16	20 21 22 23 25 28	15 16
	5-5-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	14	8 11 12 14	7 12 14	11 12	13 14 15 18 20	19 20 23	10 11 12 13	9 10 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10	10	10 12 13 14	15 17 18 21	12 13.2 14.2 16	20 21 22 23 25 28	15 16
	1-1-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	1-2-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	3-2-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	5-2-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 20 23	10 11 12 13	9 10 12 13	6 8 9 9.3	14 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	12 13 14	15 17 18 21	12 14.2 15.2 16	20 21 22 23 28	11 15 16
100pg	5-1-1	XY	14 15 16	11 15 17	10 13 14	13 14	8 12	12	11 12	13 14 15 20	19 23	10 11 12 13	9 10 13	6 8 9 9.3	14 16 17	30 31	10 11	10 12 13	6 8 11	10	10 12 13 14	15 18 21	12 16	20 23	15 16
	5-1-1	XY	14 15 16	11 15 17	10 13 14	13 14	8 12	12	11 12	13 14 15 20	19 23	10 11 12 13	9 10 13	6 8 9 9.3	14 16 17	30 31	10 11	10 12 13	6 8 11	10	10 12 13 14	15 18 21	12 16	20 23	15 16
	5-5-1	XY	12 14 15 16	11 15 17	10 11 13 14	14	8 11 12 14	12 14	11 12	13 14 18 20	19 20 23	10 12 13	9 10 12 13	6 8 9 9.3	14 16 17 19	28 30 31	10 11	8 12 13	6 8 10	10	10 12 13 14	15 18	12 14.2 16	20 21 22	15 16
	1-1-1	XY	12 14 15 16	11 12 17	11	13 14	8 11	7 12 14 18	11 12	13 14 15 18 20	19 23 25	10 11 12	9 10	8 9 9.3	15 16 18 19	28 29 30 31	10 11	10 11 12 13	8 10 11	10	10 12 13 14	17 18 21	12 14.2 15.2	23 25 28	11 16
	1-2-1	XY	12 14 15 16	12 15 17	11 13 14	13 14	8 11 12 14	7 12 14 18	11	13 14 15 18 20	19 20	10 12	9 10 13	6 8 9	15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18	12 13.2 14.2 15.2	21 25 28	15 16
	3-2-1	XY	12 14 15 16	11 12 15 17	10 14	13 14	8 12 14	7 12	11 12	13 14 15 18 20	19 20 23 25	12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 12 13	6 8 10 11	10	12 13 14	15 17 18	12 13.2 14.2	20 22 25 28	15 16
	5-2-1	XY	12 14 15 16	11 12 17	10 11 13 14	13 14	8 11 12	7 12 14	11 12	13 14 15 20	19 20 23	10 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17	28 29 30 31	10 11	8 10 12 13	6 8 10 11	10	10 12 13 14	15 18	12 14.2 16	20 21 22 28	16



Mixture Results generated for 3 person Mixture Series 1 on Newton. Red numbers indicate artifacts potentially due to pull up or elevated stutter.

		AMEL	D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E	D16S539	D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818	TPOX	DYS391	D8S1179	D12S391	D19S433	FGA	D22S1045
Mixture 1	12F	X	16	14 17	10	13 15	9 10	12 15	11	14	19 22	10 11	12	6	16 17	30 31.2	11	11 13	8	**	12 13	17 19	13 15.2	24 25	16
	13M	XY	15 16	14 16	10	13 14	8	14 17	9 13	13 14	23 24	10	10 11	67	14 20	29	11 12	13	8	10	11 14	20	15.2 16	22	11 17
	6M	XY	15 17	15	10 12	13 15	8 9	11 17	9 11	15 16	19 23	12	9	9	14 19	30	9.1 11	11 12	10	10	14	19 20	12 13	23 25	11 16
750pg	5-1-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	5-5-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	1-1-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 12 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	10 14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	1-2-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	10 14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	3-2-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 16 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	10 14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	5-2-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 13 14 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	10 14 16 17 19 20	27.1 29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
500pg	5-1-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 13	14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 18 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	5-5-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 12 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	10 14 16 17 19 20	27.1 29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	1-1-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 12 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	10 14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	1-2-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	3-2-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	10 14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	5-2-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
250pg	5-1-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2	22 23 24 25	11 16 17
	5-5-1	XY	15 16 17	14 15 16 17	10	13 14 15	8 9 10	11 12 14 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	1-1-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 12 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	1-2-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 12 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	3-2-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16 17
	5-2-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	13 15.2 16	22 23 24 25	11 16 17
100pg	5-1-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	12 15 17	9 11 13	14 15 16	19 22 23 24	10 11	9 10 11 12	6 7 9	14 16 17 20	29 30 31.2	11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	13 15.2 16	22 23 24 25	11 16
	5-5-1	XY	15 16 17	14 15 16 17	10	13 14 15	8 9	12 15 17	9 11 13	13 14	19 22 23 24	10 11 12	10 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8	10	11 12 13 14	17 19 20	13 15.2 16	22 23 24 25	11 16 17
	1-1-1	XY	15 16 17	14 15 17	10 12	13 14 15	8 9 10	11 12 14 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 11 12	6 7 9	14 16 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	13 15.2 16	22 23 24 25	11 16 17
	1-2-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	12 14 15 17	9 11 13	13 14 15 16	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 17 19 20	29 30 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2 16	22 23 24 25	11 16
	3-2-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	12 14 17	9 11 13	13 14 15	19 22 23 24	10 11 12	9 10 11 12	6 7 9	14 16 17 19 20	29 30 30.2 31.2	9.1 11 12	11 12 13	8 10	10	11 12 13 14	17 19 20	12 13 15.2	22 23 24 25	11 16 17
	5-2-1	XY	15 16 17	14 15 16 17	10 12	13 14 15	8 9 10	12 15 17	9 11	13 14 15 16	19 22 23	10 11 12	9 11 12	6 7 9	14 16 17	29 30 31.2	11	11 12 13	8 10	10	11 12 13 14	17 19 20	13 15.2 16	22 23 24 25	11 16 17



Mixture Results generated for 3 person Mixture Series 2 on Newton. Red numbers indicate artifacts potentially due to pull up or elevated stutter.

		AMEL	D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E	D16S539	D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818	TPOX	DYS391	D8S1179	D12S391	D19S433	FGA	D22S1045
Mixture 2	17M	XY	14 16	11 17	10 14	14	8 12	12	11 12	13 14	19 23	10 13	9 13	6 8	14 17	30 31	11	13	8	10	12 14	18	12 16	20 22	16
	27F	X	12 15	12 15	11 13	14	11 14	7 14	11	18 20	20	12	9 10	99.3	16 19	28 29	10	8 12	6 10	**	14	15 18	14.2	21 28	15 16
	9M	XY	15 16	17	11 14	13 14	8	12 18	11	14 15	19 25	11 12	10 12	9	15 18	30	10 11	10 11	11	10	10 13	17 21	13.2 15.2	23 25	11 15
750pg	5-1-1	XY	11 12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12 13 14	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	5-5-1	XY	9 12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	6 10 11	8 11 12 13	6 8 10 11	10	10 12 13 14	15 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	1-1-1	XY	9 12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	1-2-1	XY	9 12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	3-2-1	XY	9 12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	10 14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	5-2-1	XY	9 12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31 32	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
500pg	5-1-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	5-5-1	XY	12 14 15 16	11 12 15 16 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20 21	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	10 14 15 16 17 18 19	28 29 30 31 32	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	1-1-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14 15	7 12 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	1-2-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12 13	9 10 12 13	6.6 1 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	3-2-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 13 14	7 12 13 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	5-2-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
250pg	5-1-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 20	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	15 16
	5-5-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12 13	9 10 12 13	6.6 1 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10	10	10 12 13 14	15 17 18 21	12 13.2 14.2 16	20 21 22 23 25 28	11 15 16
	1-1-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 17 18 19 20	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	1-2-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	3-2-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	5-2-1	XY	12 14 15 16	11 12 13 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 19 20	19 20 23	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	12 13 14	15 17 18 21	12 14.2 15.2 16	20 21 22 23 25 28	11 15 16
100pg	5-1-1	XY	14 15 16	11 12 15 16 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 20	19 20 23	10 11 12 13	9 10 13	6 8 9 9.3	14 16 17	28 30 31	10 11	10 12 13	6 8 10 11	10	10 12 13 14	15 18 21	12 13.2 14.2 16	20 21 22 23	15 16
	5-5-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	14	8 11 12 14	7 12 14	11 12	13 14 18 19 20	19 20 23	10 12 13	9 10 12 13	6 8 9 9.3	14 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10	10	10 12 13 14	15 18 21	12 14.2 16	20 21 22 25	15 16
	1-1-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 18 20	19 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	21 22 23 25 28	11 15 16
	1-2-1	XY	12 14 15 16	12 15 17	11 13 14	13 14	8 11 12 14	7 12 14 18	11	13 14 15 18 20	19 20 25	10 12	9 10 12 13	6 8 9	15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 10 11	10	10 12 13 14	15 17 18	12 13.2 14.2 15.2	20 21 22 25 28	15 16
	3-2-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12 14	7 12 18	11 12	13 14 15 18 20	19 20 23 25	12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 12 13	6 8 10 11	9 10	10 12 13 14	15 17 18	12 13.2 14.2 16	20 22 25 28	15 16
	5-2-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12	7 12 14 18	11 12	13 14 15 20	19 20 23 25	10 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17	28 29 30 31	10 11	8 10 12 13	6 8 10 11	10	10 12 13 14	15 18	12 14.2 16	20 21 22 28	15 16



Mixture Results generated for 4 person on Athena. Red numbers indicate artifacts potentially due to pull up or elevated stutter.

		AMEL	D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E	D16S539	D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818	TPOX	DYS391	D8S1179	D12S391	D19S433	FGA	D22S1045
	17M	XY	14 16	11 17	10 14	14	8 12	12	11 12	13 14	19 23	10 13	9 13	6 8	14 17	30 31	11	13	8	10	12 14	18	12 16	20 22	16
	27F	X	12 15	12 15	11 13	14	11 14	7 14	11	18 20	20	12	9 10	99.3	16 19	28 29	10	8 12	6 10	**	14	15 18	14.2	21 28	15 16
	9M	XY	15 16	17	11 14	13 14	8	12 18	11	14 15	19 25	11 12	10 12	9	15 18	30	10 11	10 11	11	10	10 13	17 21	13.2 15.2	23 25	11 15
	28F	X	16 17	16 17.3	11 14	14 15	8 11	13 18	10 11	15 17	17 24	13 14	12 14	79	15 19	30 32.2	10 12	9 13	9 11	**	11 13	15 21	15.2 16.2	21 22	15 16
750pg	1-1-1-1	XY	12 14 15 16 17	11 12 15 16 17 17.3	10 11 13 14	13 14 15	8 11 12 14	7 12 13 14 18	10 11 12	13 14 15 17 18 20	17 19 20 23 24 25	10 11 12 13 14	9 10 12 13 14	6 7 8 9 9.3	14 15 16 17 18 19	28 29 30 31 32.2	10 11 12	8 9 10 11 12 13	6 8 9 10 11	10	10 11 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16 16.2	20 21 22 23 25 28	11 15 16
	1-3-3-1	XY	12 14 15 16 17	11 12 15 16 17 17.3	10 11 13 14	13 14 15	8 11 12 14	7 12 13 14 18	10 11 12	13 14 15 17 18 20 21.2	17 19 20 23 24 25	10 11 12 13 14	9 10 12 13 14	6 7 8 9 9.3	14 15 16 17 18 19	28 29 30 31 32.2	10 11 12	8 9 10 11 12 13	6 8 9 10 11	10	10 11 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16 16.2	20 21 22 23 25 28	11 15 16
	1-3-5-1	XY	12 14 15 16	11 12 15 16 17 17.3	10 11 13 14	13 14 15	8 11 12 14	7 12 14 18	10 11 12	13 14 15 17 18 20	17 19 20 23 24 25	10 11 12 13 14	9 10 12 13	6 7 8 9 9.3	14 15 16 17 18 19	28 29 30 31 32.2	10 11 12	8 9 10 11 12 13	6 8 9 10 11	10	10 11 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
500pg	1-1-1-1	XY	12 14 15 16	11 12 15 17	10 11 13 14	13 14	8 11 12	7 12 14 18	10 11 12	13 14 15 18 20	19 20 23 25	11 12 13	9 10 12 13	6 7 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	9 10 11 12 13	6 8 9 11	10	10 12 13 14	15 17 18 21	12 13.2 15.2 16	20 21 23 25	11 15 16
	1-3-3-1	XY	9 12 14 15 16	11 12 15 16 17	10 11 13 14	13 14	8 11 12 14	7 12 13 14 18	11 12	13 14 15 17 18 20	17 19 20 23 24 25	10 11 12 13	9 10 12 13 14	6 7 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11 12	8 9 10 11 12 13	6 8 9 10 11	10	10 11 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16 16.2	20 21 22 23 25 28	11 15 16
	1-3-5-1	XY	12 14 15 16 17	11 12 15 16 17	10 11 13 14	13 14	8 11 12 14	7 12 13 14 18	11 12	13 14 15 17 18 20	19 20 23 25	10 11 12 13 14	9 10 12 13 14	6 7 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11 12	8 9 10 11 12 13	6 8 9 10 11	10	10 11 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
250pg	1-1-1-1	XY	12 14 15 16	11 12 15 16 17	10 11 13 14	13 14 15	8 11 12 14	7 12 14 18	11 12	13 14 15 17 18 20 21.2	19 20 23 24 25	10 11 12 13 14	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31 32.2	10 11	8 10 11 12 13	6 8 10 11	10	10 11 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	1-3-3-1	XY	12 14 15 16	11 12 15 16 17	10 11 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 17 18 20	17 19 20 23 25	10 11 12 13	9 10 12 13	6 7 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 9 10 11	10	10 11 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 22 23 25 28	11 15 16
	1-3-5-1	XY	12 14 15 16	11 12 15 16 17	10 11 13 14	13 14	8 11 12 14	7 12 13 14 18	11 12	12 13 14 15 17 18 20 21.2	17 19 20 23 24 25	10 11 12 13	9 10 12 13 14	6 7 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11 12	8 9 10 11 12 13	6 8 9 10 11	10	10 11 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16 16.2	20 21 22 23 25 28	11 15 16
100pg	1-1-1-1	XY	12 14 15 16 17	11 12 15 16 17	10 11 13 14	13 14	8 11 12 14	7 12 13 14 18	11 12	13 14 15 17 18 20	19 20 23 24 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 9 10 11 12 13	6 8 10 11	10	10 11 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16 16.2	20 21 22 23 25 28	11 15 16
	1-3-3-1	XY	12 14 15 16 17	11 12 15 16 17	10 11 13 14	13 14 15	8 11 14	7 12 14 18	11 12	13 14 15 17 18 20 21.2	19 20 23 25	10 11 12 13	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11 12	8 9 10 11 12 13	6 8 9 10 11	10	10 11 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	1-3-5-1	XY	12 14 15 16	11 12 17	10 11 14	13 14	8 11	12 18	11 12	13 14 15 18 20	19 20 25	10 11 12 13	9 10 12	8 9	14 15 16 18	28 29 30 31	10 11	8 10 11 12 13	8 9 10 11	10	10 13 14	17 18 21	12 13.2 14.2 15.2	21 23 25	15 16



Mixture Results generated for 4 person on Newton. Red numbers indicate artifacts potentially due to pull up or elevated stutter.

		AMEL	D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E	D16S539	D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818	TPOX	DYS391	D8S1179	D12S391	D19S433	FGA	D22S1045
	17M	XY	14 16	11 17	10 14	14	8 12	12	11 12	13 14	19 23	10 13	9 13	6 8	14 17	30 31	11	13	8	10	12 14	18	12 16	20 22	16
	27F	X	12 15	12 15	11 13	14	11 14	7 14	11	18 20	20	12	9 10	9 9.3	16 19	28 29	10	8 12	6 10	**	14	15 18	14.2	21 28	15 16
	9M	XY	15 16	17	11 14	13 14	8	12 18	11	14 15	19 25	11 12	10 12	9	15 18	30	10 11	10 11	11	10	10 13	17 21	13.2 15.2	23 25	11 15
	28F	X	16 17	16 17.3	11 14	14 15	8 11	13 18	10 11	15 17	17 24	13 14	12 14	7 9	15 19	30 32.2	10 12	9 13	9 11	**	11 13	15 21	15.2 16.2	21 22	15 16
750PB	1-1-1-1	XY	12 14 15 16 17	11 12 15 16 17 17.3	10 11 13 14	13 14 15	8 11 12 14	7 12 13 14 18	10 11 12	13 14 15 17 18 20	17 19 20 23 24 25	10 11 12 13 14	9 10 12 13 14	6 6.1 7 8 9 9.3	10 14 15 16 17 18 19	28 29 30 31 32.2	10 11 12	8 9 10 11 12 13	6 8 9 10 11	10	10 11 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16 16.2	20 21 22 23 25 28	11 15 16
	1-3-3-1	XY	12 14 15 16 17	11 12 15 16 17 17.3	10 11 13 14	13 14 15	8 11 12 14	7 12 13 14 18	10 11 12	13 14 15 17 18 19 20	17 19 20 23 24 25	10 11 12 13 14	9 10 12 13 14	6 7 8 9 9.3	14 15 16 17 18 19	28 29 30 31 32.2	10 11 12	8 9 10 11 12 13	6 8 9 10 11	10	10 11 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16 16.2	20 21 22 23 25 28	11 15 16
	1-3-5-1	XY	12 14 15 16 17	11 12 15 16 17 17.3	10 11 13 14	13 14 15	8 11 12 14	7 12 14 18	10 11 12	13 14 15 17 18 20	17 19 20 23 24 25	10 11 12 13 14	9 10 12 13	6 7 8 9 9.3	14 15 16 17 18 19	28 29 30 31 32.2	10 11 12	8 9 10 11 12 13	6 8 9 10 11	10	10 11 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16 16.2	20 21 22 23 25 28	11 15 16
500PB	1-1-1-1	XY	12 14 15 16	11 12 15 17 17.3	10 11 13 14	13 14	8 11 12	7 12 13 14 18	10 11 12	13 14 15 17 18 20	17 19 20 23 25	10 11 12 13	9 10 12 13	6 7 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	9 10 11 12 13	6 8 9 10 11	10	10 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	1-3-3-1	XY	9 12 14 15 16	11 12 15 16 17	10 11 13 14	13 14	8 11 12 14	7 12 13 14 18	11 12	13 14 15 17 18 20	12 17 19 20 23 24 25	10 11 12 13	9 10 12 13 14	6 7 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11 12	8 9 10 11 12 13	6 8 9 10 11	10	10 11 12 13 14 14.1	15 17 18 21	12 13.2 14.2 15.2 16 16.2	20 21 22 23 25 28	11 15 16
	1-3-5-1	XY	12 14 15 16 17	11 12 15 16 17	10 11 13 14	13 14	8 11 12 14	7 12 13 14 18	11 12	13 14 15 17 18 20	19 20 23 25	10 11 12 13 14	9 10 12 13 14	6 7 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11 12	8 9 10 11 12 13	6 8 9 10 11	10	10 11 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
250PB	1-1-1-1	XY	11 12 14 15 16	11 12 15 16 17	10 11 13 14	13 14 15	8 11 12 14	7 12 13 14 18	11 12	13 14 15 17 18 20	17 19 20 23 24 25	10 11 12 13 14	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31 32.2	10 11	8 10 11 12 13	6 8 10 11	10	10 11 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 21 22 23 25 28	11 15 16
	1-3-3-1	XY	12 14 15 16 17	11 12 15 16 17	10 11 14	13 14	8 11 12 14	7 12 14 18	11 12	13 14 15 17 18 20	17 19 20 23 25	10 11 12 13	9 10 12 13	6 7 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11	8 10 11 12 13	6 8 9 10 11	10	10 11 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16	20 22 23 25 28	11 15 16
	1-3-5-1	XY	12 14 15 16	11 12 15 16 17	10 11 13 14	13 14	8 11 12 14	7 12 13 14 18	11 12	12 13 14 15 17 18 20	17 19 20 23 24 25	10 11 12 13	9 10 12 13 14	6 7 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11 12	8 10 11 12 13	6 8 9 10 11	10	10 11 12 13 14 14.1	15 17 18 21	12 13.2 14.2 15.2 16 16.2	20 21 22 23 25 28	11 15 16
100PB	1-1-1-1	XY	12 14 15 16 17	11 12 15 16 17	10 11 13 14	13 14	8 11 12 14	7 12 13 14 18	11 12	13 14 15 17 18 20	19 20 23 24 25	10 11 12 13 14	9 10 12 13	6 8 9 9.3	10 14 15 16 17 18 19	28 29 30 31	10 11	8 9 10 11 12 13	6 8 10 11	10	10 11 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16 16.2	20 21 22 23 25 28	11 15 16
	1-3-3-1	XY	12 14 15 16 17	11 12 15 16 17	10 11 13 14	13 14 15	8 11 14	7 12 13 14 18	11 12	13 14 15 17 18 20	19 20 23 25	10 11 12 13 14	9 10 12 13	6 8 9 9.3	14 15 16 17 18 19	28 29 30 31	10 11 12	8 9 10 11 12 13	6 8 9 10 11	10	10 11 12 13 14	15 17 18 21	12 13.2 14.2 15.2 16 16.2	20 21 22 23 25 28	11 15 16
	1-3-5-1	XY	12 14 15 16	11 12 15 17 17.3	10 11 13 14	13 14	8 11	7 12 14 18	11 12	13 14 15 18 20	17 19 20 23 25	10 11 12 13	9 10 12 13	8 9	14 15 16 17 18	28 29 30 31 32.2	10 11 12	8 10 11 12 13	6 8 9 10 11	10	10 11 12 13 14	15 17 18 21	12 13.2 14.2 15.2	21 22 23 24 25	11 15 16

Addendum to the Powerplex Fusion Validation, Genemarker HID v2.8.2 Analysis Software Validation, and STRmix Validation

Global filter and DNA input amount evaluation

A large number of artifacts were observed using a 50 RFU analytical threshold. Therefore, a Global Filter evaluation was conducted. Lowering the input amount to 500pg and diluting the instrument matrix to make it less saturated were not successful in removing these artifacts. A global filter was evaluated to determine if artifacts could be reduced without excessive loss of data. Analysis of a portion of the samples run on both Athena and Newton was performed. A Global Max filter was applied during the run wizard, as well as a Min Heterozygote Imbalance (%) filter within the panel settings across all the locations. These samples were analyzed with a 10% filter, 8% filter, 5% filter, 4% filter, and 3% filter set in both locations. After evaluation it was noticed that almost half of the lost alleles could be recovered by going down to a 3% filter, while still greatly reducing the number of artifacts. Therefore, for all evidence and exemplar samples, it was decided that a filter of 3% would be applied for the Global Max filter during the run wizard and across all locations within the panel for the Min Heterozygote Imbalance (%) filter.

However, after beginning work on the STRmix[®] validation it was determined that the Global Max filter and Min Heterozygote Imbalance filter could not be used during analysis since the filters were removing potential low-level stutter peaks which STRmix[®] needed to properly analyze the sample. Therefore, to be able to remove both filters, a lower input amount of 525pg was decided upon to achieve fewer artifacts. This is justified because samples amplified with a 525pg input amount achieved the same accuracy and comparable peak height ratios as samples amplified with a 750pg input amount.

The final decision for Powerplex Fusion @ 29 cycles stands as follows:

Global Max Filter:	0%
Min Heterozygote Imbalance Filter:	0%
Optimum DNA Input Amount:	525pg

Based on sensitivity studies and mixture studies, the minimum DNA Input Amount has been set at 37.5pg.