



Intermountain Forensics

VAL #

QTY-V02

Forensic DNA Technical Leader Approval

Issue Date

7/6/20

Quantiplex Pro– Quant Studio 5 Summary

1. Summary and Conclusions:

Summary:

Commercial quantification kit Quantiplex Pro (Qiagen) was evaluated for use to quantify DNA samples for forensic testing using the QuantStudio 5 instrument (ThermoFisher). The use of Virtual Standard Curves was also examined.

Data indicates that the quantification kit sufficiently detects human DNA and can differentiate human vs. male DNA. The kit can detect DNA at levels far below what is needed to generate DNA profiling data. Normalizing samples based on the quantification results produce expected and interpretable DNA profiles. The use of Virtual Standard Curves will give an increased degree of reproducibility over generating a new standard curve with every plate. A sample that generates a "no result" or zero quant can be considered free of DNA for forensic DNA downstream processing, will not generate a DNA profile and processing of the sample may be stopped.

Conclusion:

The Qiagen Quantiplex Pro Quantification kit utilizing the Applied Biosystem QuantStudio 5 was examined through Contamination Assessment, Reproducibility, Sensitivity, Mixture Studies, Non-Probative casework like samples and zero=zero studies and as a result of the conclusions contained within this document it is determined that it is suitable for DNA quantification processing in Forensic DNA Casework.

2. Standard Curve Study

Summary:

Standard Curve dilution series of 50ng/ul, 5ng/ul, 0.5ng/ul, 0.05ng/ul, and 0.005ng/ul was made according to the instructions in the Quantiplex Pro kit User Guide. A set of standard curves were assessed to determine the variation in order to use Virtual Standard Curves to analyze quantification data. Standard Curve data for plates run during the Validation show variation in DNA results dependent on the standard curve that is used to analyze the data, though it is not expected to significantly affect processing decisions unless stop processing thresholds are used but may be a cause for reamplifying samples when general amplification targets are used.

A single standard is required on plates where a Virtual Standard Curve is used. A comparison in variability between the different standards of the standard curve was performed. 5ng/ul had a lower degree of variation than the other dilutions and will be used. +/- (3) of the average Standard Deviations or a range of 4.20-5.80 will be used as an indicator of individual plate amplification. No assessment of how long a single standard dilution tube remains viable was performed, however, the Quantiplex Pro User Guide states that a standard dilution is viable for at least one week.

Table 1. Standard Curve reproducibility

Autosomal	Slope	R ²	Y-Intercept	Efficiency
Standard Set 1	-3.11	1	25.751	109.653
Standard Set 2	-3.267	0.999	26.27	102.352
Standard Set 3	-3.234	1	26.194	103.791
Standard Set 4	-3.148	0.999	26.172	107.832
Standard Set 5	-3.163	1	26.106	107.093
Standard Set 6	-3.262	1	26.072	102.568
Standard Set 7	-3.221	1	26.134	104.405



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Average	-3.20071	0.9997	26.10	105.38
STDEV	0.06069	0.000488	0.1667	2.821
CV	0.0190	0.0005	0.0064	0.0268
Male	Slope	R²	Y-Intercept	Efficiency
Standard Set 1	-3.155	0.999	23.837	107.457
Standard Set 2	-3.292	1	24.354	101.243
Standard Set 3	-3.308	1	24.361	100.601
Standard Set 4	-3.132	1	24.188	107.625
Standard Set 5	-3.378	0.999	24.174	97.712
Standard Set 6	-3.298	0.998	23.986	101.013
Standard Set 7	-3.26	0.999	24.014	102.646
Average	-3.26043	0.9993	24.13	102.61
STDEV	0.087658	0.000756	0.1953	3.676
CV	0.0269	0.0008	0.0081	0.0358
Degradation	Slope	R²	Y-Intercept	Efficiency
Standard Set 1	-3.047	0.998	26.312	112.903
Standard Set 2	-3.248	0.998	26.847	103.194
Standard Set 3	-3.22	1	26.689	104.441
Standard Set 4	-3.118	0.999	26.544	109.261
Standard Set 5	-3.133	1	26.426	108.552
Standard Set 6	-3.288	0.999	26.443	101.431
Standard Set 7	-3.11	1	26.364	109.345
Average	-3.16629	0.9991	26.52	107.02
STDEV	0.086808	0.000900	0.1908	4.081
CV	0.0274	0.0009	0.0072	0.0381

Conclusion:

The CV values in the sample sets are extremely low and thus the variability in quant metrics from run to run is also very low. This suggests that use of a virtual standard curve is appropriate as run to run variability of these metrics is unlikely to significantly affect results. The use of a virtual standard curve will allow an even further degree of replication so that each quant value will result in the same actions for similar "true" DNA amounts across every plate and provide a more consistent analysis and use of standard amplification targets. This will also decrease costs and increase efficiency.

From these results, the QuantStudio 5 and Qiagen Quantiplex Quantification Kit:

- 1) Has shown the ability to reproduce standard curves very consistently with an extremely low level of variation
- 2) Allows use of virtual standard curves to be utilized
 - a. As different manufacture lots may have slight variability, each newly received manufacture lot should have a new Virtual Standard Curve created as per vendor recommendations



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3. Contamination Assessment

Summary:

The NTC from all quantification runs were examined for DNA. One NTC with Quantiplex Pro showed a very low value.

Table 2: Non template control (NTC) results

Quant Plate	Human	Y
2020-02-14-DH-1	0	0
2020-05-07-135907	0	0
QPCR200221AM-2	0	0
QPCR200420-AM-QP1	0	0
QPCR200421-AM	0	0
Q200512DC-QPP-1	0.0001	0

Conclusion:

With the majority of NTC quantifications detecting 0 DNA and the one non-zero sample generating a miniscule result that is not conducive to generating a usable DNA profile, the protocol and quantification process is shown to be able to be performed without introducing contamination.

From these results, the QuantStudio 5 and Qiagen Quantiplex Quantification Kit:

- 1) Has shown to yield results that are clear of contamination
 - a. The QuantStudio 5 does not appear to cross contaminate
 - b. The Qiagen Quantiplex Pro Quantification kit does not appear to introduce reagent-based contamination
 - c. The quant setup / lab processes associated with QuantStudio 5 and Qiagen Quantiplex pro quantification kit do not appear to introduce contamination

4. Sensitivity

Summary:

A dilution series for 6 samples was made with dilutions of 1ng/ul, 0.5ng/ul, 0.125ng/ul, 0.0625ng/ul, 0.03125ng/ul, and 0.015625ng/ul. Each was quantified and amplified, as the total input DNA amount, with Globalfiler and Investigator 24Plex.

An assessment to determine amplification activity at lower quantification values and determine whether any stop-at-quantification thresholds can be established.

Table 3. Sensitivity Data

Sample ID	QP	GF	AVG. PHR	Comparable	INV	AVG. PHR	Comparable
ND1-0.015625	0.0028	2 (6%)	N/A	No	0 (0%)	N/A	No
ND1-0.03125	0.0093	10 (29%)	47%	No	4 (11%)	N/A	No
ND21-0.015625	0.0102	0 (0%)	N/A	No	2 (6%)	N/A	No
ND18-0.015625	0.0172	10 (25%)	53%	No	8 (20%)	N/A	No
ND12-0.015625	0.0184	14(37%)	47%	Yes	18 (47%)	54%	Yes



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ND21-0.03125	0.0241	2 (6%)	N/A	No	8 (24%)	N/A	No
ND11-0.015625	0.0363	10 (26%)	N/A	Yes	37 (95%)	70%	Yes
ND1-0.0625	0.0401	31 (89%)	60%	Yes	32 (91%)	60%	Yes
ND12-0.03125	0.0404	25 (66%)	70%	Yes	31 (82%)	70%	Yes
ND18-0.03125	0.0478	32 (80%)	62%	Yes	20 (50%)	68%	Yes
ND11-0.03125	0.0643	---	---	---	34 (87%)	61%	Yes
ND21-0.0625	0.0649	29 (85%)	64%	Yes	26 (76%)	56%	Yes
ND11-0.0625	0.0662	34 (87%)	81%	Yes	39 (100%)	77%	Yes
ND1-0.125	0.0774	35 (100%)	72%	Yes	34 (97%)	62%	Yes
ND12-0.0625	0.0845	38 (100%)	70%	Yes	38 (100%)	71%	Yes
ND9-0.015625	0.1156	40 (100%)	81%	Yes	39 (98%)	69%	Yes
ND21-0.25	0.124	34 (100%)	74%	Yes	33 (97%)	73%	Yes
ND1-0.25	0.1291	35 (100%)	81%	Yes	35 (100%)	73%	Yes
ND18-0.0625	0.1374	39 (98%)	68%	Yes	36 (90%)	68%	Yes
ND21-0.125	0.1743	31 (91%)	70%	Yes	27 (79%)	74%	Yes
ND12-0.125	0.184	38 (100%)	86%	Yes	38 (100%)	72%	Yes
ND9-0.0625	0.2005	40 (100%)	85%	Yes	40 (100%)	84%	Yes
ND18-0.125	0.2152	40 (100%)	82%	Yes	40 (100%)	69%	Yes
ND18-0.25	0.2941	40 (100%)	87%	Yes	40 (100%)	78%	Yes
ND11-0.125	0.3197	33 (85%)	75%	Yes	39 (100%)	78%	Yes
ND12-0.25	0.3581	38 (100%)	86%	Yes	38 (100%)	86%	Yes
ND9-0.03125	0.3584	40 (100%)	85%	Yes	40 (100%)	81%	Yes
ND1-0.5	0.4309	35 (100%)	87%	Yes	35 (100%)	85%	Yes
ND11-0.25	0.4391	36 (92%)	82%	Yes	39 (100%)	89%	Yes
ND12-1	0.4563	38 (100%)	87%	Yes	38 (100%)	89%	Yes
ND11-0.5	0.4609	39 (100%)	89%	Yes	39 (100%)	89%	Yes
ND9-0.125	0.4612	40 (100%)	88%	Yes	40 (100%)	88%	Yes
ND21-0.5	0.4931	34 (100%)	86%	Yes	34 (100%)	75%	Yes
ND1-1	0.519	35 (100%)	89%	Yes	35 (100%)	89%	Yes
ND18-1	0.5489	40 (100%)	90%	Yes	40 (100%)	89%	Yes
ND9-0.25	0.7493	40 (100%)	90%	Yes	40 (100%)	86%	Yes
ND12-0.5	0.7806	38 (100%)	91%	Yes	---	---	---
ND18-0.5	0.8386	40 (100%)	87%	Yes	40 (100%)	85%	Yes
ND9-0.5	1.1988	40 (100%)	88%	Yes	40 (100%)	91%	Yes



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ND21-1	1.2466	34 (100%)	88%	Yes	34 (100%)	86%	Yes
ND9-1	2.0083	40 (100%)	93%	Yes	40 (100%)	89%	Yes
ND11-1	2.5375	39 (100%)	87%	Yes	39 (100%)	90%	Yes

Conclusion:

Data indicates that the quantification kit is capable of detecting DNA at a lower level than will produce amplification profile results. Full profiles can be obtained with high quality DNA template as low as 0.080 ng and comparable DNA profiles obtained as low as 0.020 ng. Average Peak Height Ratio decreases with the decrease of input DNA with average PHR above 60% down to 0.04ng/ul. These values are not applicable with degraded and/or inhibited DNA profiles.

By obtaining a comparable DNA profile with only 20pg of input DNA, a Stop-at-Quant threshold will not be determined at this time. Data will be collected from casework samples and an assessment from that will be made at a later time.

From these results, the QuantStudio 5 and Qiagen Quantiplex Quantification Kit:

- 1) Is able to detect miniscule amounts of DNA that may be usable to downstream processes
- 2) Has a limit of detection that exceeds that of downstream processes, thus is able to obtain results even in circumstances where the sample was not able to generate a DNA profile
 - a. The kit does not have a clear delineation of when a DNA profile is obtained, even at low levels, thus no stop at quant value is recommended (i.e. the lab will amplify samples that yield any result from the quant kit)
- 3) Is able to accurately reflect the amount of DNA within the sample and is capable of estimates that can be normalized effectively to generate usable DNA profiles
 - a. Samples above approximately 200pg appear to show consistently full profiles
 - b. Samples above approximately 350pg appear to show profiles with PHR greater than 80%

5. Mixture Samples

Summary:

A 1:1,000 dilution of saliva was combined with a 1:1,000 dilution of semen and a 1:10,000 dilution of blood was combined with a 1:10,000 dilution of semen and was extracted non-differentially with an addition of 40ul of DTT for sperm cell lysis. These samples were quantified with Quantiplex Pro.

Table 4: Semen/Saliva Mixture

Sample	Human	Male
SP7 1:1000	6,750	0.011
SP8 1:10000	7,562	1.520

To assist in detection of mixed samples, single source male samples were evaluated to determine the High, Low, and Average Auto:Y ratio. 21 Quantifications from 3 male donors yielded a low of 0.76, a high of 1.94, and an average of 1.32 for Quantiplex Pro. Outside of this range should indicate mixed samples for downstream processing.

Table 5: Single Source Male Auto/Y Ratios

Sample	Auto:Y Ratio
ND1-0.015625	1.655629
ND1-0.03125	1.412535
ND1-0.0625	1.350893
ND1-0.125	1.506666
ND1-0.25	1.376459
ND1-0.5	1.409404
ND1-1	1.374264



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ND18-0.01525	1.581728
ND18-0.03125	1.127642
ND18-0.0625	1.116338
ND18-0.125	1.201597
ND18-0.25	1.276141
ND18-0.5	1.310012
ND18-1	1.305477
ND21-0.01525	1.623168
ND21-0.03125	1.942375
ND21-0.0625	1.459941
ND21-0.125	1.419336
ND21-0.25	1.428193
ND21-0.5	1.561111
ND21-1	1.655153

Conclusion:

Quantiplex Pro displays the ability to detect male DNA separately of total human DNA. The Autosomal:Y ratio can give an indication when a mixture of male and female DNA is present. DNA mixtures below can be visualized at quantification:

Table 6: summary data

Female DNA	Male DNA	Visualized
High	High	Yes
High	Low	Yes
Low	High	No
Low	Low	Yes

Mixtures of same gender individuals or number of contributors cannot be visualized from quantification data.

From these results, the QuantStudio 5 and Qiagen Quantiplex Quantification Kit:

- 1) Exhibits the ability to detect Female/Male mixtures from the Auto:Y ratio
- 2) Single Source male profiles can present with some variability in the Auto:Y ratio
 - a. Auto:Y ratios above 1.75 to 1 should be considered indicative of a possible mixture
 - b. Auto:Y ratios above 2.0 to 1 should be considered a likely mixture
 - c. Low female contributor DNA may not be visualized in the quant data
- 3) Does not allow for detection of Male/Male or Female/Female mixture detection

6. Reproducibility and Precision

Summary:

A plate was run with 6 sets of a standard curve standards. The plate was analyzed using one set of standards to create the standard curve, treating the others as unknowns. This was repeated for all standard curve sets. The data indicates that the concentration levels are less variable in the 5ng/ul range and 0.05ng/ul range and most variable in the highest (50ng/ul) and lowest (0.005ng/ul) range. Also indicated is additional support for the use of a virtual standard curve over using a new standard curve for each plate. A consistent curve will improve the consistency of the amplification data obtained and the quantification results.

Table 7: Reproducibility data and CV



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	Sample Name	Standard 1 (50ng/ul)	Standard 2 (5ng/ul)	Standard 3 (0.5ng/ul)	Standard 4 (0.05ng/ul)	Standard 5 (0.005ng/ul)
1		47.756	5.46705	0.52874	0.05965	0.00512
		44.354	4.97158	0.63479	0.05624	0.00635
		50.659	5.03732	0.58785	0.06368	0.00649
		56.440	5.60847	0.57888	0.06399	0.00537
		55.198	5.03257	0.53005	0.06083	0.00590
	Average	50.9	5.22	0.572	0.0609	0.00585
	STDEV	5.05	0.292	0.0444	0.00318	0.000595
	CV	0.10	0.06	0.08	0.05	0.10
2		46.8	4.85	0.475	0.0543	0.00394
		43.7	4.79	0.599	0.0518	0.00572
		49.9	4.85	0.554	0.0587	0.00585
		55.7	5.41	0.546	0.0590	0.00483
		54.5	4.85	0.499	0.0561	0.00531
	Average	50.1	4.97	0.543	0.0560	0.00508
	STDEV	5.06	0.291	0.0513	0.00350	0.000887
	CV	0.10	0.06	0.09	0.06	0.17
3		51.2	4.98	0.458	0.0494	0.00333
		51.5	5.43	0.481	0.0499	0.00391
		54.7	4.99	0.537	0.0534	0.00500
		61.2	5.58	0.528	0.0537	0.00410
		59.8	4.98	0.482	0.0510	0.00453
	Average	55.7	5.13	0.492	0.0509	0.00408
	STDEV	4.65	0.257	0.0405	0.00221	0.000848
	CV	0.08	0.05	0.08	0.04	0.21
4		47.9	4.71	0.438	0.0477	0.00326
		48.1	5.13	0.460	0.0483	0.00383
		44.6	4.65	0.555	0.0454	0.00477
		57.2	5.27	0.505	0.0519	0.00402



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		55.9	4.71	0.461	0.0493	0.00443
	Average	50.7	4.83	0.484	0.0472	0.00395
	STDEV	5.50	0.262	0.0624	0.00151	0.000763
	CV	0.11	0.05	0.13	0.03	0.19
5		41.6	4.39	0.438	0.0511	0.00379
		41.8	4.77	0.460	0.0517	0.00442
		38.8	4.33	0.552	0.0487	0.00548
		44.3	4.39	0.511	0.0552	0.00561
		48.3	4.39	0.461	0.0527	0.00510
	Average	43.0	4.50	0.483	0.0505	0.00456
	STDEV	3.57	0.236	0.0603	0.00157	0.000854
	CV	0.08	0.05	0.12	0.03	0.19
6		45.6	4.67	0.454	0.0514	0.00369
		45.8	5.08	0.476	0.0520	0.00431
		42.5	4.62	0.572	0.0490	0.00536
		48.6	4.68	0.530	0.0556	0.00549
		54.3	5.22	0.521	0.0559	0.00453
	Average	47.4	4.79	0.501	0.0508	0.00445
	STDEV	4.43	0.255	0.0633	0.00160	0.000844
	CV	0.09	0.05	0.13	0.03	0.19

Conclusion:

Quantiplex Pro displays the ability to obtain similar results from the same sample. The variation observed allows a small window of processing differences for if any stop-processing thresholds are established. A greater variation occurs due to which standard curve is used for analysis giving strong support for the use of a virtual standard curve

All data appears accurate to expected result and consistent, replicate to replicate. Only one CV above .20 was observed (.21). The most variable samples were that of the lowest DNA concentration which is an expected outcome.

From these results, the QuantStudio 5 and Qiagen Quantiplex Quantification Kit:

- 1) Yields consistent (precise) and reproducible quant results
- 2) Quantifies an extract that produces a reproducible DNA profile that is consistent with expected yield

7. Non-Probative Samples

Quant sample sources used:



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Table 8: Non Probative Samples

Item	Quantification Result
Envelope Seal and Stamps (1920's -1960's)	17% 0 DNA 43% <250pg 22% 250pg-1ng 17% >1ng
Buccal Swabs	255-1851.2ng
Semen dilutions	0.0276-1.285ng male
Discarded Clothing (Found Outside)	0.85ng
Soda Can	0.00ng
Shoes	0.00ng
Jewelry	0.01-0.07ng
Hair root	5.17ng
Hair shafts (swabbed)	0.011-0.026ng
Soccer Shin Guard	0.072-0.455ng
Beer Tap Handle	0.47ng
Cigarette Butt	45.7ng
Blood	8.96-461ng
M-Vac Filter	0.07-6.20ng

From these results, we conclude that the QuantStudio 5 and Qiagen Quantiplex Quantification Kit:

- 1) Provides robust quantification capabilities of extracts on a variety of different substrates
- 2) Confirms the ability to obtain quantification results of DNA extracts for forensically common items and biological materials

8. Zero equals Zero study

Summary:

Low level quantification samples were compared with their associated amplification e-grams to determine at which input amount consistently results in no DNA peaks calling after amplification with Globalfiler and Investigator 24Plex.

Table 9: Zero = Zero data

	QP (Total Amp Input)	GF (Called Peaks)	INV (Called Peaks)
E200618DC- END-RB1	0	0	0
D1 SPRB	0	0	0
RB 200505	0	0	-
AMRB1	0.0003	0	0
DCRB1	0.0006	0	0
DCRB4	0.00075	0	0
AMRB2	0.00105	0	0
AMRB4	0.0015	0	0
RBSP 2	0.0015	4	0
RBEP 4	0.0015	6	0
AMRB3	0.00285	1	0



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RBSP 1	0.003	1	2
AMRB7	0.00345	0	0
AMRB6	0.00585	0	0
ND1-0.015625	0.0028	2	0
ND1-0.03125	0.0093	10	4
ND21-0.015625	0.0102	0	2
RBSP 5	0.0165	1	0
ND18-0.015625	0.0172	10	8
ND12-0.015625	0.0184	14	18
ND21-0.03125	0.0241	2	8



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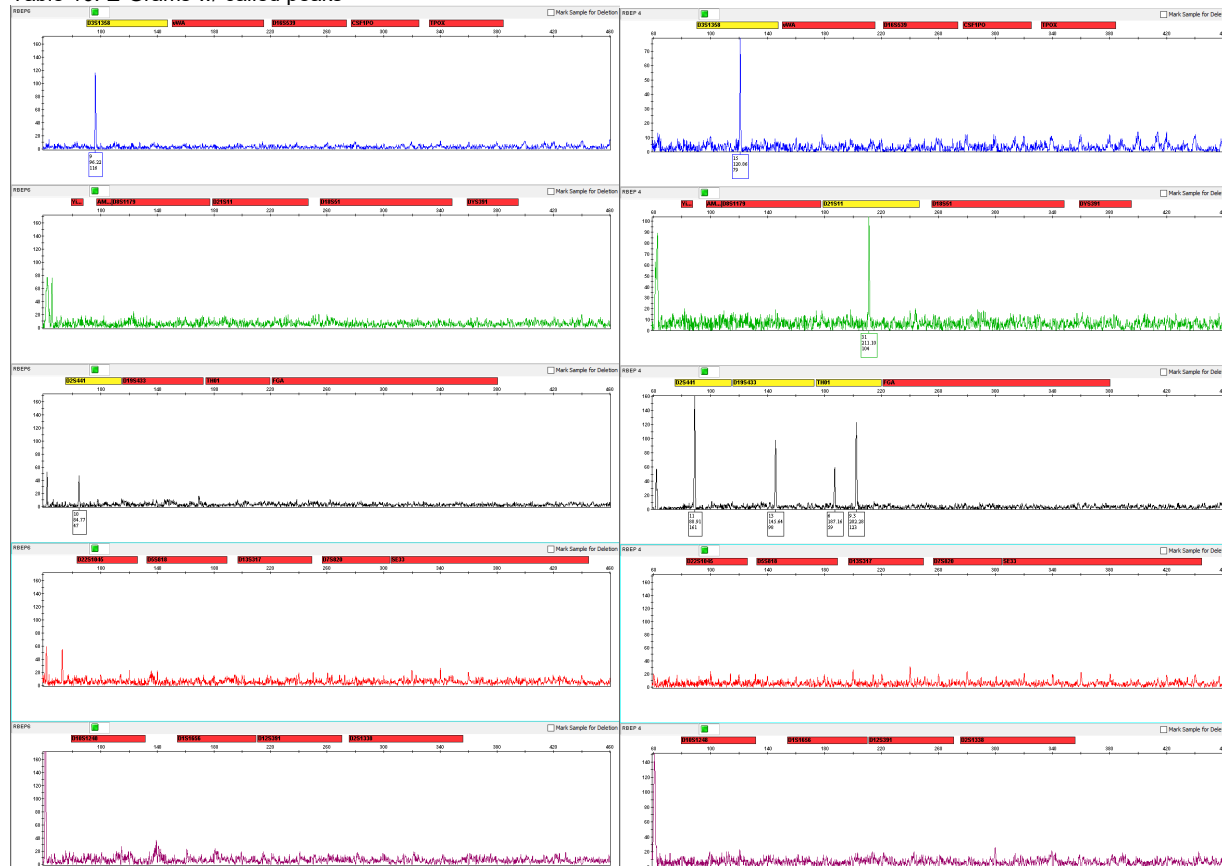
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Table 10: E-Grams w/ called peaks





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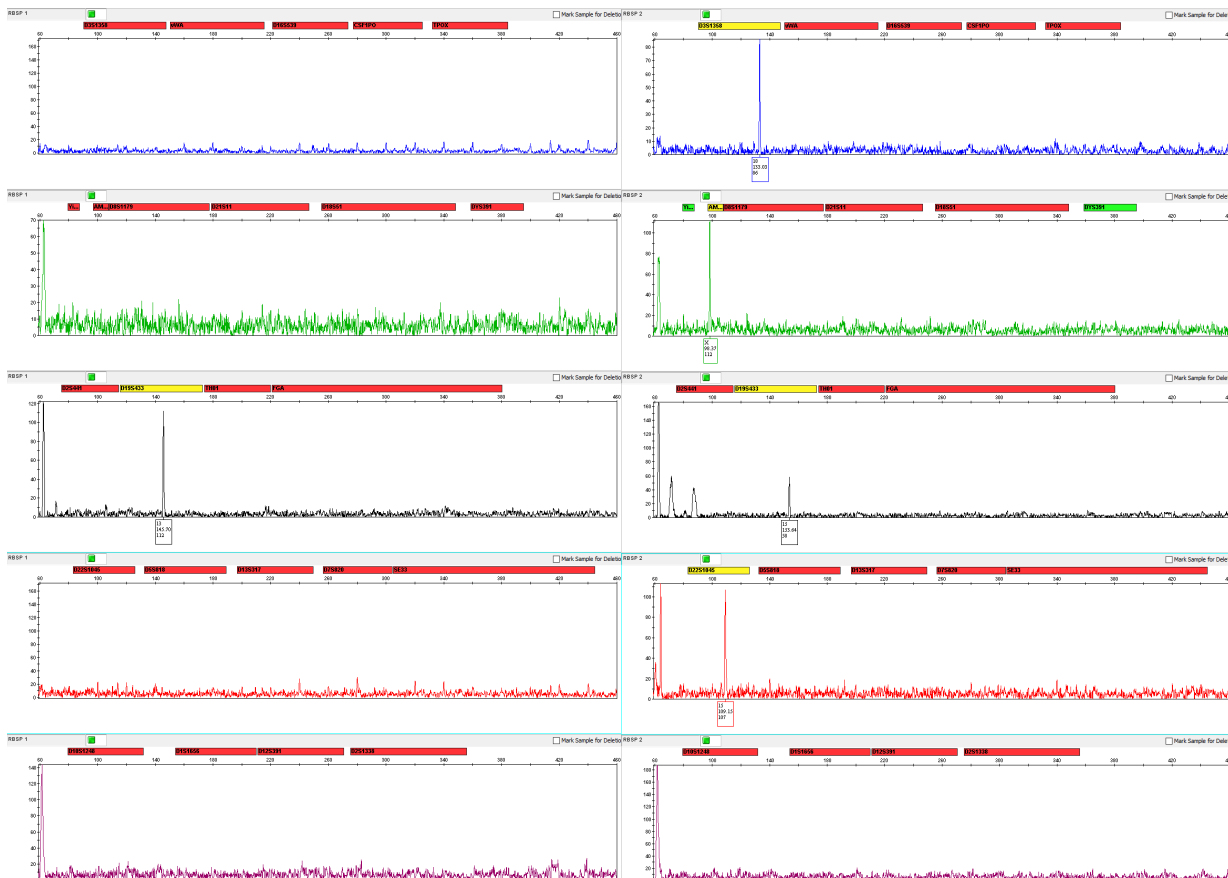
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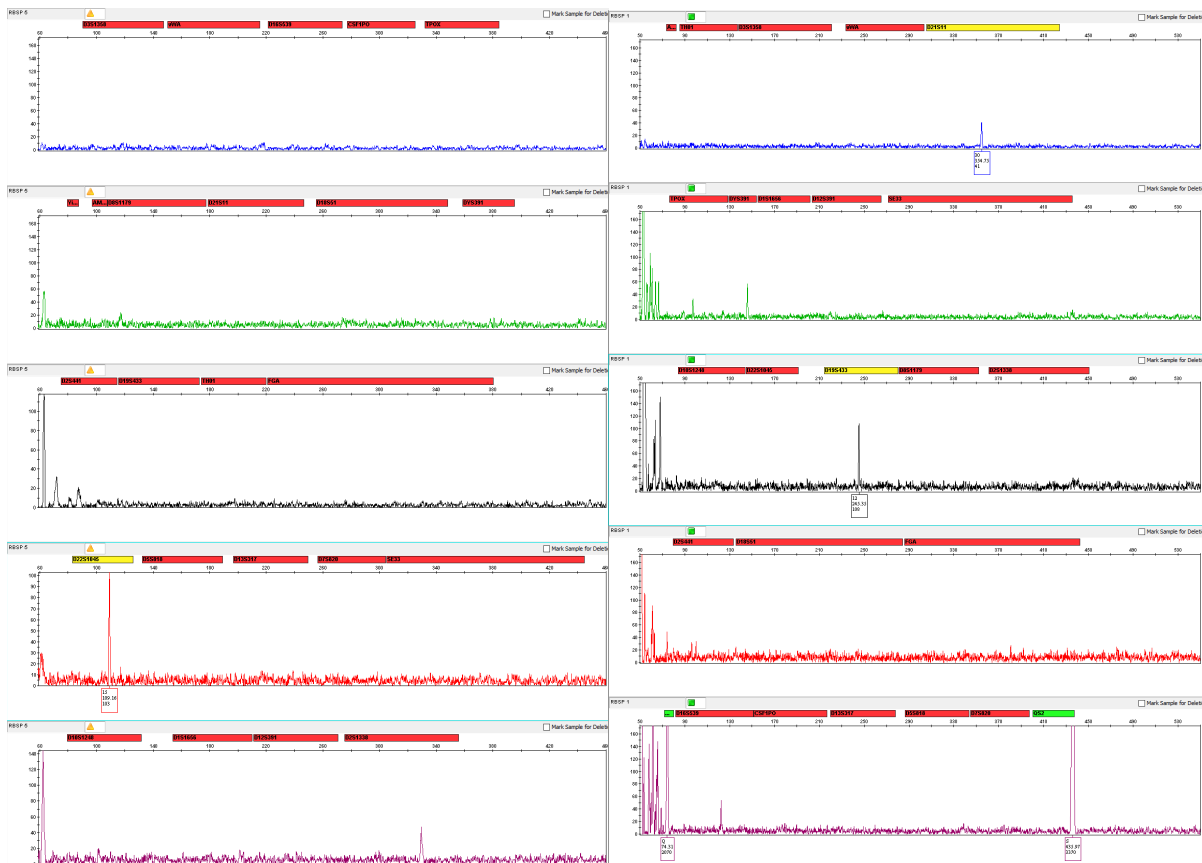
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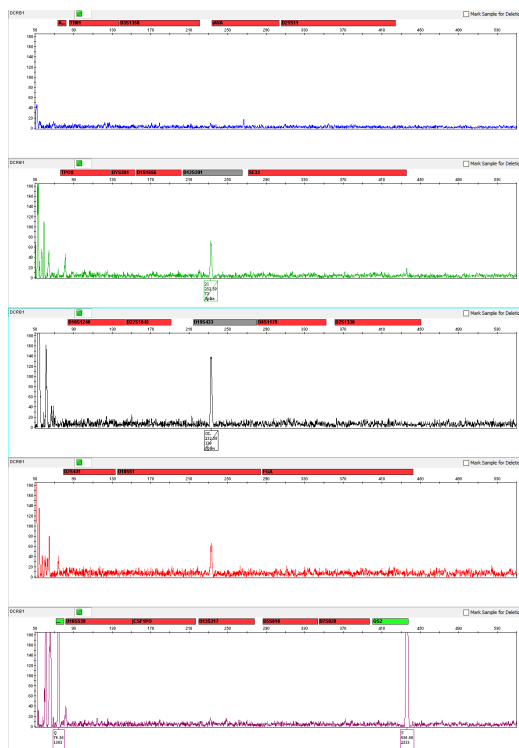
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Conclusion:

No DNA peaks were consistently observed below an input amount of 0.001ng. Subset of zero quant RB's that quanted zero were amplified with no peaks present.

From these results, we conclude that the QuantStudio 5 and Qiagen Quantiplex Quantification Kit:

- 1) Is unlikely to get a usable DNA profile from results below .001ng/ul or less
- 2) Was unable to obtain any peaks or usable DNA profiles from results lower than .0015ng/ul or less including No Result or Zero quants
 - a. As a result of this and out of an abundance of caution, the laboratory will stop processing (Stop at Quant, SAQ) when samples yield a No Result or Zero quant

9. Validation Personnel

(upon successful completion of a competency test, integral individuals are deemed to have completed training in the below listed components)

The following individuals were performed the integral to the validation and have met all training requirements as a result of this validation:

Alyssa McElreath
Derek Cutler
Daniel Hellwig

10. Validation Components

Quant Studio 5 (SN: 272523070)



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Qiagen Quantiplex Pro Quantification Kit