WHITE PAPER

Modifications to TaqMan® Universal PCR Master Mix, No AmpErase® UNG, have no effect on functional performance or stability

Abstract

Beginning in April 2014, minor modifications will be made to the TaqMan® Universal PCR Master Mix and TaqMan® Universal PCR Master Mix, No AmpErase® UNG. These modifications include transfer of manufacturing from Branchburg, NJ to Warrington, UK and changes in source/vendor for some raw material components. In recognition of the importance of these products in existing protocols, we have conducted extensive studies to demonstrate functional equivalency. These changes have been shown to have no effect on the overall functional characteristics of the master mixes. This paper describes the testing process and test results for dynamic range, sensitivity, specificity, discrimination, C_t and ΔRn comparisons, genotyping, and pre- and post-PCR stability. The results show no differences between the current TaqMan® Universal PCR Master Mix manufactured in Branchburg, NJ and validation lots manufactured in Warrington, UK with the described changes.

Introduction

As part of the ongoing efforts at Life Technologies to provide the highest quality qPCR master mixes, modifications will be made to the TaqMan® Universal PCR Master Mix and TaqMan® Universal PCR Master Mix, No AmpErase® UNG, beginning in April 2014. The impacted catalog numbers are listed in the Appendix (Table 6). These modifications include the transfer of manufacturing from Branchburg, NJ (Roche Molecular Systems, third party manufacturer) to Warrington, UK (Thermo Fisher Scientific Inc., formerly Life Technologies). The formulation remains unchanged; however, there is a change in source/vendor for some raw material components such as dNTPs and AmpliTaq Gold® DNA Polymerase.

The execution of the transfer is tightly controlled in a vigilant manner to minimize disruption to the many laboratories that rely on these reagents every day. The most important aspect of this process is to maintain consistent performance and reliability. This paper describes the testing process, involving multiple lots, with a number of gene expression and genotyping assays, to assess dynamic range, sensitivity, specificity, discrimination, and pre- and post-PCR stability. Results show no functional difference between current TaqMan® Universal PCR Master Mix, No AmpErase® UNG manufactured in Branchburg, NJ and validation lots manufactured in Warrington, UK with the implemented changes. A separate document is available describing testing and results for TaqMan® Universal PCR Master Mix.

Materials and methods

Material lots tested

For performance comparisons, three unique lots of TaqMan® Universal PCR Master Mix, No AmpErase® UNG made in Branchburg, NJ (designated R1, R2, and R3) were ordered in 50 mL kits directly from the Life Technologies website.

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Three validation lots (designated L1, L2, and L3) were formulated in full-scale volumes at the Warrington, UK manufacturing site.

Validation material QC

Validation lots passed analytical QC specifications set for Mg^{2+} concentration (HPIC), dNTP concentrations (HPLC), DNase/RNase activity, *E. coli* contamination, and pH. They also passed functional tests with RNase P and ß-actin gene expression assays.

Functional performance

Dynamic range

Four TaqMan® Gene Expression Assays (FAM™-MGB, 20X) were tested across a 5-log concentration dynamic range: FN1 (Hs00277509_m1), PGK1 (Hs99999906_m1), RPLP0 (Hs99999902_m1), and B2M (Hs00187842_m1). The serial dilution spanned final concentrations of 100 ng to 1 pg of cDNA per reaction. The cDNA template was synthesized from Universal Human RNA (Stratagene) and the SuperScript® VILO™ cDNA Synthesis Kit (Cat. No. 11754250). Additionally, duplex performance was tested with B2M and an exogenous internal positive control (IPC) (VIC®-TAMRA™ probe; Cat. No. 4308323). Reactions were run in the 384-well format on a ViiA™ 7 Real-Time PCR System using universal cycling conditions (95°C, 10 min; 95°C, 15 sec; and 60°C, 1 min for 40 cycles). Each reaction was tested with six technical replicates and was further repeated across three PCR runs. ViiA™ 7 software v1.2.2 was used to generate amplification plots and determine C_t values (analysis settings: auto-baseline; threshold set at 0.1).

Gene expression panel

138 TaqMan® Gene Expression Assays (FAM™-MGB, 20X) and six TaqMan® endogenous controls were functionally tested. Refer to Table 4 in the Appendix for assay information. The six endogenous controls were primer-limited (150 nM instead of 900 nM) and had VIC®-TAMRA™ probes. Reactions followed the standard product protocol for a 10 µL reaction volume. 1 ng of cDNA synthesized from Universal Human RNA (Stratagene) and the SuperScript® VILO™ cDNA Synthesis Kit was used as the final template amount for all reactions. Reactions were run in the 384-well format on a ViiA™ 7 Real-Time PCR System using universal cycling conditions (95°C, 10 min; 95°C, 15 sec; and 60°C, 1 min for 40 cycles). Each reaction was tested with six technical replicates. ViiA™ 7 software v1.2.2 was used to generate amplification plots and determine C_t values (analysis settings: auto-baseline; threshold: 0.1).

Pre-PCR stability

The 144-assay gene expression panel was also tested for pre-PCR stability of 24 and 72 hours. Reactions were assembled as described above and the sealed 384-well plates were stored on the benchtop at room temperature, exposed to intermittent light before being run on the ViiA™ 7 Real-Time PCR System. Thermal cycling conditions and analysis settings were identical to those previously described.

Specificity

The 144-assay gene expression panel was also tested for specificity by running "no-template control" (NTC) reactions. Reactions were assembled as described above, except that water replaced the sample volume. Thermal cycling conditions and analysis settings were identical to those previously described.

Sensitivity

The RNase P assay from the TaqMan® RNase P Detection Reagents Kit (Cat. No. 4316831) was tested with CEPH gDNA (Cat. No. 403062) diluted to a final concentration of 2 copies per 20 µL reaction. Reactions were run in the 384-well format on a ViiA™ 7 Real-Time PCR System using universal cycling conditions (95°C, 10 min; 95°C, 15 sec; and 60°C, 1 min for 40 cycles). Each reaction was tested with 20 technical replicates to overcome sampling error at the low concentration. NTC reactions were tested with six technical replicates. Each set of replicates was further repeated across three PCR runs. ViiA™ 7 software v1.2.2 was used to determine C_t values (analysis settings: auto-baseline; threshold set at 0.1).

Discrimination

The RNase P assay from the TaqMan® RNase P Detection Reagents Kit (Cat. No. 4316831) was tested with CEPH gDNA (Cat. No. 403062) diluted to final concentrations of either 1,600 or 800 copies per 20 µL reaction. Reactions were run in the 384-well format on a ViiA™ 7 Real-Time

PCR System using universal cycling conditions (95°C, 10 min; 95°C, 15 sec; and 60°C, 1 min for 40 cycles). Each reaction was tested with 10 technical replicates. NTC reactions were tested with six technical replicates. Each set of replicates was further repeated across three PCR runs. ViiA™ 7 software v1.2.2 was used to determine C_t values (analysis settings: auto-baseline; threshold set at 0.1).

Genotyping panel

20 TaqMan® Drug Metabolism Genotyping Assays (4362691) and 28 TaqMan® SNP Genotyping Assays (4351379) were tested against a panel of 22 purified gDNA samples diluted to a final concentration of 1 ng per 5 µL reaction. Refer to Table 5 in the Appendix for assay information. Reactions were run in the 384-well format: standard thermal cycling on a GeneAmp® PCR System 9700 Dual 384-well, and post-read on a ViiA™ 7 Real-Time PCR System. Each reaction was tested with four technical replicates. ViiA™ 7 software run files were imported into TaqMan® Genotyper™ Software v1.3 for auto-calling analysis.

Post-PCR stability

The 48-assay genotyping panel was also tested for post-PCR stability over 24 and 72 hours. After PCR cycling and post-reads described in the previous section, plates were stored in the dark at room temperature. At the 24- and 72-hour time points, each plate was post-read again and results were compared with time 0.

Results

Dynamic range

With each of the four TaqMan® Gene Expression Assays—FN1, PGK1, RPLP0, and B2M—no difference was observed between master mix lots for the mean C_t values calculated from the 18 data points at each dilution point. Three validation lots (L1–L3) were compared against three lots of the current product (R1–R3).

The following graphs depict the mean C_t vs. log concentration for the four tested assays (Figures 1A–4A) and a representation of the amplification plots for each assay (Figures 1B–4B), showing strong linearity (R^2 values ⩾ 0.999), tight clustering across the series, and clean amplification curves.

Figures 5A and 5B depict the dilution plot and amplification curve, respectively, of the duplex B2M assay with IPC, once again displaying strong linearity (R^2 ⩾ 0.999) and amplification.

PCR efficiency values were determined for the three tested lots using the slope of the mean C_t values for each of the four assays and the duplex assay (see Figure 6). Efficiencies across lots were consistent (<2% difference) for each tested assay.

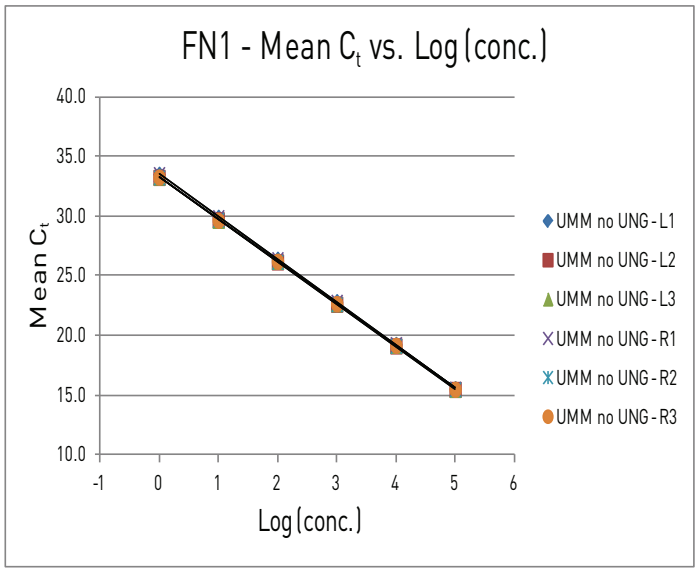


Figure 1A. FN1 (Hs00277509_m1) mean C_t values plotted across 5 logs of cDNA sample for all six lots of Universal PCR Master Mix, No AmpErase® UNG.

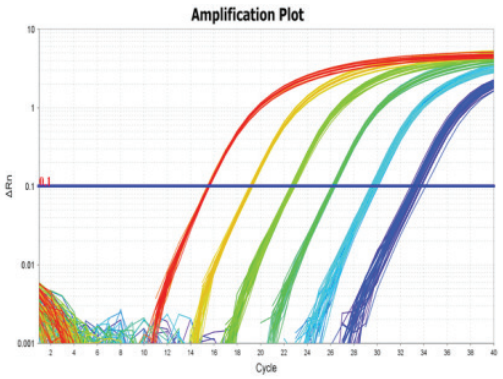


Figure 1B. PCR amplification plot overlaying technical replicates from all six master mix lots at each concentration point.

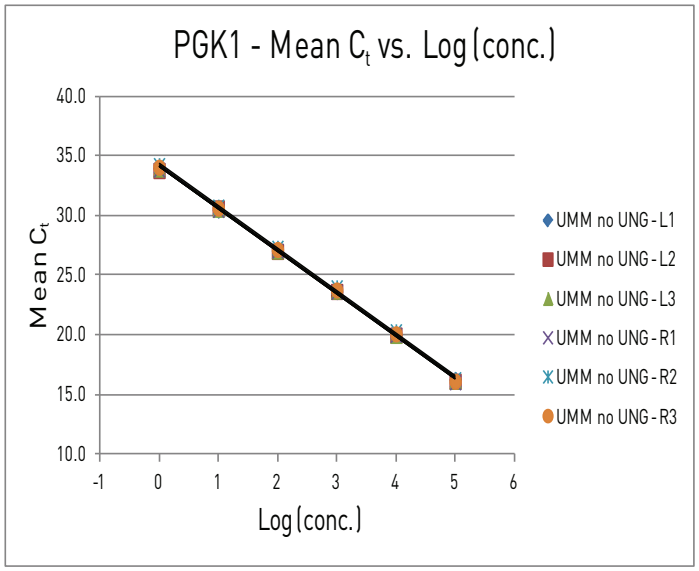


Figure 2A. PGK1 (Hs99999906_m1) mean C_t values plotted across 5 logs of cDNA sample for all six lots of Universal PCR Master Mix, No AmpErase® UNG.

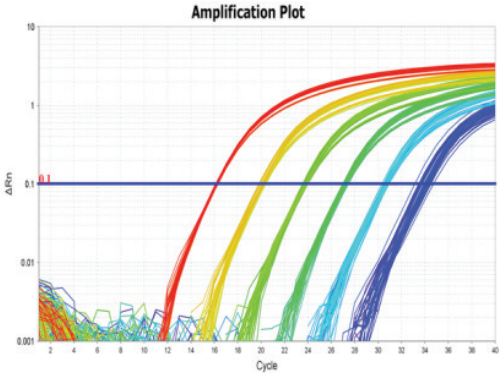


Figure 2B. PCR amplification plot overlaying technical replicates from all six master mix lots at each concentration point.

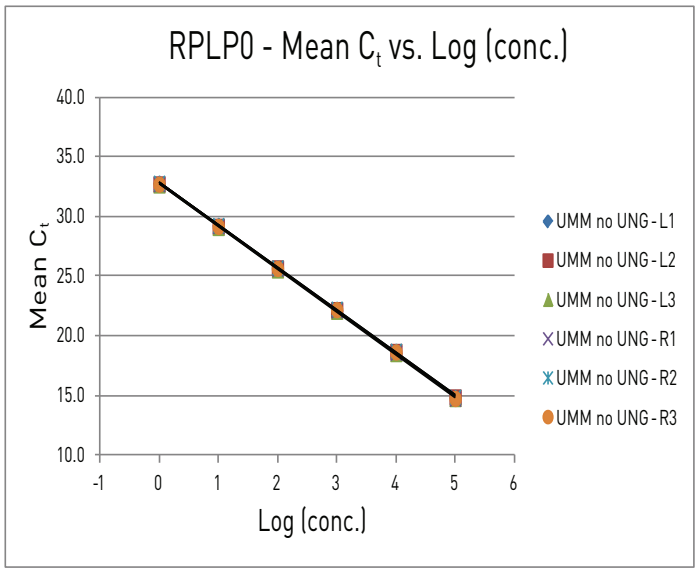


Figure 3A. RPLP0 (Hs99999902_m1) mean C_t values plotted across 5 logs of cDNA sample for all six lots of Universal PCR Master Mix, No AmpErase® UNG.

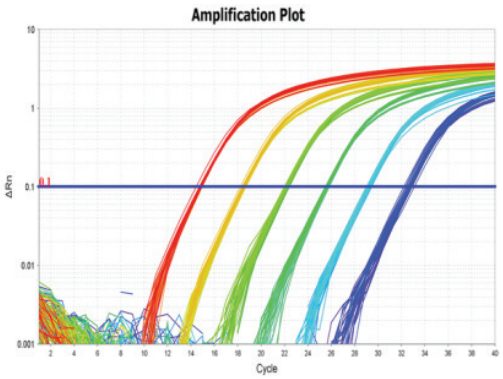


Figure 3B. PCR amplification plot overlaying technical replicates from all six master mix lots at each concentration point.

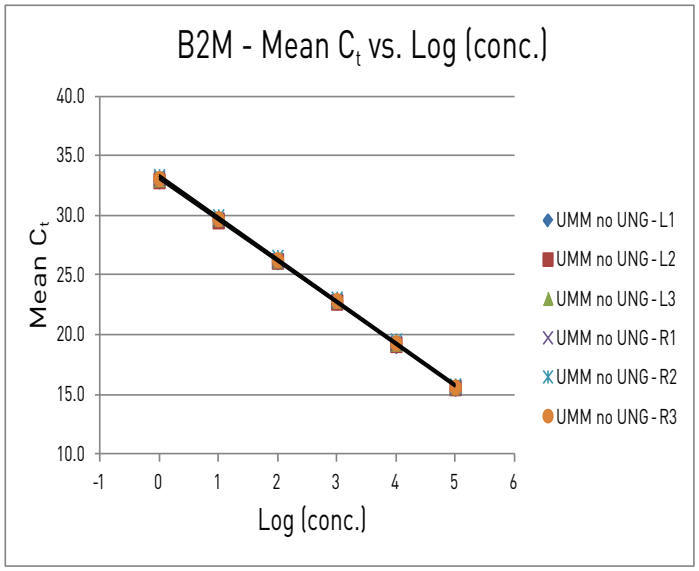


Figure 4A. B2M (Hs00363670_m1) mean C_t values plotted across 5 logs of cDNA sample for all six lots of Universal PCR Master Mix, No AmpErase® UNG.

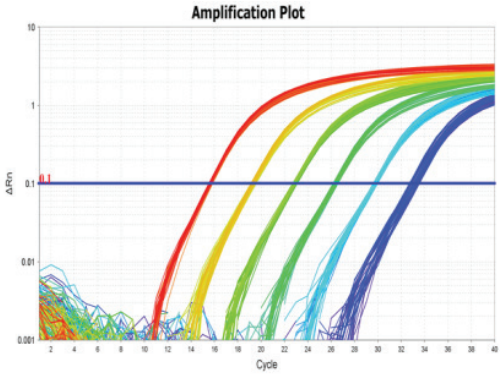


Figure 4B. PCR amplification plot overlaying technical replicates from all six master mix lots at each concentration point.

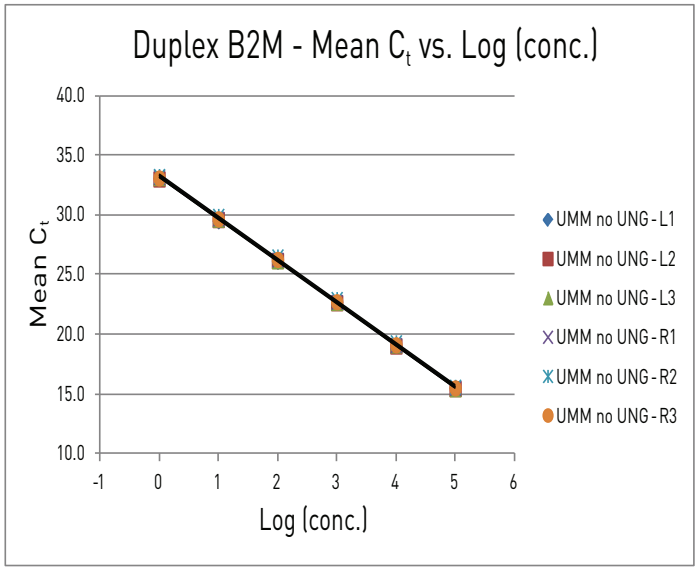


Figure 5A. B2M (Hs00363670_m1) mean C_t values when duplexed with an exogenous IPC (4308323); plotted across 5 logs of cDNA sample for all six lots of Universal PCR Master Mix, No AmpErase® UNG.

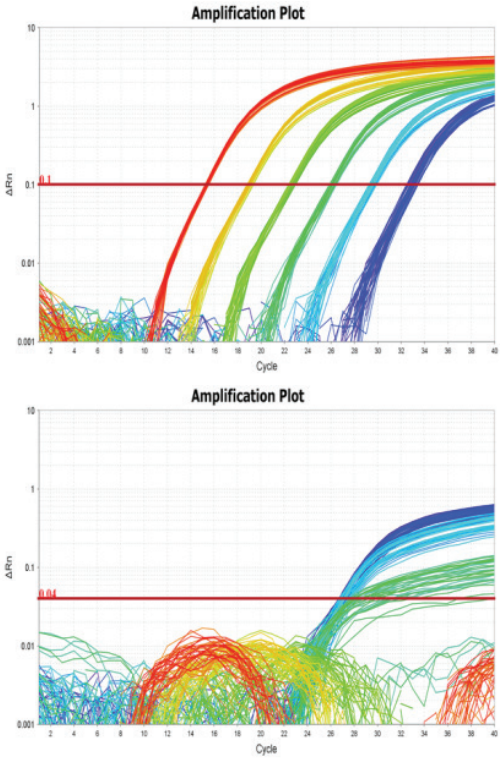


Figure 5B. PCR amplification plot overlaying technical replicates from all six master mix lots at each concentration point and for the exogenous IPC (bottom). Note that at high concentrations the B2M assay out-competes the exogenous IPC, accounting for the observed variability in the IPC amplification plot.

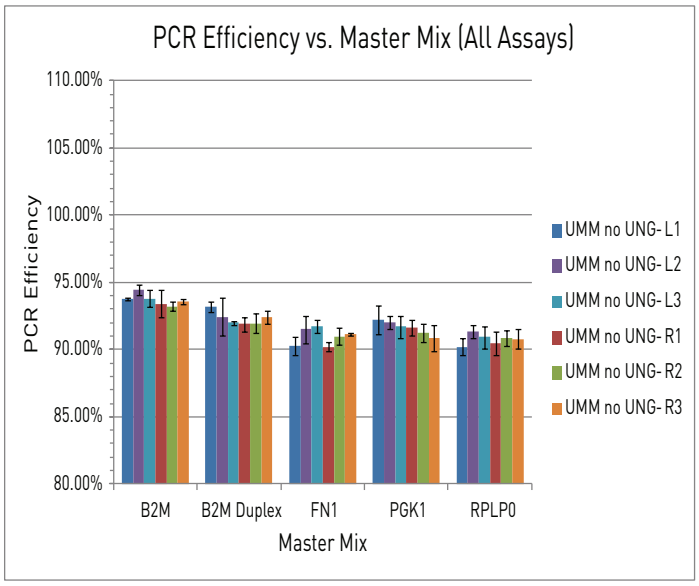


Figure 6. PCR efficiency values for each gene expression assay, calculated from the slope of the mean C_t vs.log(conc.) plot. Each assay exhibited consistent efficiencies between lots of Universal PCR Master Mix, No AmpErase® UNG (differences <2%).

Gene expression panel

Performance in gene expression assays was tested across the six lots using 138 TaqMan® Gene Expression Assays and six endogenous control assays with average C_t values calculated from six technical replicates. The plots in Figure 7 show clear consistency of C_t values (average difference: <0.4%) within each assay across the six lots from low-, medium-, and high-expressing genes.

Figure 8 depicts the mean ΔRn values for the same 144 TaqMan® Gene Expression Assays. No real differences are observed, with 99% of assays showing between-lot differences of <20% (calculated with the mean ΔRn of each population for each assay).

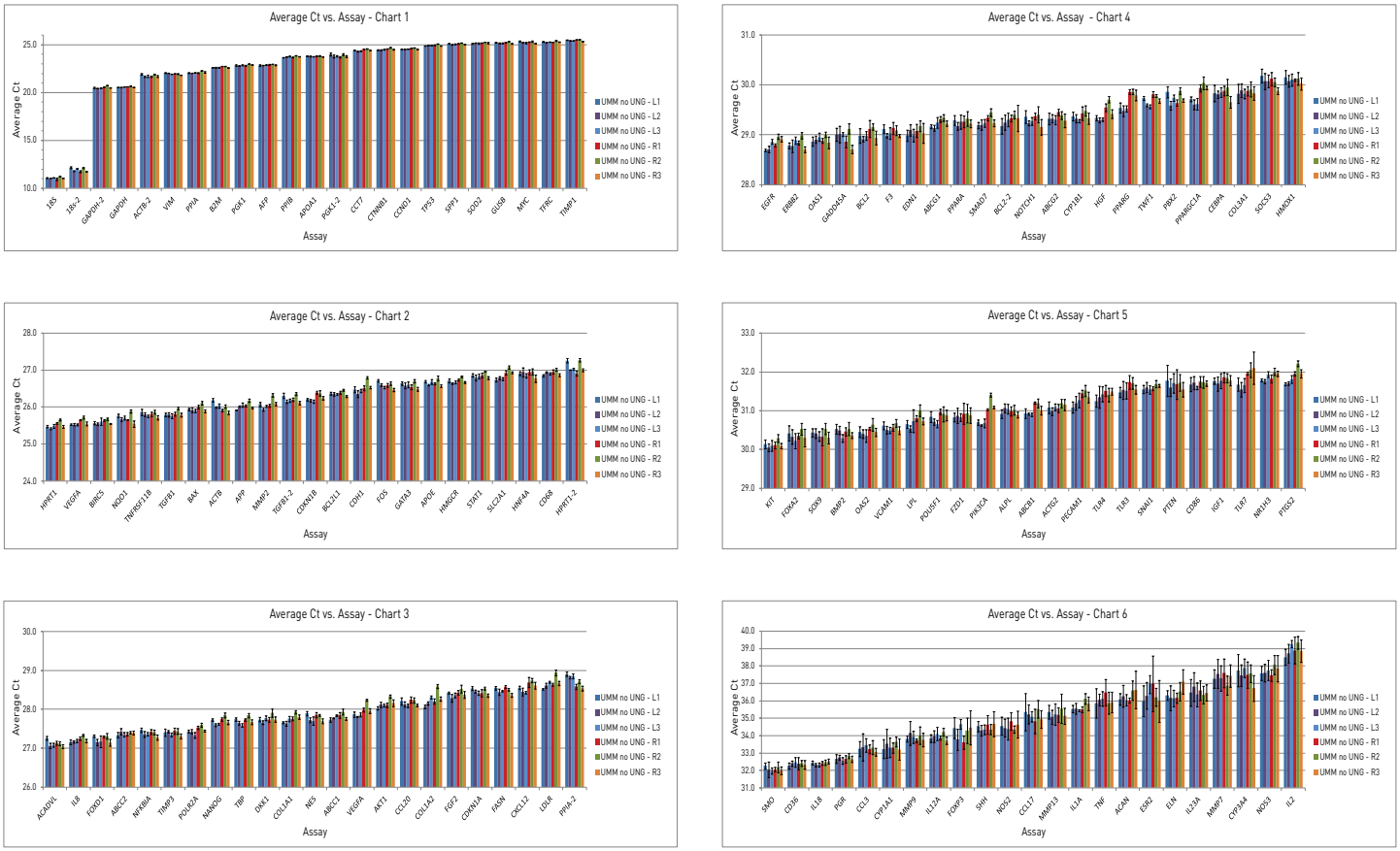


Figure 7. Mean C_t values obtained from each master mix (lots L1–L3 of Universal Master Mix and current lots R1–R3 of Universal Master Mix) across all 138 TaqMan® Gene Expression Assays and 6 endogenous control assays. Six assays were excluded because of no amplification with any lot groups: ADIPOQ, CYP2B1, CYP2C9, IL12B, FABP4, and FASLG. Six technical replicates per assay were run with each master mix on the same PCR plate (n = 6; error bars are ±1σ).

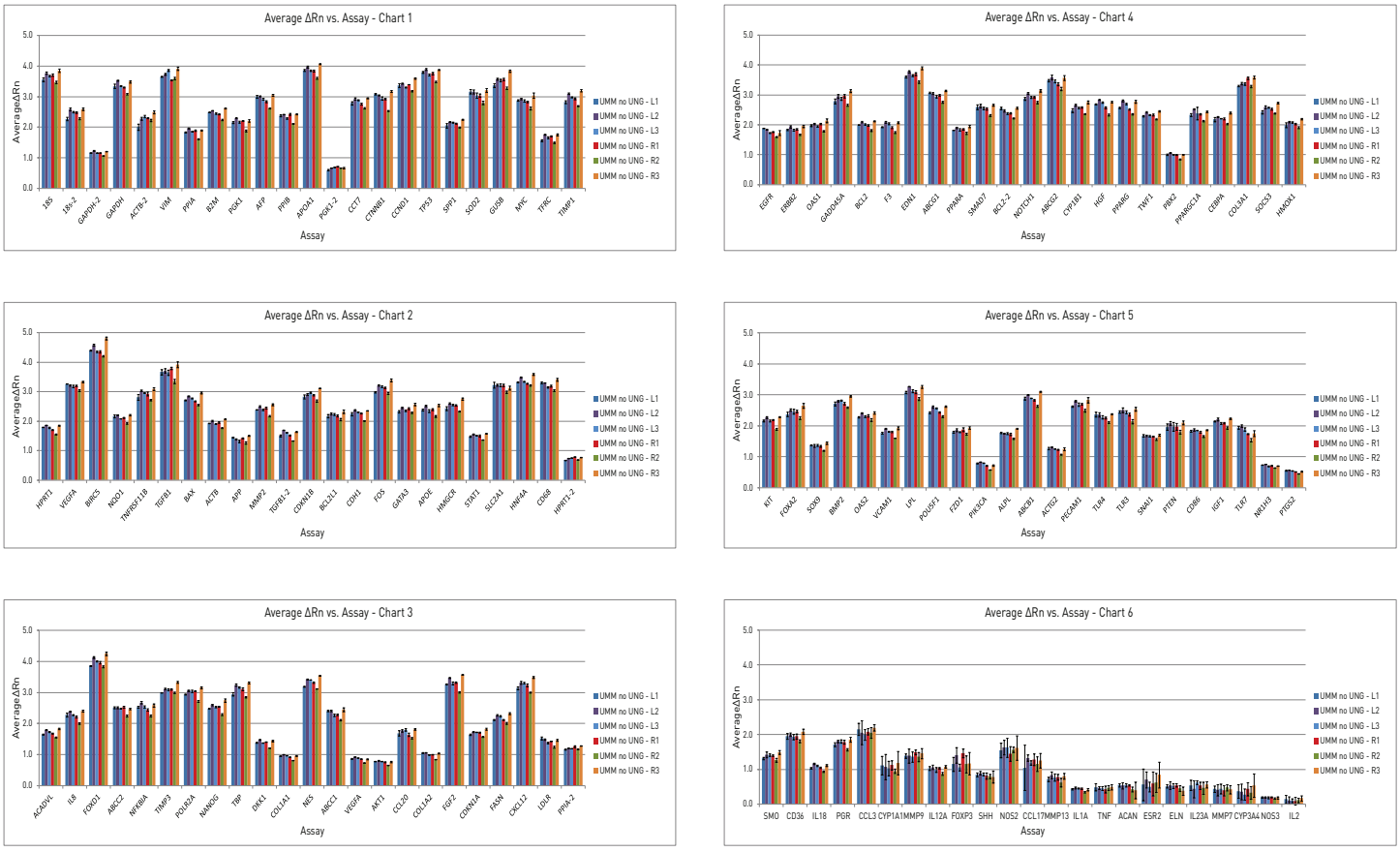
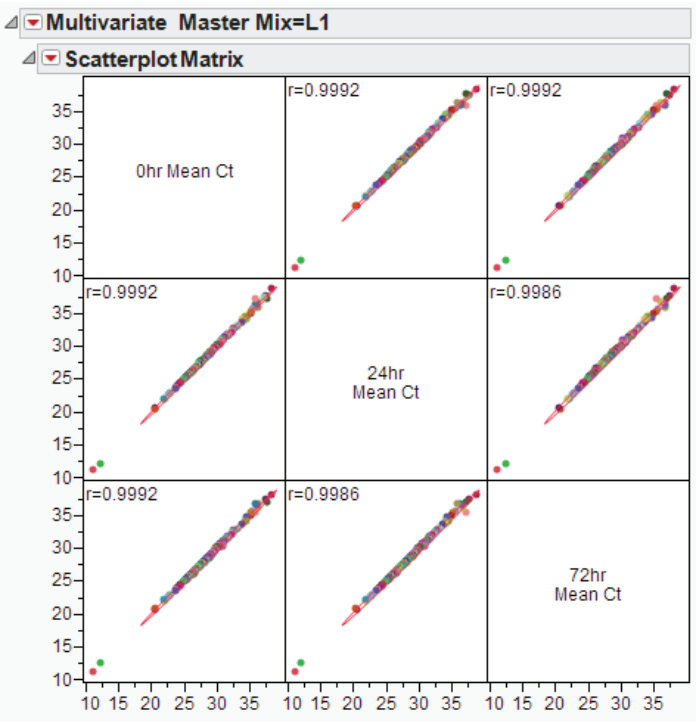


Figure 8. Mean ΔRn values obtained from each master mix (lots L1–L3 of Universal Master Mix and current lots R1–R3 of Universal Master Mix) across all 138 TaqMan® Gene Expression Assays and 6 endogenous control assays. Six assays were excluded because of no amplification with any lot groups: ADIPOQ, CYP2B1, CYP2C9, IL12B, FABP4, and FASLG. Six technical replicates per assay were run with each master mix on the same PCR plate (n = 6; error bars are ±1σ).

Pre-PCR stability

Extensive stability testing was performed on all 144 assays used for the performance testing above. Assembled reaction plates were sealed and left at room temperature for 24 and 72 hours, and all results calculated and collated. Figure 9 is a representation from a single validation lot (left) and current lot (right), showing mean C_t values for each assay with clear correlation across time points (Pearson's value correlation scores were ≥0.997 for validation lots and current lots). Similarly, Figure 10 shows data for mean ΔRn values for the same two lots (Pearson's r-value correlation scores were ≥0.98 for both validation lots and current lots).



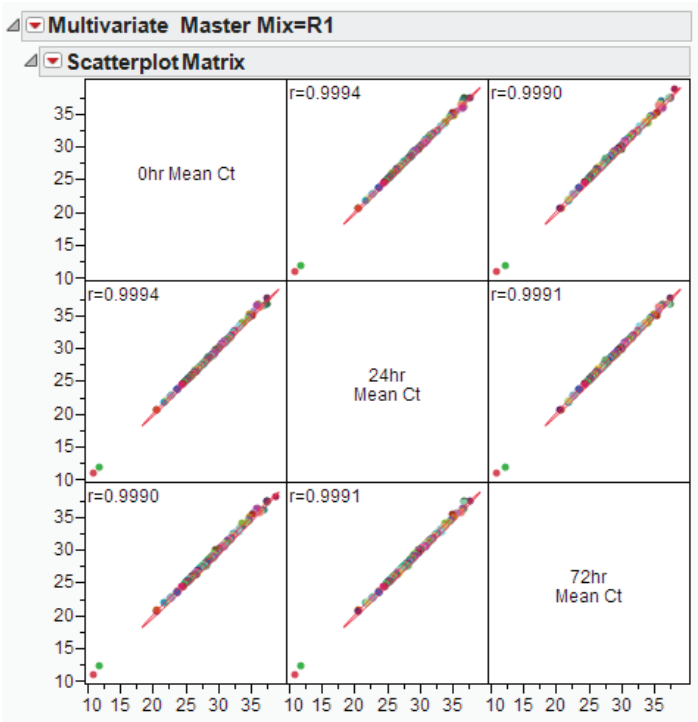
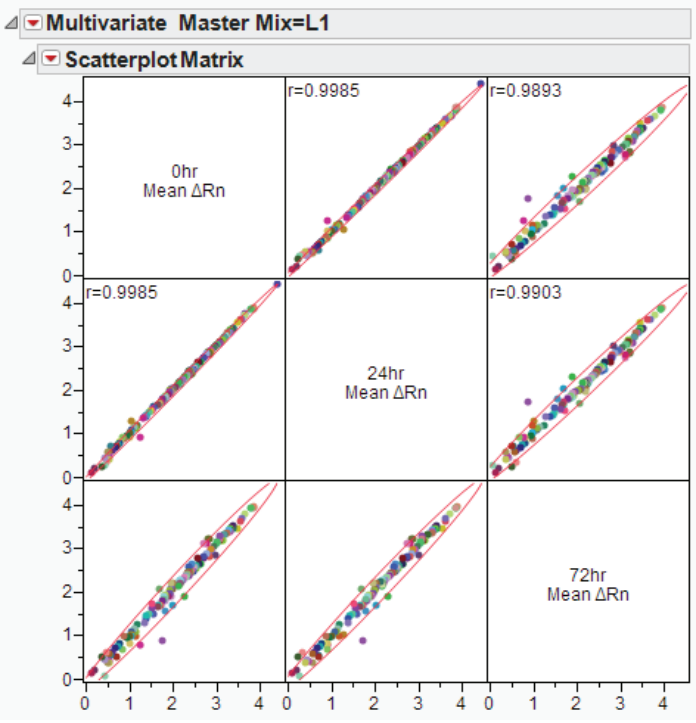


Figure 9. Mean C_t values compared between benchtop stability time points of 0, 24, and 72 hours (JMP 10, SAS Inc.). Each point represents a single assay out of the 144 gene expression assay panel (138 included). Data are displayed for validation lot L1 (left) and a representative lot R1 of current UMM (right). Correlation values were ≥0.998, with red oval boundaries representing the 95% confidence curves (α = 0.05).



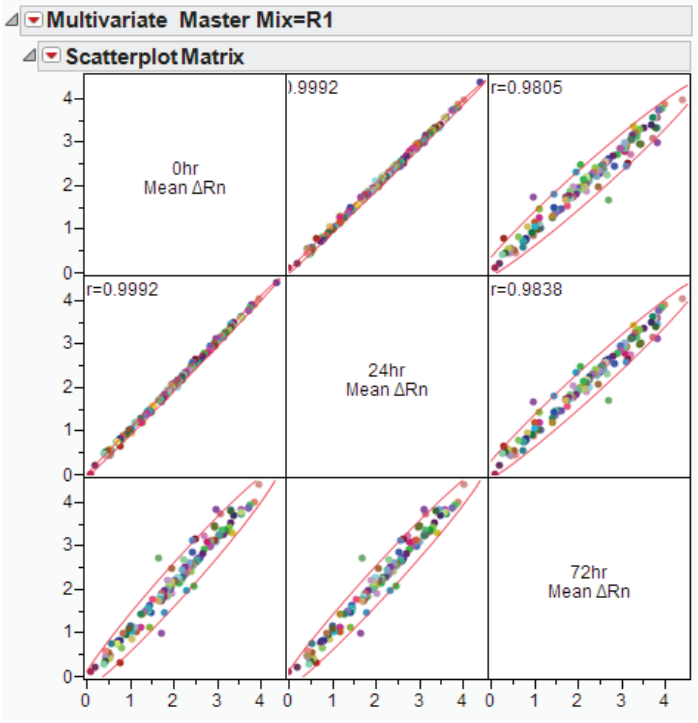


Figure 10. Mean ΔRn values compared between benchtop stability time points of 0, 24, and 72-hours (JMP 10, SAS Inc.). Each point represents a single assay out of the 144 gene expression assay panel (138 included). Data is displayed from validation UMM L1 (left) and a representative lot R1 of current UMM (right). Correlation values were ≥ 0.98 with red oval boundaries representing the 95% confidence curves (α = 0.05).

Specificity

To test performance with regards to nonspecific amplification, primer-dimer formation, and other specificity concerns, the same 144 TaqMan® Gene Expression Assays were run using water in place of template. Greater than 98% of NTC reactions across all assays exhibited no amplification. Table 1 shows the NTC amplification percentage statistics (C_t values <40) for the current and validation lots, with no statistical difference (p = 0.671) observed between the two populations.

Sensitivity

Sensitivity performance was tested using the TaqMan® RNase P assay on CEPH gDNA diluted to a 2-copy final dilution. Figure 11 shows distribution graphs for a total of 60 data points (collected across three runs of 20 replicates) for a single, representative master mix lot. Fitted normal distribution curves are shown in red.

NTC Amplification Percentage.

	Mean	St. Dev.	95% Conf.	Interval
Validation	1.04%	0.00%	–	–
Current	98%	0.20%	0.76%	1.21%

Table 1. Percentage of NTC reactions containing positive amplification (nonspecific amplification) vs. master mix (three validation lots (n = 3) and three current lots (n = 3)). All 144 gene expression assays were run with four technical replicates each (total n = 576), and the percentage was calculated for each master mix lot. Positive amplifications were largely from both 18S assays (Hs99999901_s1; 4310893E).

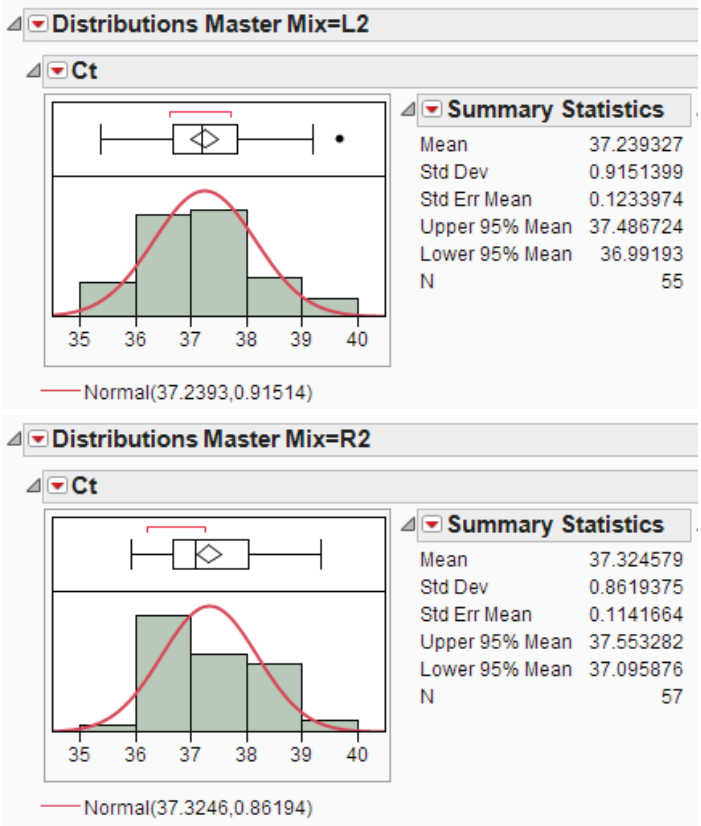


Figure 11. Distribution of RNase P C_t values obtained from 2 copies/rxn of CEPH gDNA. Data from three PCR setups were combined (20 technical replicates each, total n = 60). Data are displayed using the JMP 10 Statistical Software package (SAS Institute Inc.) for a single, representative master mix lot. Reactions that did not amplify were considered to have 0 copies of CEPH gDNA, a result of sampling error near the digital concentration range.

Discrimination

Two-fold discrimination performance was tested using the TaqMan® RNase P assay on CEPH gDNA diluted to either 1,600 or 800 copies per reaction. Figure 12 shows distribution graphs for a total of 30 data points (collected across three runs of 10 replicates) for a single, representative master mix lot. Fitted normal distribution curves are shown in red for each mean copy number. Mean C_t values for the two concentrations were separated by >6 SD, providing sufficient separation for 2-fold copy number resolution.

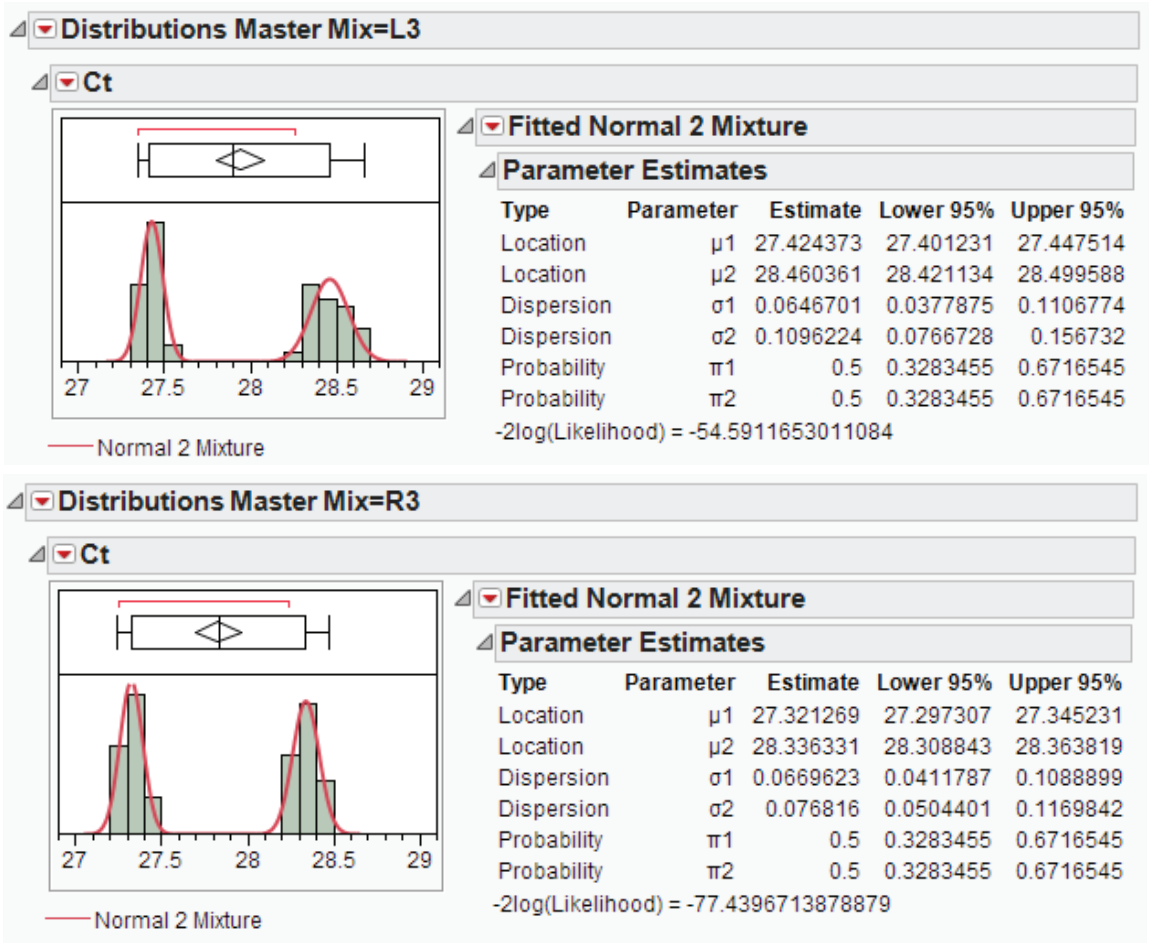


Figure 12. Distributions of RNase P C_t values obtained from 1,600 and 800 copies/rxn of CEPH gDNA. Data from three PCR setups were combined (10 technical replicates for each concentration, total n = 30). Data are displayed using the JMP 10 Statistical Software package (SAS Institute Inc.) for a single, representative master mix lot.

Genotyping panel

Functional performance in genotyping was compared between validation and current lots. A total of 48 TaqMan® Genotyping Assays were used across 22 gDNA samples to assess accuracy, clustering, and separation. All assays performed equally well, and data are displayed from a representation of assays (Figure 13). Cluster plots for all lots were plotted together, showing good clusters with similar profiles. Validation lots and current lots have similar placement, grouping, and separation; no significant differences are seen between the lot populations, and there were no differences in the genotyping calls displayed below.

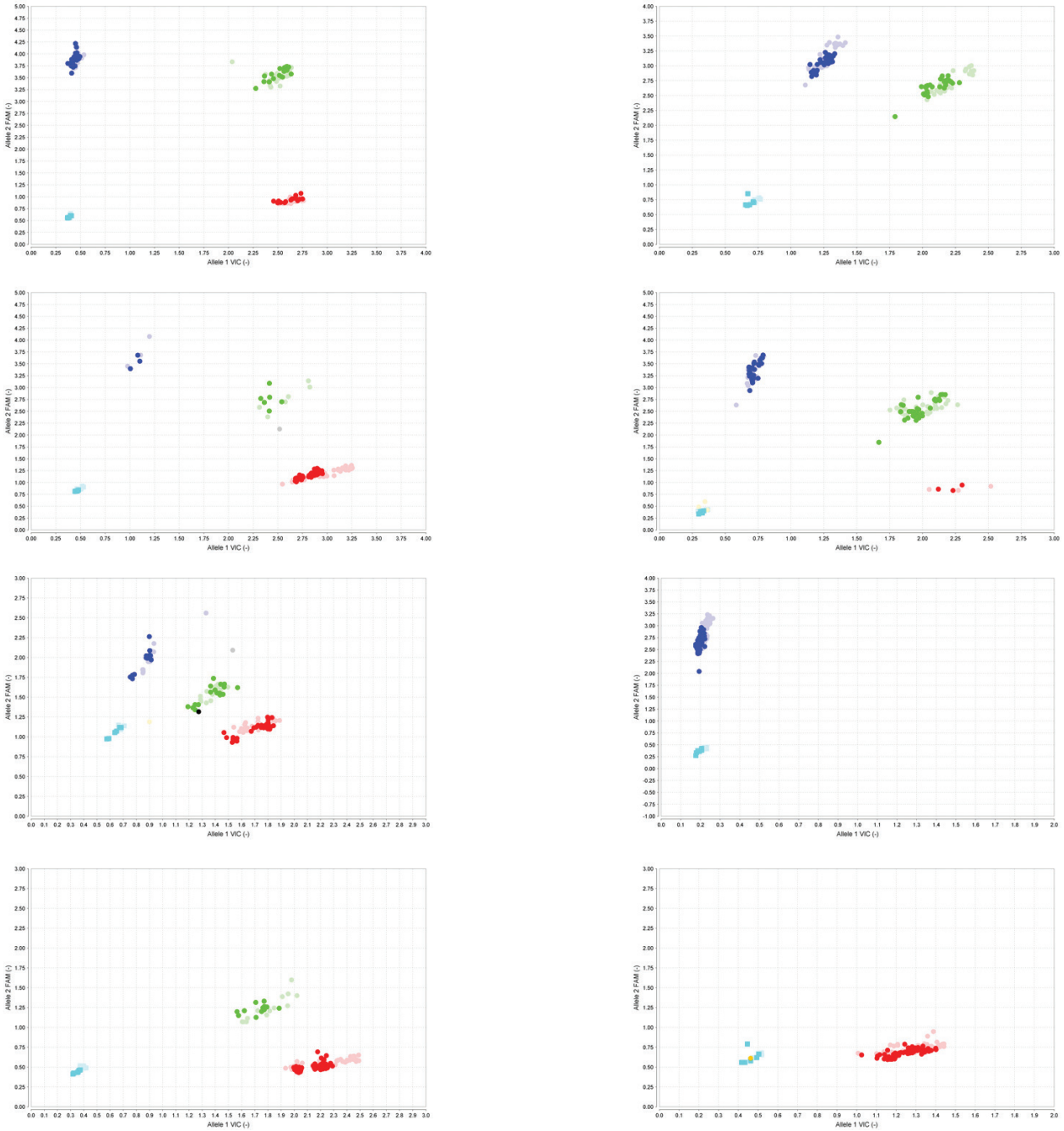


Figure 13. Genotyping plots for 8 assays run on the GeneAmp® PCR System 9700 Dual 384-well and post-read on the ViiA™ 7 Real-Time PCR System, representing the entire panel of 48 assays. Data from the three validation lots of Universal PCR Master Mix, No AmpErase® UNG, are shown in bold colors; data from the three current lots are in faded colors. Validation and current lots both clustered samples consistently. Orange dots represent poor/no amplification for a single sample, which occurred randomly for both validation and current lots, likely a result of pipetting small volumes (5 µL).

Post-PCR stability

To determine post-PCR stability, the same plates used for the genotyping analysis were post-read again after 24 and 72 hours of room temperature storage. Plotted values represent average concordance values across all 48 assays. Comparisons were made within validation and current lots, in addition to between the two populations. A significant difference in the concordance percentage of genotyping calls was detected in three cases, although actual concordance percentage differences were <3.2% (see Table 2 below for p-values).

Concordance percentage p-values.

0 hr		
	Validation:Current	Current:Current
Validation:Validation	1.0000	0.8637
Validation:Current	–	0.8037
24 hr		
	Validation:Current	Current:Current
Validation:Validation	0.0001*	0.2411
Validation:Current	–	0.0009*
72 hr		
	Validation:Current	Current:Current
Validation:Validation	0.2053	0.0315*
Validation:Current	–	0.2217

Table 2. Calculated p-values (All Pairs, Tukey-Kramer HSD test) for concordance values from Figure 13. Statistical differences existed in three cases (denoted with "*"), although actual differences in concordance were <3.2% (calculated using JMP version 10; SAS Inc.)

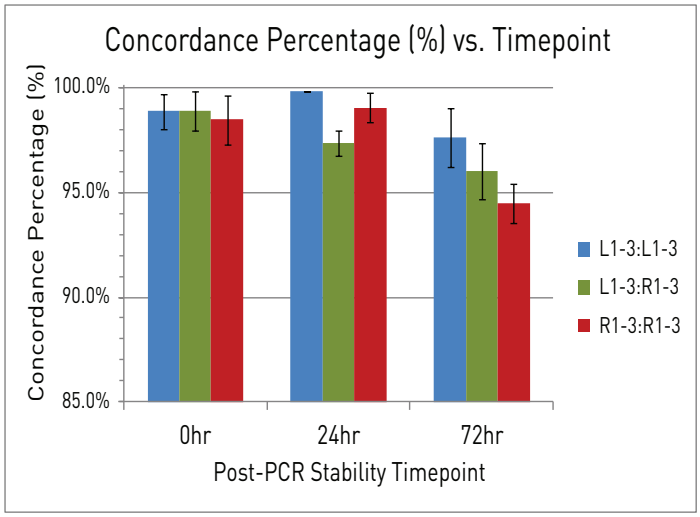


Figure 14. Genotyping call concordance among lots of Universal PCR Master Mix, No AmpErase® UNG. Concordance percentages were calculated for each of the 48 genotyping assays (22 gDNA samples each) and then averaged. Post-PCR reads were taken after reactions were left for 0, 24, and 72 hours at room temperature. Validation lots (L1–L3:L1–L3, n = 3), between populations (L1–L3:R1–R3, n = 9), and current lots (R1–R3:R1–R3, n = 3). Error bars represent ±1σ.

Conclusion

Validation lots incorporating the manufacturing changes were extensively tested against current lots of TaqMan® Universal PCR Master Mix, No AmpErase® UNG to ascertain whether the changes would impact the functionality of the reagents. Tests showed no discernible differences in dynamic range, sensitivity, specificity, discrimination, genotyping, and pre- and post-PCR stability as well as general assay performance for gene expression assays selected to cover a representative range.

We are confident that the TaqMan® Universal PCR Master Mix, No AmpErase® UNG offered after April 2014 will continue to perform with the same quality, integrity, and functional performance as exists today.

Appendix

Table 3. Linear dynamic range assays.

Number	Assay ID	Gene symbol
1	Hs00277509_m1	FN1
2	Hs99999906_m1	PGK1
3	Hs99999902_m1	RPLP0
4	Hs00187842_m1	B2M
Duplex	4308323	Exo IPC

Table 4. Gene expression assays.

Number	Assay ID	Gene symbol
1	Hs99999901_s1	18S
2	Hs00184500_m1	ABCB1
3	Hs00219905_m1	ABCC1
4	Hs00245154_m1	ABCG1
5	Hs00153936_m1	ACAN
6	Hs99999903_m1	ACTB
7	Hs00242273_m1	ACTG2
8	Hs00173490_m1	AFP
9	Hs00178289_m1	AKT1
10	Hs00163641_m1	APOA1
11	Hs00171168_m1	APOE
12	Hs99999907_m1	B2M
13	Hs00180269_m1	BAX
14	Hs00153353_m1	BIRC5
15	Hs00277039_m1	CCND1
16	Hs00154355_m1	CD68
17	Hs00170423_m1	CDH1
18	Hs00355782_m1	CDKN1A
19	Hs00153277_m1	CDKN1B
20	Hs00269972_s1	CEBPA
21	Hs00164004_m1	COL1A1
22	Hs00171022_m1	CXCL12
23	Hs00164383_m1	CYP1B1
24	Hs00604506_m1	CYP3A4
25	Hs00183740_m1	DKK1
26	Hs00174961_m1	EDN1
27	Hs00230957_m1	ESR2
28	Hs00266645_m1	FGF2
29	Hs00170630_m1	FOS
30	Hs00232764_m1	FOXA2
31	Hs00203958_m1	FOXP3
32	Hs00268943_s1	FZD1
33	Hs00169255_m1	GADD45A
34	Hs99999908_m1	GUSB
35	Hs00168352_m1	HMGCR
36	Hs00157965_m1	HMOX1

Gene expression assays, *continued*

Number	Assay ID	Gene symbol
37	Hs00168405_m1	IL12A
38	Hs00155517_m1	IL18
39	Hs00174092_m1	IL1A
40	Hs00174103_m1	IL8
41	Hs00174029_m1	KIT
42	Hs00234422_m1	MMP2
43	Hs00159163_m1	MMP7
44	Hs02387400_g1	NANOG
45	Hs00707120_s1	NES
46	Hs00167166_m1	NOS3
47	Hs00242943_m1	OAS1
48	Hs00855025_s1	PBX2
49	Hs99999906_m1	PGK1
50	Hs00172183_m1	PGR
51	Hs00180679_m1	PIK3CA
52	Hs00172187_m1	POLR2A
53	Hs00742896_s1	POU5F1
54	Hs00173304_m1	PPARGC1A
55	Hs99999904_m1	PPIA
56	Hs00168719_m1	PPIB
57	Hs00197884_m1	SLC2A1
58	Hs00170665_m1	SMO
59	Hs00269575_s1	SOCS3
60	Hs00167093_m1	SPP1
61	Hs00234829_m1	STAT1
62	Hs00427620_m1	TBP
63	Hs99999911_m1	TFRC
64	Hs00171257_m1	TGFB1
65	Hs99999918_m1	TGFB1
66	Hs00171558_m1	TIMP1
67	Hs00174128_m1	TNF
68	Hs00171068_m1	TNFRSF11B
69	Hs00173626_m1	VEGFA
70*	4310893E	18s-2
71*	4310881E	ACTB-2
72*	4310884E	GAPDH-2
73	Hs00166123_m1	ABCC2
74	Hs00184979_m1	ABCG2
75	Hs00817723_g1	ACADVL
76	Hs00605917_m1	ADIPOQ
77	Hs00758162_m1	ALPL
78	Hs00169098_m1	APP
79	Hs00153350_m1	BCL2
80	Hs00608023_m1	BCL2
81	Hs00236329_m1	BCL2L1
82	Hs00154192_m1	BMP2
83	Hs00171074_m1	CCL17
84	Hs00171125_m1	CCL20

Gene expression assays, *continued*

Number	Assay ID	Gene symbol
85	Hs00234142_m1	CCL3
86	Hs00362446_m1	CCT7
87	Hs00169627_m1	CD36
88	Hs00199349_m1	CD86
89	Hs00164099_m1	COL1A2
90	Hs00164103_m1	COL3A1
91	Hs00170025_m1	CTNNB1
92	Hs00153120_m1	CYP1A1
93	Hs00167937_g1	CYP2B6
94	Hs00426397_m1	CYP2C9
95	Hs00193306_m1	EGFR
96	Hs00355783_m1	ELN
97	Hs00170433_m1	ERBB2
98	Hs00175225_m1	F3
99	Hs00609791_m1	FABP4
100	Hs00181225_m1	FASLG
101	Hs00188012_m1	FASN
102	Hs00270117_s1	FOXD1
103	Hs02758991_g1	GAPDH
104	Hs00231122_m1	GATA3
105	Hs00300159_m1	HGF
106	Hs00230853_m1	HNF4A
107	Hs99999909_m1	HPRT1
108	Hs00153126_m1	IGF1
109	Hs00233688_m1	IL12B
110	Hs00174114_m1	IL2
111	Hs00372324_m1	IL23A
112	Hs00181192_m1	LDLR
113	Hs00173425_m1	LPL
114	Hs00233992_m1	MMP13
115	Hs00234579_m1	MMP9
116	Hs00153408_m1	MYC
117	Hs00153283_m1	NFKBIA
118	Hs00167248_m1	NOS2
119	Hs00413187_m1	NOTCH1
120	Hs00168547_m1	NQO1
121	Hs00172885_m1	NR1H3
122	Hs00159719_m1	OAS2
123	Hs00169777_m1	PECAM1
124	Hs00231882_m1	PPARA
125	Hs00234592_m1	PPARG
126	Hs00829813_s1	PTEN
127	Hs00153133_m1	PTGS2
128	Hs00179843_m1	SHH
129	Hs00178696_m1	SMAD7
130	Hs00195591_m1	SNAI1
131	Hs00167309_m1	SOD2
132	Hs00165814_m1	SOX9

Gene expression assays, *continued*

Number	Assay ID	Gene symbol
133	Hs00165949_m1	TIMP3
134	Hs00152933_m1	TLR3
135	Hs00152939_m1	TLR4
136	Hs00152971_m1	TLR7
137	Hs00153340_m1	TP53
138	Hs00702289_s1	TWF1
139	Hs00365486_m1	VCAM1
140	Hs00900054_m1	VEGFA
141	Hs00185584_m1	VIM
142*	4310890E	HPRT1-2
143*	4310885E	PGK1-2
144*	4310883E	PPIA-2

* TaqMan® endogenous control assays; primer-limited (150 nM instead of 900 nM) with VIC™-TAMRA™ probes.

Table 5. Genotyping assays.

Number	Assay ID	Assay type	dbSNP	Gene symbol
1	C___7586657_20	DME	rs1045642	ABCB1
2	C____479128_40	DME	rs3749442	ABCC5
3	C__15854163_70	DME	rs2231142	ABCG2
4	C__25746809_50	DME	rs4680	COMT, ARVCF
5	C___7817765_60	DME	rs3745274	CYP2B6, CYP2A7P1
6	C__27861809_10	DME	rs4986893	CYP2C19
7	C__25625805_10	DME	rs28371674	CYP2C9
8	C__32407232_50	DME	rs35742686	CYP2D6
9	C__11484460_40	DME	rs1065852	CYP2D7P1
10	C__30203950_10	DME	rs10264272	CYP3A5
11	C___3237198_20	DME	rs1695	GSTP1
12	C___1204093_20	DME	rs1801280	NAT2
13	C____572770_20	DME	rs1799931	NAT2
14	C_____19567_20	DME	rs1142345	NHLRC1, TPMT
15	C____263841_20	DME	rs1523127	NR1I2
16	C___2548962_20	DME	rs662	PON1
17	C___1129864_10	DME	rs1801282	PPARG
18	C__30633906_10	DME	rs4149056	SLCO1B1
19	C__30634116_20	DME	rs1800460	TPMT
20	C__12091552_30	DME	rs1800462	TPMT
21	C___2084765_20	Functionally Tested	rs1042714	ADRB2
22	C___1985481_20	Functionally Tested	rs699	AGT
23	C___3084793_20	Functionally Tested	rs429358	APOC1, TOMM40, APOE
24	C__11841860_10	Functionally Tested	rs10757278	CDKN2BAS
25	C___8726802_20	Functionally Tested	rs1799963	CKAP5, F2
26	C___1202883_20	Functionally Tested	rs1801133	CLCN6, C1orf167, MTHFR
27	C___2415786_20	Functionally Tested	rs231775	CTLA4
28	C__30090620_10	Functionally Tested	rs9939609	FTO
29	C___1085595_10	Functionally Tested	rs1800562	HFE
30	C__11748116_10	Functionally Tested	rs4073	IL8
31	C__11717468_20	Functionally Tested	rs2066844	NOD2
32	C___3219460_20	Functionally Tested	rs1799983	NOS3
33	C__29347861_10	Functionally Tested	rs7903146	TCF7L2
34	C__11722238_20	Functionally Tested	rs4986790	TLR4
35	C___8311602_10	Functionally Tested	rs699947	VEGFA
36	C___2403545_10	Functionally Tested	rs1042522	WRAP53, TP53

Genotyping assays, *continued*

Number	Assay ID	Assay type	dbSNP	Gene symbol
37	C__11654065_10	Validated	rs5219	ABCC8, KCNJ11
38	C__11592758_10	Validated	rs6265	BDNFOS, BDNF
39	C___9077561_20	Validated	rs1801274	FCGR2A
40	C__25815666_10	Validated	rs396991	FCGR3A
41	C____245448_10	Validated	rs1959959	FLJ43390
42	C____940460_1_	Validated	rs513349	GGNBP1, BAK1
43	C___1085600_10	Validated	rs1799945	HFE
44	C___1747363_10	Validated	rs1800872	IL10
45	C___7514879_10	Validated	rs1800629	LTA, TNF, LTB
46	C___8950074_1_	Validated	rs1799971	OPRM1
47	C___9384501_10	Validated	rs2960422	PPARG
48	C___8311614_10	Validated	rs2010963	VEGFA

Table 6. Affected catalog numbers.

List of all catalog numbers TaqMan® Universal PCR Master Mix and TaqMan® Universal Master Mix, No AmpErase® UNG impacted by the manufacturing site change.

Product name	Cat. No.
TaqMan® Universal PCR Master Mix, 1-Pack (1 x 5 mL)	4304437
TaqMan® Universal PCR Master Mix, 10-Pack (10 x 5 mL)	4305719
TaqMan® Universal PCR Master Mix, 10 Unit Pack (10 x 5 mL)	4318157
TaqMan® Universal PCR Master Mix, 1 Bulk Pack (1 x 50 mL)	4326708
TaqMan® Universal PCR Master Mix, 2-Pack (2 x 5 mL)	4364338
TaqMan® Universal PCR Master Mix, 5-Pack (5 x 5 mL)	4364340
TaqMan® Universal PCR Master Mix, No AmpErase® UNG, 1-Pack (1 x 5 mL)	4324018
TaqMan® Universal PCR Master Mix, No AmpErase® UNG, 10 Unit (10 x 5 mL)	4324020
TaqMan® Universal PCR Master Mix, No AmpErase® UNG, 1 Bulk Pack (1 x 50 mL)	4326614
TaqMan® Universal PCR Master Mix, No AmpErase® UNG, 2-Pack (2 x 5 mL)	4364341
TaqMan® Universal PCR Master Mix, No AmpErase® UNG, 5-Pack (5 x 5 mL)	4364343