Brilliant III Ultra-Fast QPCR Master Mix Provides Superior Performance with the Roche LightCycler480

Application Note

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Abstract

Brilliant III Master Mix has been shown to provide faster cycling times, higher reproducibility and lower limits of detection (LODs) than the master mixes from twelve competitive suppliers. It enables the scientists at GeneWake GmbH to transform borderline positive results into definitively measurable ones.

Introduction

PCR hardware capable of very fast cycling has been available for a number of years. However, qPCR assay speed has been limited by the time required for the DNA polymerase in the master mix to anneal to the target template and extend the amplicon, making the master mix the limiting factor for very fast cycling times. The Brilliant III Ultra-Fast QPCR and QRT-PCR master mixes are designed to provide the fastest cycling times on very rapid cycling systems such as the Roche LC480 real-time PCR instrument. These ultra-fast reagents give researchers access to their data faster, without compromising data quality.

The Brilliant III Ultra-Fast QPCR Master Mix uses a new fast and highly processive polymerase that delivers rapid results without compromising qPCR data quality. A novel hot start technology reduces non-specific amplification products and primer-dimer formation for increased specificity. The end result is greater reproducibility and sensitivity within an assay and across multiple assays and templates, at very low copy number.

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Researchers are often looking for very low levels of DNA or RNA sequences in a background of more predominant sequences, as for example when attempting to detect rare cell types.

Elevating borderline positive results to detectable and measurable ones, without prior enrichment on a cellular level, is essential for these studies to decipher the impact of these cells of interest. In addition, reducing protocol run time by 50% or more compared to other master mixes enables the generation of more data in the same time frame.

This application note details a comparison of Brilliant III to competitive master mixes that was conducted at GeneWake GmbH, a company that provides a comprehensive panel of genomic services and technologies for genomics, transcriptomics, epigenetics and proteomics. GeneWake offers diagnostic kit development, companion diagnostics, and biomarker detection as part of contract research or within the scope of clinical studies. Rapid and reliable detection of very low levels of DNA and RNA sequences is essential to many of GeneWake's endeavors. For example, the analysis of genes expressed at very low levels, such as tumor-associated genes in whole blood, is a challenge that must be met and can lead to novel diagnostic tests.

To meet this need, GeneWake scientists analyzed multiple SYBR green master mixes from a variety of vendors to find robust, reliable and ideally fast options. This comparison with 13 competitive master mixes from twelve suppliers demonstrated that Brilliant III provided the fastest, most sensitive, and most reproducible results. Total run time for 45 cycles was up to 50% shorter, and sensitivity was increased as much as 7-fold.

Materials and Methods

Master Mixes

In addition to Brilliant III, 13 master mixes were obtained from 12 different suppliers and used according to the manufacturers' instructions.

Brilliant III Cycling Conditions

Pre-incubation	95 °C	3 min
Amplification	95 °C	5 sec
	60 °C	10 sec
Melting curve	95 °C	5 sec
	65 °C	1 min
	97 °C	continuous
Number of cycles	45	
Run duration	47 min	

cDNA

QPCR Human Reference Total RNA (Agilent, #750500) was used as the substrate for reverse transcription (1 µg of input RNA). The High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems, #4374967) was used according to the manufacturer's protocol to generate cDNA.

Amplified Genes

Gene	Cq Range	Melting Peak Appearance
LOC100008588 (18S ribosomal RNA)	6-9	Single distinct peak
ACTB (Beta actin)	13-17	Two peaks merging into one peak (primers "detect" polymorphism)
CHEK1 (Serine/threonine-protein kinase)	20-24	Single distinct peak
KRT19 (Keratin 19)	26-31	Two peaks (two distinctly different splice variants are expressed)
HMGB1 (High-mobility group protein B1)	18-21	Single distinct peak
RPL32 (60S ribosomal protein L32)	16-20	Single distinct peak
MLH1 (MutL homolog 1)	22-25	Single distinct peak
PMS2 (Postmeiotic segregation increased 2)	22-26	Single distinct peak
ADA (Adenosine deaminase)	22-24	Single distinct peak
FLOT1 (Flotillin 1)	20-24	Single distinct peak
JUN (JUN Proto-Oncogene)	22-26	Single distinct peak
PDPN (Podoplanin)	22-25	Single distinct peak

Standard Dilution Curve

In order to assure meaningful qPCR results, a cDNA dilution series was performed as previously described [1]. The cDNA solution was diluted in water as follows: 1:10, $1:10^2$, $1:10^3$, $1:10^4$, $1:10^5$, $1:10^6$, $1:10^7$, $1:10^8$

Each cDNA dilution was further diluted in water containing tRNA (5 ng/µl) from brewer's yeast (Roche, #10109525001) as a carrier, and the total nucleic acid concentration (5 ng/µl) was equivalent in all samples prior to amplification.

If the limit of detection (LOD) was reached for a dilution step, additional dilution steps were introduced between the LOD concentration and the 10-fold higher concentration, and the amplifications were repeated to determine the LOD more definitively. All amplifications were done in triplicate.

Results and Discussion

Run Times

An initial test was done with a 1:10 dilution of the cDNA and all 14 master mixes for 45 cycles to assure that all the master mixes worked. Brilliant III provided the shortest run time for 45 cycles, at 47 minutes (Table 1). Only two other master mixes provided run times close to this (supplier Q, at 50 minutes, and supplier B at 52 minutes), while all the other master mixes provided run times ranging from one hour to almost two hours.

The initial testing provided expected Cq values for all the gene amplification results in all of the mixes except for suppliers B, F and K, which gave Cq values for KRT19 that were lower than those for CHEK1.

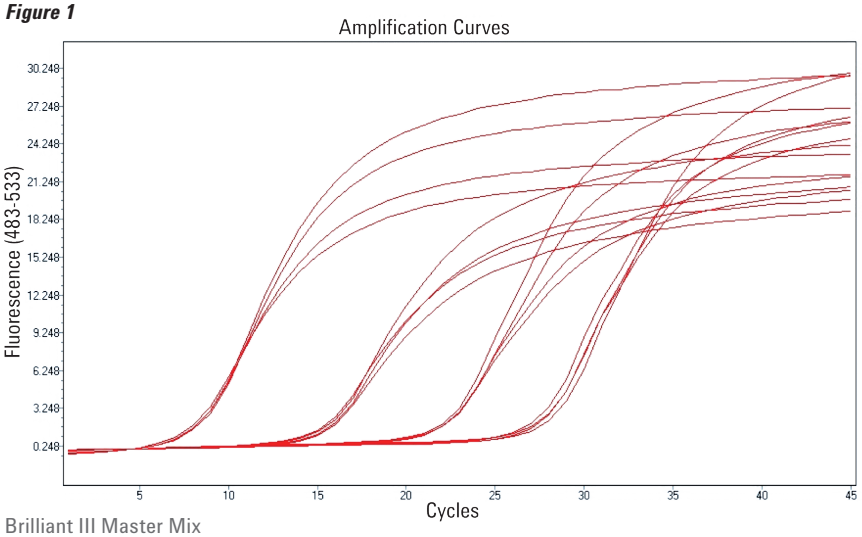
Reproducibility and Limits of Detection

Dilution curves were determined for all the master mixes, using triplicate determinations for each point on the curve. Comparison of Brilliant III master mix to supplier R master mix reveals that Brilliant III provided lower Cqs, higher reproducibility and lower limits of detection (LOD) values (Table 2, Figure 1, and Figure 2).

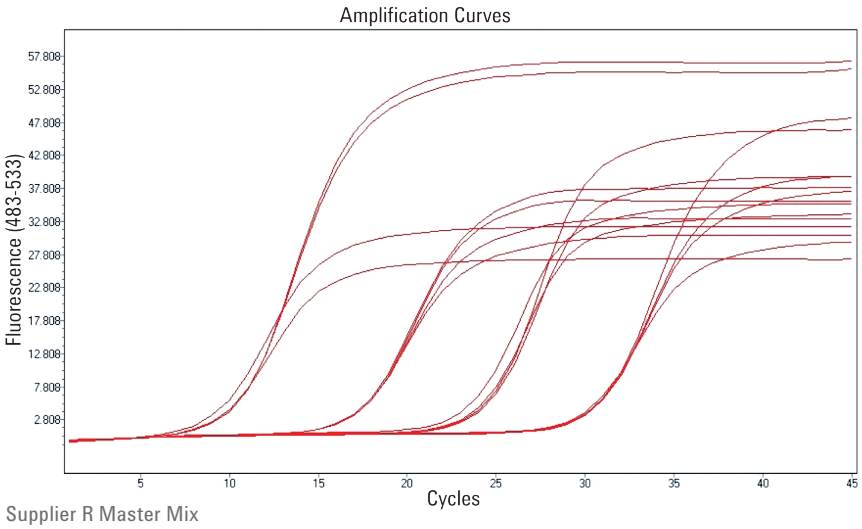
Table 1. Time Required to Complete 45 Cycles

Master Mix	Time (min)
Agilent Brilliant III	47
Supplier B	52
Supplier BR	84
Supplier F	100
Supplier FI	73
Supplier I	78
Supplier K	61
Supplier P	60
Supplier Q	50
Supplier R	83
Supplier R Fast Start	83
Supplier S	90
Supplier T	107
Supplier U	115

Figure 1



Brilliant III Master Mix



Supplier R Master Mix

For those genes with dilution curves that have r^2 values ≥0.99, the reproducibility (r^2 value) was very comparable with both master mixes, with the exception of genes MLH1 and ADA. For these two genes, Brilliant III showed higher r^2 values and reproducibility. For those genes with r^2 values <0.99, Brilliant III always provided higher reproducibility (Table 2).

Conclusion

Agilent Brilliant III Master Mix provided faster cycling times, higher reproducibility and lower limits of detection (LODs) than the master mixes from twelve competitive suppliers. The time to 45 cycles was as much as an hour shorter, and LODs were as much as an order of magnitude lower, enabling efficient detection of very low levels of RNA and DNA in a much shorter time.

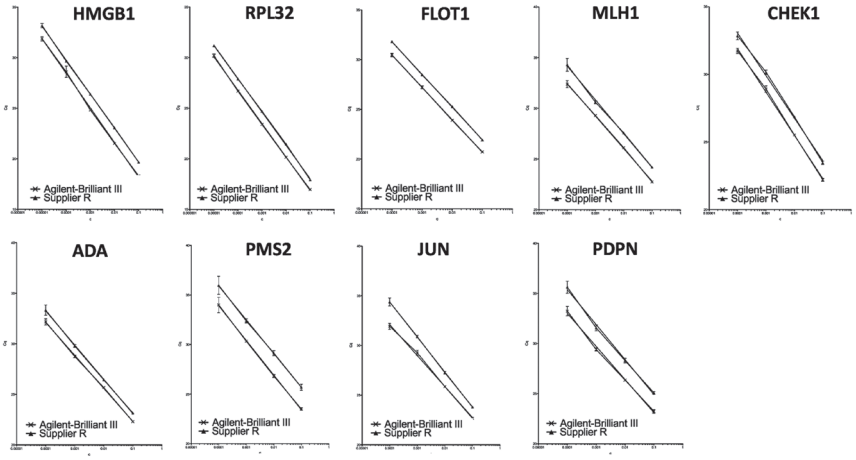
Brilliant III Ultra-Fast Master Mix with ROX

Agilent now offers convenient Brilliant III formulations pre-blended with ROX for SYBR or sequence-specific probe detection assays. Available with low or high ROX concentrations, Brilliant III with Rox maintains the superior specificity, robustness, and reproducibility of Agilent's trusted Brilliant III master mixes.

Table 2. Efficiency, Reproducibility and LOD Values for Brilliant III versus Supplier R Master Mix

Gene	Supplier	Slope	Amplification	Efficiency	r^2	SE Y intercept	LOD
HMGB1	Agilent	–3.417	1.961798396	96.18%	0.993	0.2633	1:4M
HMGB1	Supplier R	–3.358	1.985163618	98.52%	0.9989	0.1031	1:600k
RPL32	Agilent	–3.298	2.010083363	101.01%	0.9984	0.1204	1:6M
RPL32	Supplier R	–3.294	2.011788266	101.18%	0.9994	0.07522	1:1M
FLOT1	Agilent	–3.257	2.027827508	102.78%	0.9977	0.1352	1:100k
FLOT1	Supplier R	–3.286	2.01521489	101.52%	0.9987	0.1018	1:200k
MLH1	Agilent	–3.209	2.049385026	104.94%	0.9943	0.2106	1:20k
MLH1	Supplier R	–3.331	1.996228019	99.62%	0.9839	0.3685	1:20k
CHEK1	Agilent	–3.199	2.053986982	105.40%	0.9941	0.2133	1:80k
CHEK1	Supplier R	–3.143	2.080498256	108.05%	0.9919	0.2457	1:40k
ADA	Agilent	–3.264	2.024755321	102.48%	0.9951	0.1988	1:40k
ADA	Supplier R	–3.385	1.974335593	97.43%	0.9895	0.3018	1:60k
PMS2	Agilent	–3.499	1.931060773	93.11%	0.9789	0.4451	1:60k
PMS2	Supplier R	–3.407	1.965682426	96.57%	0.9653	0.5598	1:60k
JUN	Agilent	–3.118	2.092755101	109.28%	0.9905	0.2647	1:70k
JUN	Supplier R	–3.53	1.919933202	91.99%	0.9937	0.2426	1:50k
PDPN	Agilent	–3.303	2.007960065	100.80%	0.9869	0.3302	1:40k
PDPN	Supplier R	–3.49	1.934340618	93.43%	0.9799	0.4335	1:40k

Figure 2



References

1. B. D'haene, J. Vandesompele, J. Hellemans. "Accurate and objective copy number profiling using real-time quantitative PCR." Methods 50, 262-270 (2010).

www.agilent.com/genomics/brilliantIIIROX

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