

qPCR Reagents



Quantitative Real-Time PCR Solutions for Your Needs

Continuously improving qPCR solutions

Over the years, we have built our experience on real-time quantitative PCR (qPCR) thanks to the continuing search for improvement. The qPCR technology combines RNA, DNA amplification, and cDNA synthesis with accurate and fast real-time monitoring of the amplified product.

Release of first single-tube multiplex reagent kit:
Brilliant Probe Multiplex Master Mix.

2006

2010

Launch of fastest Brilliant III qPCR kits:
Brilliant III Ultra-fast Master Mix.

Big year for Agilent's qPCR range: AriaMx qPCR instrument is launched. Also that year, the introduction of SureDirect blood PCR kit and Brilliant HRM for high resolution melt capacities.

2014

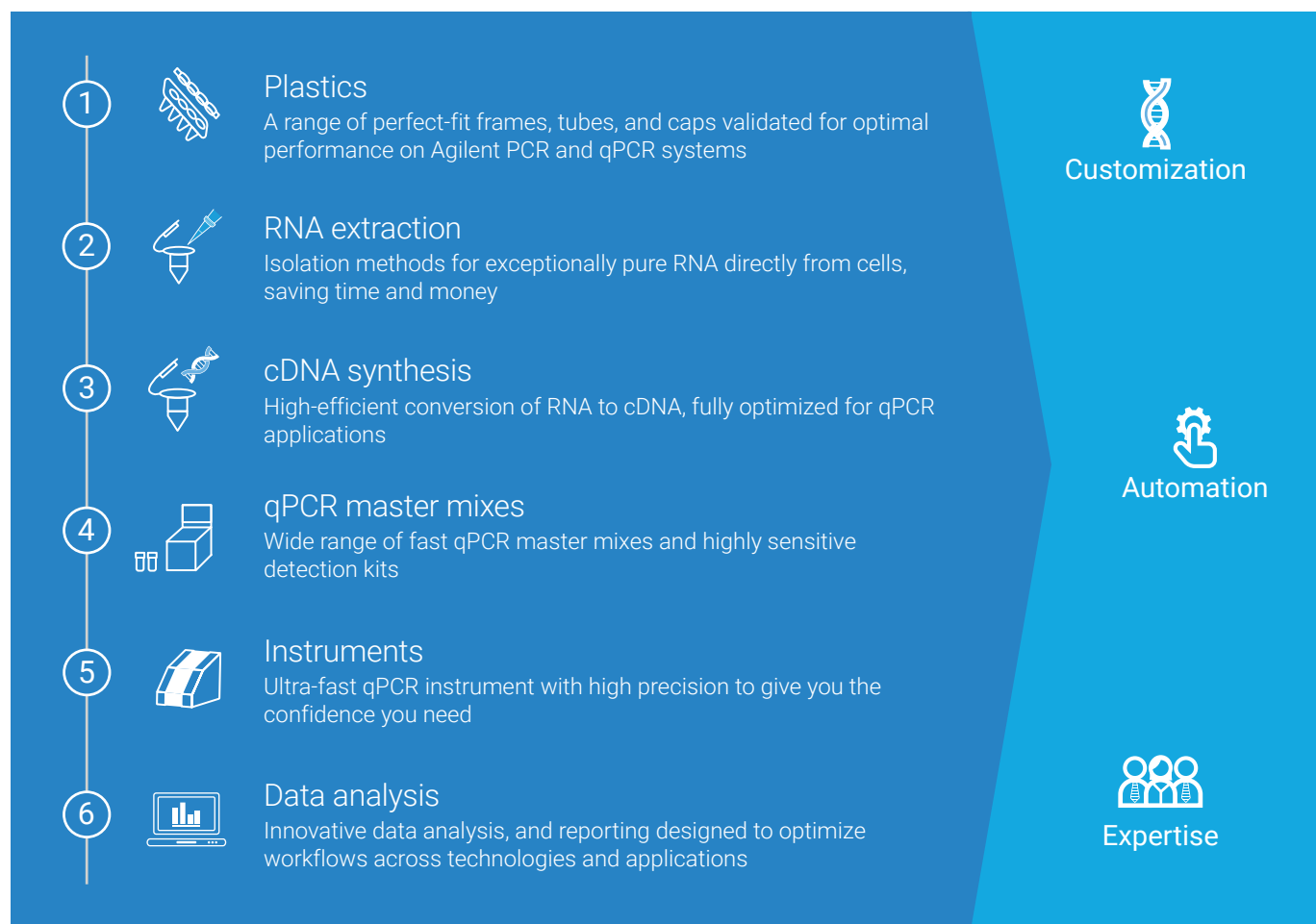
2018

Cannabis Microbial Testing is optimized and validated on the AriaMx.

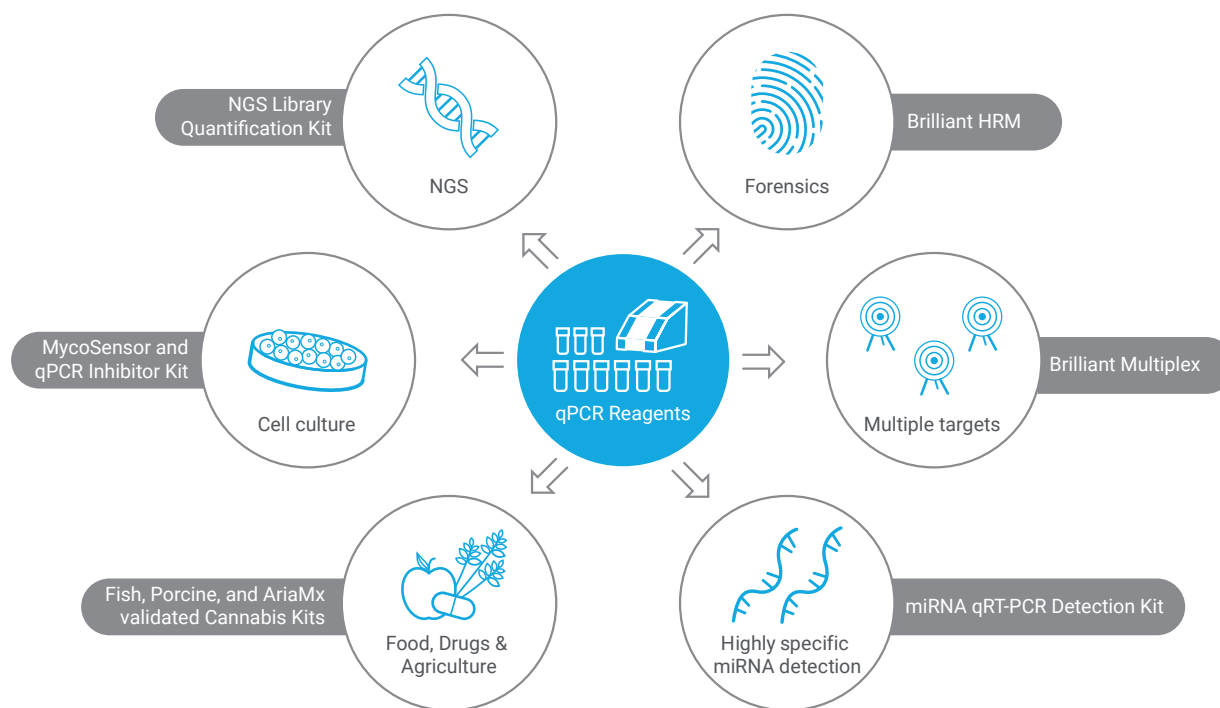
4150 TapeStation System launched, supporting qPCR applications.

Agilent Solutions for qPCR

We offer your lab a total solution approach to real-time PCR by simplifying the challenges you face from sample preparation to data analysis and validation. Whether you are new or experienced in qPCR, your individual needs are met with our comprehensive range of products and support. Those getting started in qPCR can benefit from web-based training programs, premixed reagent kits, and turnkey instrument installation. More experienced qPCR users appreciate the flexibility of our powerful, user-friendly software as well as reagent kits that support user customization and optimization of even the most challenging assays.



Best qPCR Tools, No Matter the Application



Real-Time PCR can meet the needs for various applications, such as next-generation sequencing, food, forensic, agricultural and drug research, miRNA detection, and many more. For example, Agilent's qPCR reagents are utilized in our NGS library quantification kit for these various applications. The Brilliant III enzyme, a mutant derivative of our Taq polymerase, has been adapted for NGS library quantification. The Brilliant Multiplex and qPCR MycoSensor kits have our modified SureStart Taq polymerase at their core, perfect for your cell culture research. Finally, the qPCR Porcine Detection kit is a multiplex probe-based kit designed for food and agricultural research. While our reagent kits are platform-independent, they have also been validated for use on the AriaMx real-time qPCR instrument, making the AriaMx suitable for Medicinal Genomics' cannabis microbial detection kits.

RNA Extraction and cDNA Synthesis Tools

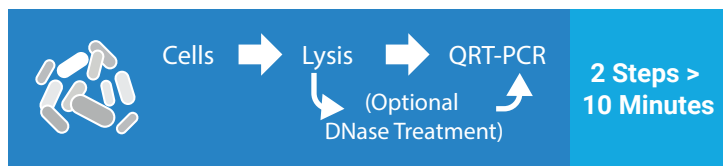
Extract RNA in only two steps with SideStep II

Extract RNA with the SideStep II Cell Lysis analysis kit. This single-tube, RNA Extraction kit offers high-efficient analysis thanks to the immediate stabilization of the nucleic acid without needing further processing steps. It prevents sample loss and degradation, ensuring accurate gene quantification in downstream QRT-PCR.

Conventional method



SideStep method



Reverse transcriptase: From Cells and RNA to cDNA

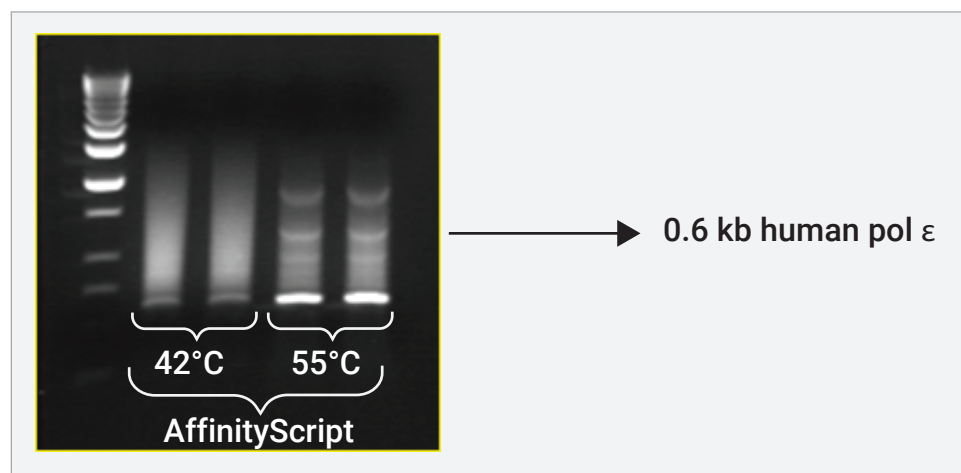
Need to make cDNA from RNA? Our cDNA synthesis kits are designed for the highest efficiency conversion of RNA to high-quality cDNA, either via one-step or two-step workflow. The AccuScript kits provide high fidelity transcripts up to 20 kb long. Complementary to this, the AffinityScript kits provide the best sensitivity over a wide range of temperatures. Together, this range enables read-through of difficult-to-read RNA.

	Fidelity	cDNA Length
AccuScript and AffinityScript Reverse Transcriptases	1 error / 1,500 to 30,000 nt during cDNA synthesis	0 to 20 kb

From RNA to Quantitative PCR with AffinityScript

AffinityScript Multi-Temperature Reverse Transcriptase exhibits improved specific activity over a broad range of cDNA synthesis temperatures, from 42 to 60 °C. Performing cDNA synthesis at higher temperatures minimizes the interference from RNA secondary structure and also increase specificity by minimizing nonspecific priming that may occur at lower temperatures. Several applications for more thermostable reverse transcriptases include cloning, end-point RT-PCR, real-time RT-PCR, microarrays, cDNA library construction, and Rapid Amplification of cDNA ends (RACE). We have shown that by comparison to competitor reverse transcriptases, AffinityScript has a longer half-life, generates longer transcripts, is more sensitive, and is better at reading through RNA with greater secondary structure.

Brand and Transcriptase		Half-life at 55 °C (min)	cDNA Yield at 42/50 + °C (by Target Length)			Specific Activity of High 2° Structure RNA (X Fold)		RT-PCR at 42 °C , High and Low Abundance Targets	
			1.5 kb	> 5 kb (X fold yield)	9 kb	42 °C	50 °C	High (Polymerase ε)	Low (Dystrophin)
Agilent	AffinityScript	35	Yes/Yes	2–4 X	Yes/Yes	1 X	1 X	Yes	Yes
Competitor	Superscript II	10	No/No	1 X	Yes/No	0.2 X	0.2 X	NA	NA
	Superscript III	20	Yes/Yes	1 X	No/No	0.5 X	0.5 X	No	No



Improved RT-PCR yield at high temperature. Reactions contained AffinityScript and 100 ng human HeLa RNA. Reactions were incubated at indicated temperatures for 30 minutes, followed by 15 minutes at 70 °C. 2 µl of cDNA was used to amplify a 0.6 kb fragment of polymerase using 2.5 U PicoMaxx.

Quantitative PCR following AccuScript Transcriptase

We have shown that when compared to competitor reverse transcriptases, AccuScript scores the highest fidelity RT combined with highest fidelity PCR enzyme (PfuUltra II).

Table 1. Compared to competing reverse transcript kits, AccuScript combined with the PfuUltra PCR enzyme shows the highest fidelity.

Reverse Transcriptase (RT) Kit	PCR Enzyme	Calculated Frequency of Mutant Clones (RT + PCR)*, 1 kb Amplicon
Agilent AccuScript RT	PfuUltra II Enzyme	2.5% (1.6% + 0.9%)
Company T, SuperScript II RT	PfuUltra II Enzyme	7.3% (6.4% + 0.9%)
	Taq Polymerase	22.4% (6.4% + 16%)

* Mutation frequencies were calculated using the equation $mF = ER \times bp \times d$, where mF is mutation frequency, ER is error rate, bp is target length in base pairs, and d is number of target doublings (10^6 -fold amplification or $d=20$ assumed here).

Quantitative Real-Time PCR Reagents

qPCR and qRT-PCR Master Mixes for all applications

Get the best quantitative data with one of our Brilliant qPCR or quantitative Reverse Transcriptase-PCR (qRT-PCR) kits. You can choose between our platform-independent SYBR and probe-based kits. Kits have been validated on the AriaMx, ABI, and BioRad RT-PCR instruments.

For High Performance & Ultrasensitive Reactions!

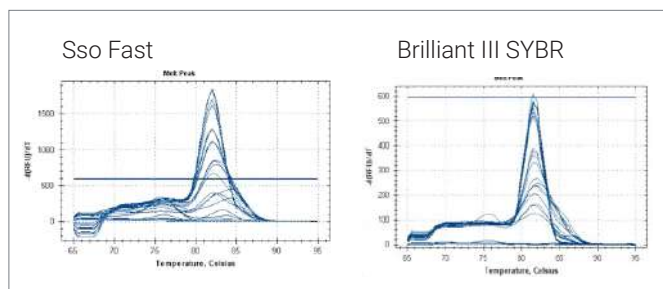
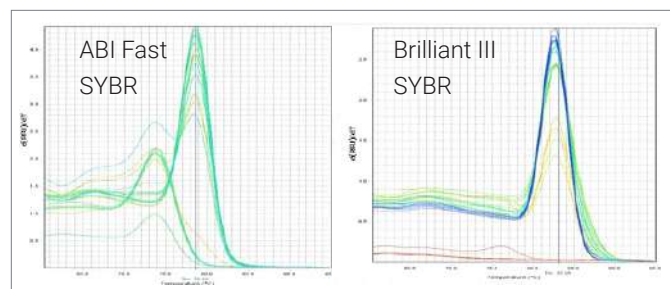
- Brilliant III Ultra-Fast qPCR master mixes for DNA
- Brilliant III Ultra-Fast qRT-PCR master mixes for RNA

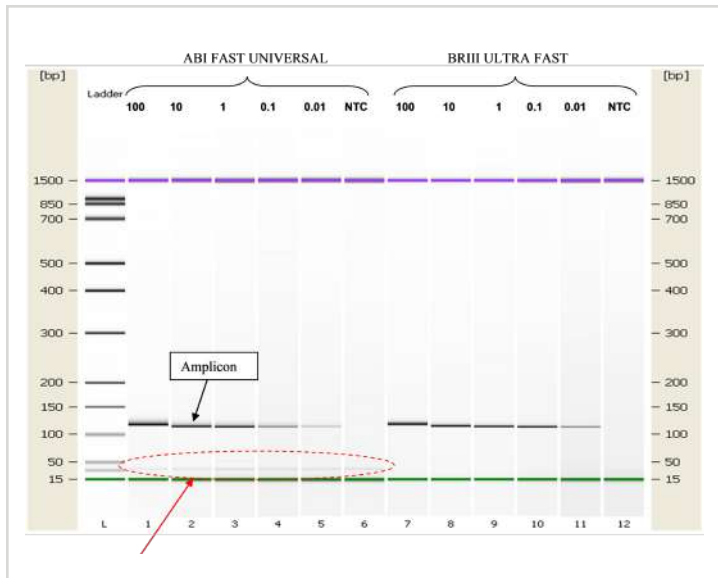
Brilliant III qPCR and qRT-PCR reagents: the industry's best in class

The Agilent Brilliant III qPCR and qRT-PCR reagents score top marks in contrast to competing brands. The Brilliant III reagents show higher Rsq values and amplification efficiency. Furthermore, Brilliant III generates fewer primer-dimers than competitor qPCR and qRT-PCR SYBR and probe kits.

Table 2. Brilliant III exhibits higher efficiency and RSq with fewer primer-dimers than competitor qPCR SYBR and probe kits.

	Competing Reagents	Agilent Reagents
Rsq Value	Express OneStep GreenER RT-qPCR SYBR kit 0.976	Brilliant III RT-qPCR SYBR kit 0.993
Rsq Value	ExpressOneStep SuperScript RT-qPCR probe kit 0.984	Brilliant III RT-qPCR probe kit 0.999
Rsq Value	ABI and Sso FAST qPCR SYBR kits 0.964 and 0.977	Brilliant III qPCR SYBR kit 0.996
Efficiency	TaqMan Universal qPCR probe kit 85.54%	Brilliant III qPCR probe kit 99.7%





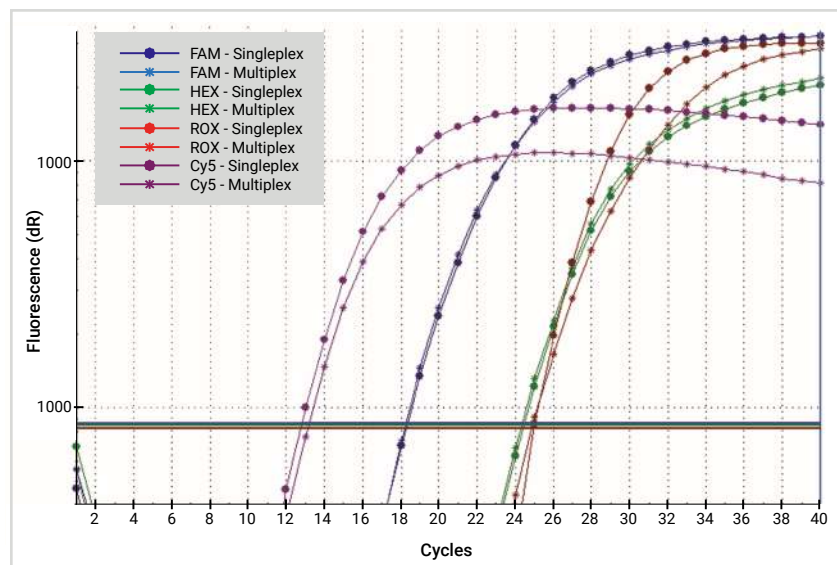
To find out what causes lower amplification efficiency with the ABI TaqMan Fast Universal formulation, reactions were run on an Agilent 2100 Bioanalyzer. Presence of primer-dimers/non-specific products is evident when ABI Fast Universal is used for amplification.



qPCR for Multiple Targets

Go beyond single-reaction qPCR with Brilliant Multiplex qPCR MASTR Mix

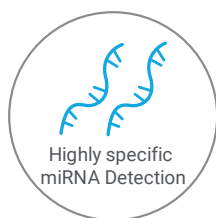
Successfully multiplex thanks to the Brilliant Multiplex qPCR Master Mix without having bias concerns due to target abundance level. The Brilliant Multiplex qPCR Master Mix allows researchers at least a four-target amplification in a single real-time PCR reaction.





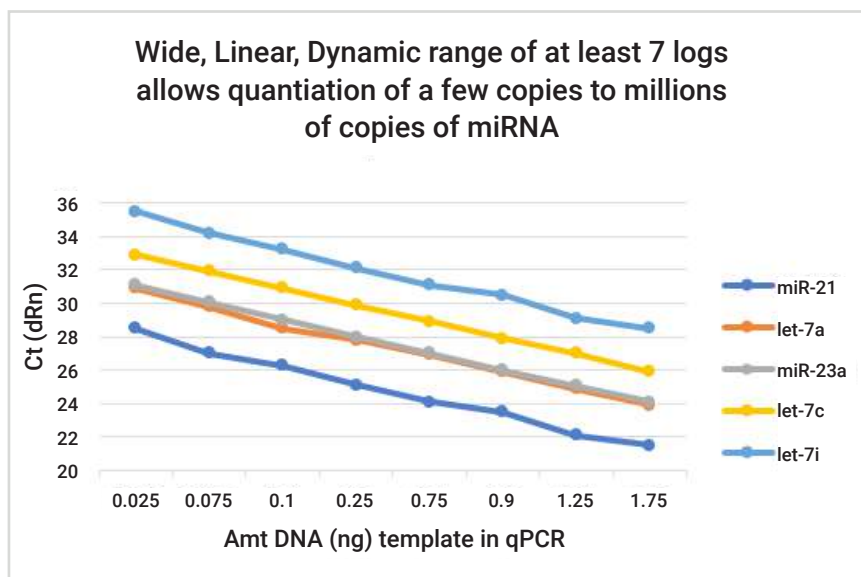
This HotStart-modified Taq polymerase enzyme ensures that same target Cts closely align when comparing them in single versus multiplexed reactions. The master mix provides enough reaction components to accurately quantify both low and high abundance targets in the same tube. Importantly, the sensitivity remains equivalent to that observed in single-plex reactions. The data quality of the multiplex kit has enabled it to be adapted into forensic kits requiring superior precision and accuracy.*

**InnoGenomics Innoquant® and Innoquant® HY kits using Brilliant Multiplex: Highly sensitive and robust assessment of human DNA quantity, and quality. Highly Informative assays, including a Degradation Index and male DNA Detection with HY. <https://innogenomics.com/>*



miRNA Detection kits

Agilent offers three assays for an accurate and sensitive microRNA detection workflow: miRNA 1st-Strand cDNA Synthesis kit, miRNA qRT-PCR master mix detection kit, and miRNA qPCR kit. The miRNA 1st-Strand cDNA Synthesis kit elongates microRNAs from a total RNA population with the addition of a polyA-tail followed by cDNA synthesis, from as little as 15 ng or 10 copies of miRNA. The two other kits provide reagents for quantitative PCR amplification of this cDNA as either a master mix or with kit reagents available individually. These amplify low abundance miRNAs and distinguish between ones with high homology of only one nucleotide difference.

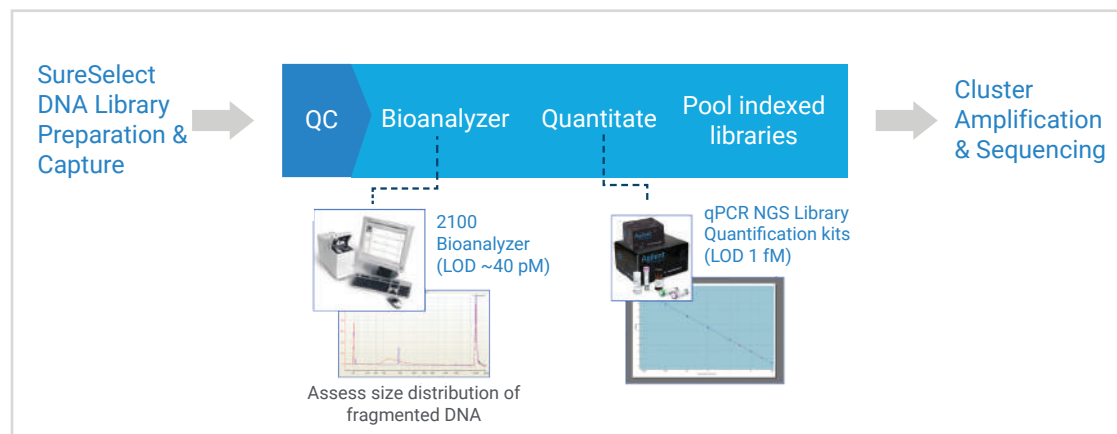


Linearity was demonstrated with two-fold dilutions of HeLa cDNA and detection of five different miRNAs of varying abundance: miR-21, miR-23a, let-7a, let-7c, and let-7i. This wide range in signal linearity allows for accurate quantitation of miRNAs of varying abundance.



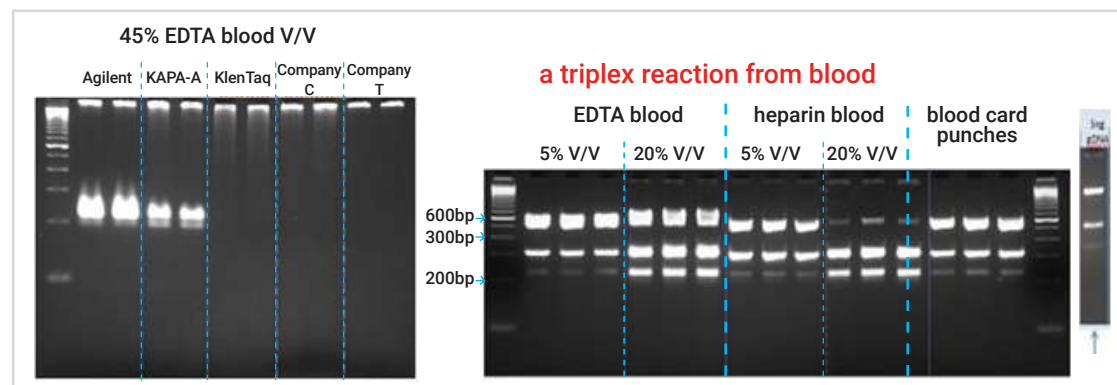
qPCR Library Quantification Kits for Next-Generation Sequencing

Next-Generation Sequencing (NGS) is gaining ground in all fields and accurate library quantification is fundamental in attaining accurate amplification and sequencing data. The Agilent NGS library quantification kit is optimized for the Illumina platforms and provides 4×10^4 greater sensitivity than the Bioanalyzer, for quantitation of samples down to 1 fM.



Streamline your workflow with SureDirect Blood PCR kit

Amplify DNA from liquid, frozen and dried blood, as well as serum, and plasma with inhibitors using the SureDirect Blood PCR kit. Eliminate the cumbersome DNA extraction step and streamline your workflow. The SureDirect Blood PCR kit's outstanding robust performance will enable you to multiplex up to three targets and amplify long targets up to 2.6 kb with high GC content (77%).



In comparative data, SureDirect amplifies target in 45% EDTA, while competitor enzymes are not able to produce amplicon.

Multiple applications with Brilliant High Resolution Melt analysis kit

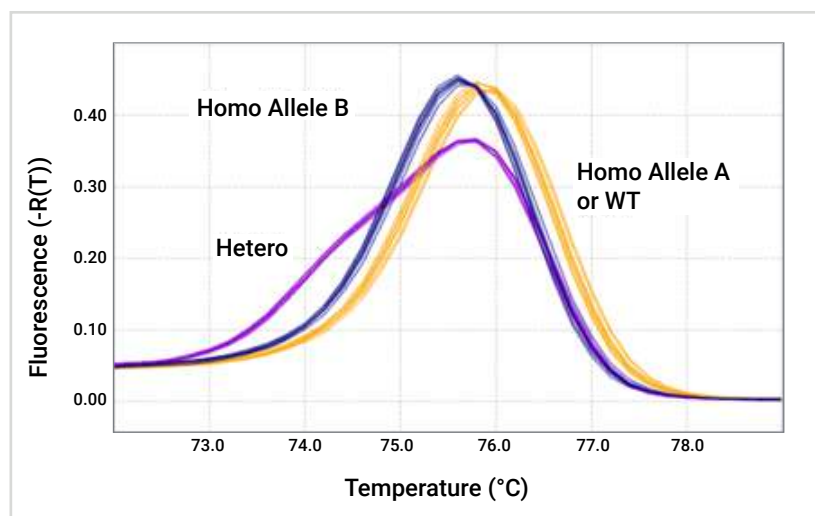
High Resolution Melt (HRM) analysis is a technique used primarily for genotyping samples that include a single nucleotide polymorphism (SNP) in the DNA sequence. Brilliant HRM is a platform-independent mix for HRM analysis validated for use on the AriaMx and third party HRM-capable thermal cyclers. The kit combines a mutant fast-start Taq polymerase, optimized MgCl₂ and dNTPs concentrations, and EvaGreen, a release-on-demand dye for fast results for the most difficult genotypes.

Use Brilliant HRM for:

- DNA methylation
- SNP analysis
- Heterozygosity screening
- Genotyping
- Viral or bacterial population diversity
- HLA compatibility typing
- Species ID

Table 3. HRM Data from AriaMx Real-Time PCR Instrument.

SNP Class	Base Change	Typical Change in T _m	Occurrence in Human Genome
I	C/T and G/A	Large (< 0.5 °C)	64%
II	C/A and G/T	Large (< 0.5 °C)	20%
III	C/G	Small (0.2 - 0.5 °C)	9%
IV	A/T	Very Small (< 0.2 °C)	7%



Agilent developed an HRM assay to resolve a Class IV SNP (A/T), Rs9939609 FTO (142 bp fragments).

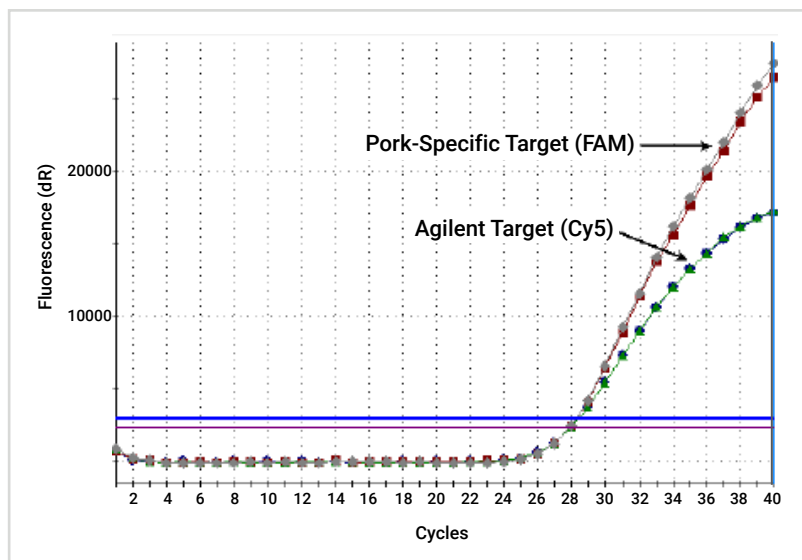


qPCR Reagents for Food, Drug, and Agriculture

Get the best quantitative analysis data for fish and porcine detection, and detection of a microbial panel in cannabis samples. Discover our reagents for food, drugs, and agriculture today!

Quantitative analysis data with Porcine Detection kits

The qPCR Porcine Detection kit can detect as low as 300 fg of DNA in food and pharmaceutical samples, including gel caps. Because isolated DNA may contain contaminants that inhibit PCR, the Porcine qPCR Multiplex kit amplifies and detects two distinct targets in a single reaction: a porcine-specific DNA sequence and an external alien DNA control that enables detection of PCR inhibition.

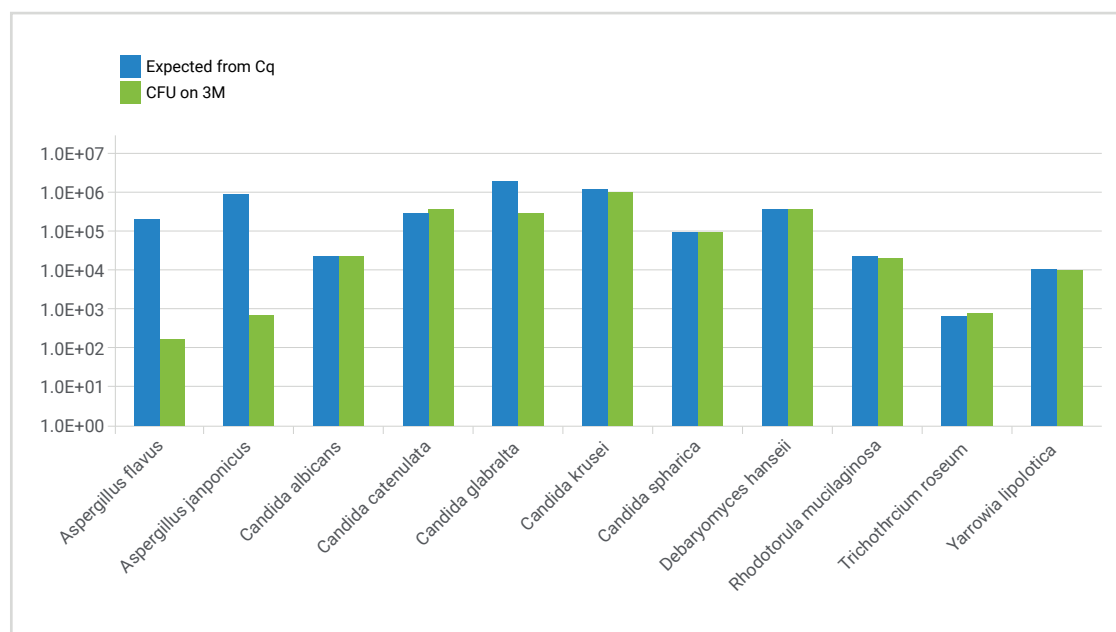


Simplified Cannabis microbial testing using AriaMx qPCR Instrument

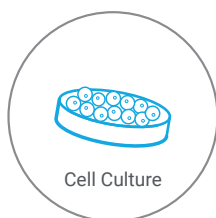
Cannabis microbial testing is challenging, due to many administration methods. While culture-based methods have long been in use, there are a substantial number of microbial species that cannot be cultured easily. Molecular methods, such as qPCR, detect unculturable organisms that clump and distort during plating. These organisms include heterogeneous microcolonies that can occur with various aspergillus species. We have partnered with Medicinal Genomics and their microbial isolation and detection kits have been validated for use on our AriaMx Real-Time qPCR instrument for the best, most sensitive quantitative data.



Assay workflow. DNA decontamination means use of a restriction enzyme to digest the potential contamination amplicon DNA from a previous qPCR. For more details on this method see www.ncbi.nlm.nih.gov/pmc/articles/PMC4008621/.



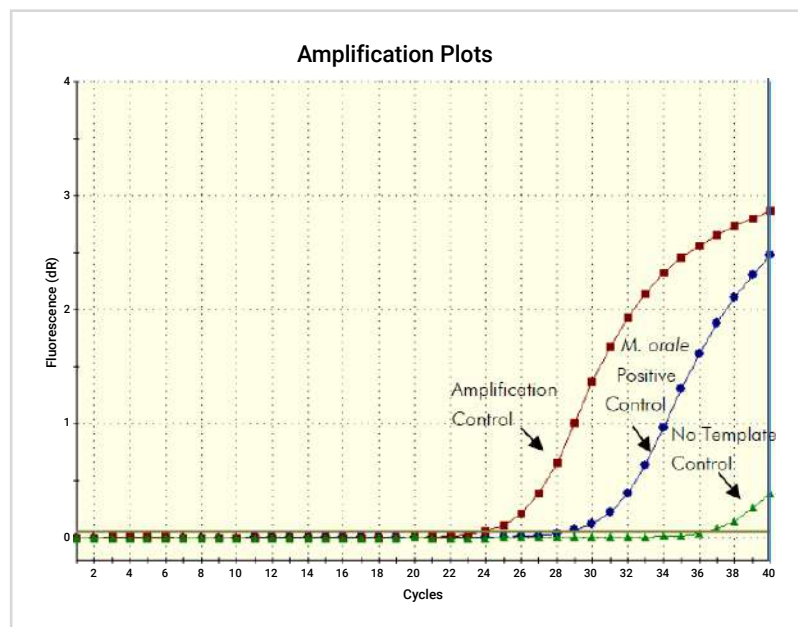
AriaMx qPCR products can detect unculturable organisms and organisms that clump and distort CFU/g enumeration such as aspergillus species (as measured by Cq values). Chart shows comparative growth of Aspergillus species and other fungi on 3M Petrifilm.



Cell Culture contaminants and qPCR inhibitors

Fast Mycoplasma detection in tissue and cell culture, and qRT-PCR Inhibitor kits

The easy-to-use MycoSensor qPCR assay kits specifically detect the eight most common Mycoplasma species in under two hours. It comes with SYBR green dye detection, to confirm the SYBR green dissociation profile of your test samples.



Amplification curves for reactions with and without the indicated MycoSensor kit control template DNA.

Monitoring the overall performance of your qRT-PCR assays

Monitor the overall performance of QRT-PCR assays with the Agilent reference, total RNA and Inhibitor detection control kits. Find our entire range of high-quality complementary qPCR and qRT-PCR products such as the Alien Reference RNA QRT-PCR detection kit, human and mouse reference RNA, MVP total RNA for human, mouse, and rat, and many more on our website.

Learn more:

www.agilent.com/en/promotions/life-science

Agilent products and solutions are intended to be used for **cannabis quality control and safety testing** in laboratories where such use is permitted under state/country law.

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PR7000-2292
© Agilent Technologies, Inc. 2019
Published in the USA, October 09, 2019
5994-1166EN

