How a powerful multiplex PCR system simplifies laboratory workflows

Maximizing efficiency, reducing costs, and elevating throughput in molecular labs

The following data was provided by the manufacturer: NIPPON Genetics EUROPE GmbH

Product

FastGene® qPYR

FG-QPTC01, 4+1 Channel System

FastGene® qPYR PLUS

FG-QPTC02, 6 Channel System

Manufacturer

NIPPON Genetics EUROPE GmbH

Summary

Laboratories today face increasing demands for higher throughput, efficient workflows, and accurate genetic analysis. Quantitative PCR (qPCR), also known as real-time PCR, is a widely used method for detecting and quantifying nucleic acids. Traditionally, qPCR is performed in a singleplex format, where one target is detected per reaction. However, the need to detect multiple targets simultaneously has driven the adoption of multiplex qPCR.

Multiplex qPCR enables the simultaneous detection of multiple genetic targets in a single reaction using spectrally distinct fluorophores. This powerful technique reduces reagent and plastic use, minimizes sample consumption, and increases throughput—all while maintaining data quality.

This white paper explores how multiplex qPCR improves day-to-day work in molecular biology labs, highlights the core advantages of multiplexing, and demonstrates how the FastGene® qFYR series are designed to meet the growing needs of modern laboratories.

What is Multiplex qPCR?

Quantitative PCR (qPCR) is a widely used method for amplifying and measuring DNA targets in real time. In a typical singleplex setup (Figure 1, left), only one target is detected per well. While this works well for simple assays, it quickly becomes inefficient when working with limited sample material or when time and reagent costs are a concern.

That's where multiplex qPCR comes in (Figure 1, right). Using different fluorescent dyes for each target allows multiple sequences to be amplified and quantified in the same reaction. This approach is useful for applications such as pathogen detection panels, SNP genotyping, and gene expression analysis, as well as other assays that require more data from fewer samples.

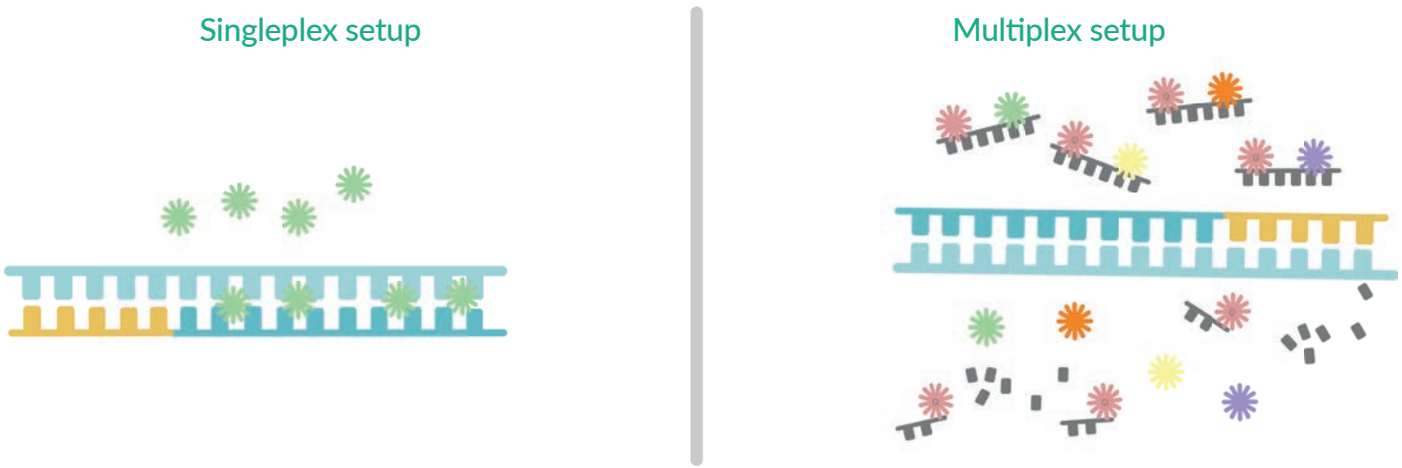

Figure 1: Comparison of singleplex and multiplex setup. *In a singleplex qPCR setup (left) , only one target is amplified per well, requiring separate reactions for each sequence of interest; in a multiplex qPCR setup (right), several targets are amplified simultaneously in the same well using probes designed to bind specific sequences.*

The challenge: complex workflows and high sample volumes

In many academic and research labs, PCR workflows are still slowed down by limited throughput. Running multiple singleplex reactions not only increases reagent and plastic use, but also adds to pipetting workload, raises the risk of manual errors, and consumes more of often limited sample material.

Consider gene expression studies, for example. Analyzing several genes across multiple conditions typically requires setting up a large number of individual qPCR reactions. This quickly becomes time-consuming and resource-intensive. As research projects grow in complexity and scale, these bottlenecks can make it more difficult to keep up.

The solution: multiplexing

Multiplex qPCR allows you to detect multiple targets in the same well using different fluorescent dyes (see Figure 1, right). This allows you to perform fewer reactions overall, reducing reagent use and plastic waste. This method is especially useful when working with limited or valuable samples in research or clinical studies.

Time savings are also substantial. With fewer pipetting steps and quicker runs, experiments can be completed faster. Since there's less manual handling, the likelihood of errors decreases, improving the reliability of your results.

When set up properly, multiplex qPCR makes it easier to gather more data per sample while significantly cutting down on cost and workload.

Choosing the right multiplexing system

Not all qPCR instruments are equally equipped for multiplexing. To fully realize the benefits of this approach, such as clean signal separation, fast analysis, and workflow flexibility, labs need systems specifically optimized for complex, multi-target assays.

When evaluating a qPCR system for multiplexing, key features to consider include:

- **number of optical channels**
- **compatibility with a broad range of fluorescent dyes**
- system's ability to **minimize spectral crosstalk**
- **software tools** available to assist with automated color compensation and complex assay setup
- **thermal uniformity** and **sensitive detection** when quantifying low-abundance targets alongside highly expressed ones.

The FastGene® qFYR series was developed with these exact needs in mind.

FastGene® qFYR series: designed for efficient multiplexing

The FastGene® qFYR Real-Time PCR serie is built for accurate and sensitive nucleic acid quantification. Its ability to multiplex allows for the simultaneous detection of multiple targets within a single reaction. Various channel configurations are available to optimize workflows.

The FastGene® qFYR series includes two models: FastGene® qFYR (FG-QPTC01) and FastGene® qFYR Plus (FG-QPTC02) (Table 1).

The effectiveness of multiplexing depends on the detection system. The unique FastGene® qFYR's patented optical system combines a high-end photomultiplier (PMT) and Fresnel lens to shorten the distance between the sample and the detector. This configuration enhances signal sensitivity and reduces cross-talk.

Table 1: FastGene® qFYR series: FastGene® qFYR (FG-QPTC01) and FastGene® qFYR Plus (FG-QPTC02).

	FastGene® qFYR (FG-QPTC01)	FastGene® qFYR Plus (FG-QPTC02)
Excitation source	Long-life, high-performance LEDs	
Detector	Highly sensitive PMT with Frensel lens	
Number of channels	4	6
Max multiplexing level	4-plex	6-plex
Multi-target detection	Medium	High
Automated color compensation	Yes	

Assay and dye compatibility

The FastGene® qFYR series is compatible with a wide range of commonly used fluorescent dyes (Table 2). This flexibility ensures that most commercial kits, existing probe-based assays applications can be directly transferred or adapted for use without extensive revalidation.

Table 2: Dye compatibility of FastGene® qFYR series according to the number of channels available.

	FastGene® qFYR (FG-QPTC01)	FastGene® qFYR Plus (FG-QPTC02)
Dye compatibility	FAM/SYBR Green VIC/HEX/TET/JOE ROX/Texas Red, Mustang Purple Cy5/LIZ	FAM/SYBR Green VIC/HEX/TET/JOE ROX/Texas Red, Mustang Purple Cy5/LIZ Cy5.5/Quasar 705/Alexa Fluor 680 ATTO 425

Impact of multiplexing on daily lab work

Multiplexing not only accelerates workflows, it transforms them:

Singleplex workflow	6-plex workflow	Time and resource savings with 6-plex
• 6 samples × 6 targets = **36 wells** • Requires careful pipetting into multiple wells or plates • Increased risk of error and contamination	• 6 samples × 1 well = **6 wells** • Reduced handling time and simplified setup • More efficient use of qPCR consumables	• Highly reduced hands-on time • Reagent consumption: **6× fewer master mix reactions** • Plate usage: **minimal**, even for complex panels

Real-world example: validating reference genes

In gene expression studies, it‘s important to use stable reference (housekeeping) genes to ensure accurate and reliable results. However, commonly used reference genes like *GAPDH*, *ACTB*, or *B2M* don‘t always show consistent expression in every cell type or condition.

To address this, we designed a multiplex qPCR experiment targeting **six widely used mammalian housekeeping genes**: *GAPDH*, *B2M*, *ACTG1*, *PGK1*, *HPRT1*, and *ACTB* (Figure 2). The goal was to test how consistently these genes are expressed, and to evaluate whether some genes are more stable than others for use as internal controls.

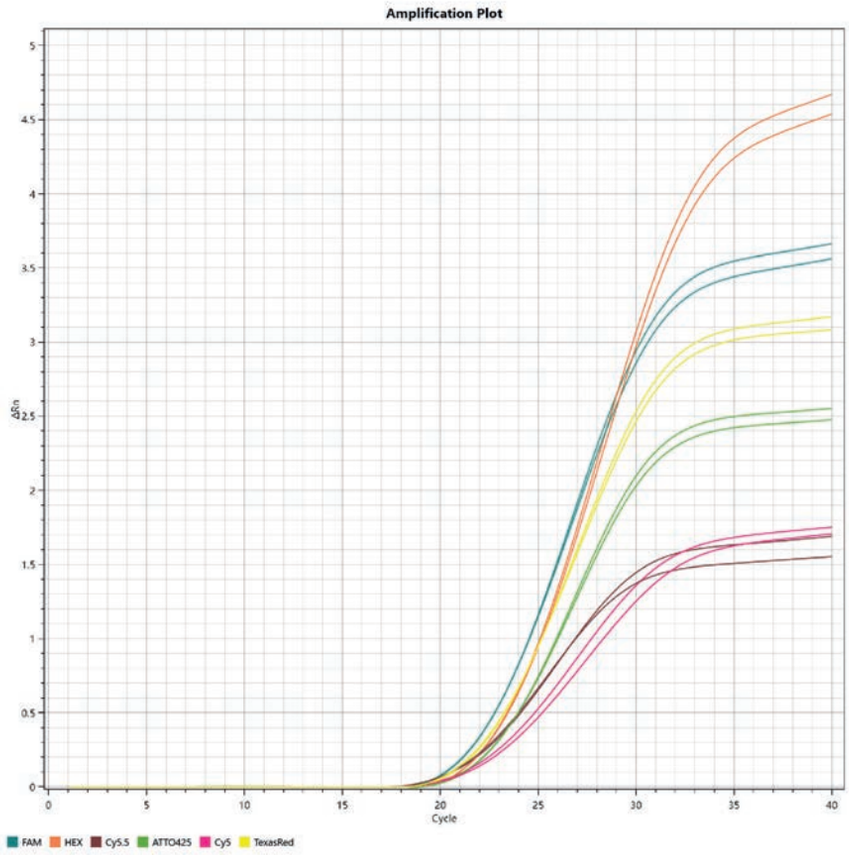

Figure 2: Expression consistency of mammalian housekeeping genes. *The expression of six widely used mammalian housekeeping genes (GAPDH, B2M, ACTG1, PGK1, HPRT1, and ACTB) were analyzed. Each gene was detected using a different fluorescent probe: FAM - GAPDH, HEX - B2M, TexasRed - ACTG1, Cy5.5 - PGK1, ATTO425 - HPRT1, Cy5 - ACTB.*

This experiment helps identify which reference genes are most reliable and also shows that multiplex qPCR can be an efficient way to assess gene expression stability in a single reaction.

Smart software for confident multiplexing

Multiplex assays can be complex, particularly with regard to data interpretation and signal discrimination. With multiple fluorophores emitting signals simultaneously, the system must accurately distinguish between them without signal bleed-through. Even slight spectral overlap can lead to false positives or inaccurate quantification if not properly managed.

Maintaining consistent baselines and threshold settings across channels while normalizing multiple targets in a single well is yet another challenge.

qFYR Analyzer Studio Software was designed to enhance the qPCR workflow, from experiment setup to data analysis. It ensures an easier and faster multiplex setup:

- **Automatic analysis with integrated algorithms** delivers reliable quantification without manual data processing.
- **Guided experiment setup** walks users through plate setup and target assignment to ensure everything is configured correctly.
- **Automated color compensation** minimizes spectral overlap between fluorophores, improving accuracy in multiplex detection.
- **Multi-instrument control** from a single computer enables coordinated, high-throughput multiplex workflows; ideal for labs running multiple panels or assay types in parallel.
- **Multi-plate analysis** tools merge data from different instruments or runs, streamlining interpretation of complex multiplex experiments.

The software is intuitive, helping users detect weak signals even in the presence of strong ones and confidently interpret multiplex results.

Conclusion

Multiplexing not only saves time, but also makes lab processes more efficient. In situations where sample volume is limited, the sample is complex, or results are needed quickly, the FastGene® qFYR (FG-QPTC01) and qFYR Plus (FG-QPTC02) offer a reliable solution.

By making better use of each sample, FastGene® qFYR serie reduces the number of reactions needed, minimize plastic use, and ensure consistent performance across a range of applications.

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