QIAGEN® Multiplex PCR *Plus* Kit

For straightforward and successful multiplex PCR in advanced applications

The QIAGEN Multiplex PCR *Plus* Kit is designed for easy and sensitive multiplex PCR without the need for optimization. The kit is based on proprietary multiplex PCR technology and delivers successful results at the first attempt, and with far greater speed and ease than ever before.

Benefits of the QIAGEN Multiplex PCR *Plus* Kit:

- Successful multiplex PCR for advanced applications
- Consistent and reliable results
- Rapid establishment of multiplex PCR assays without optimization
- Faster reaction times and greater convenience

Figure 1. QIAGEN Multiplex PCR *Plus* Kit.

Critical factors for successful multiplex PCR — obtain consistent and reliable results for your applications

Multiplex PCR saves time and reagents for researchers performing large numbers of PCRs and is widely used in genotyping and DNA or cDNA testing applications in research, forensic and molecular testing laboratories. Applications include typing and analysis of transgenic organisms and pathogens, amplification and analysis of microsatellites, and detection of regions for SNPs or mutations, as well as metagenomics studies (Table 1). However, multiplex PCR in general is a highly demanding technique. Factors influencing the success or failure of multiplex PCR are mainly the establishment of optimal PCR parameters. The varying hybridization kinetics of different primer pairs in multiplex PCR can lead to problems such as amplification bias. Primers that bind with high efficiency utilize more of the amplification reaction components, thereby reducing the yield of other PCR products. Due to the larger number of primers, there is also a greater risk of primer–dimer formation and nonspecific priming. Not addressing these challenges leads to poor sensitivity, nonspecific amplification, and biased amplification of selected targets — and therefore inconsistent and unsatisfactory results. Overcoming these bottlenecks requires tedious and time-consuming optimization steps, resulting in increased costs. The QIAGEN Multiplex PCR *Plus* Kit ▷

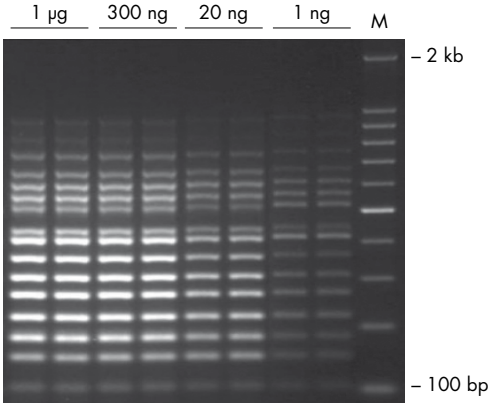

Figure 2. Successful 16-plex PCR over a wide range of template amounts. The QIAGEN Multiplex PCR *Plus* Kit was used to amplify 16 targets (99–955 bp) from various amounts of human genomic DNA, ranging from 1 ng to 1 µg. Successful multiplex PCR results were achieved in each case. **M**: GelPilot® 100 bp Plus Ladder.

eliminates these challenges and easily works at the first attempt. It enables rapid and successful establishment of all multiplex PCR assays, significantly saving time and costs (Figure 3).

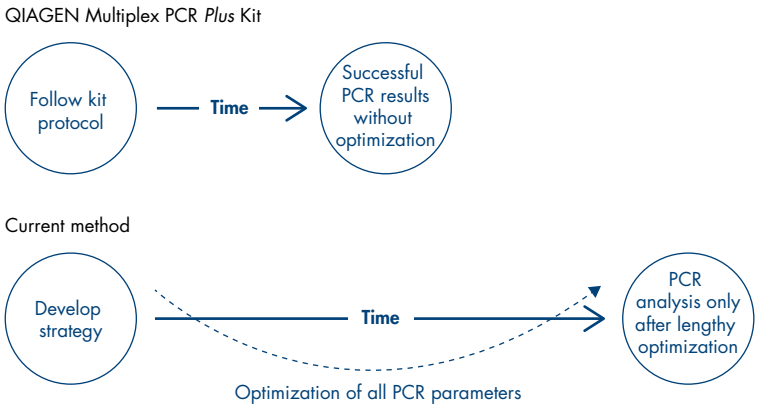

Figure 3. Successful multiplex PCR without the need for optimization. The QIAGEN Multiplex PCR *Plus* Kit is based on patented QIAGEN Multiplex Technology and provides a single, straightforward procedure for rapid and reliable results every time. In contrast to current methods, this kit eliminates the need for optimization of PCR parameters.

High specificity and sensitivity, without the need for optimization

The QIAGEN Multiplex PCR *Plus* Kit is based on QIAGEN's proprietary multiplex PCR technology and is provided in an easy-to-use master mix format. The Multiplex PCR Master Mix contains preoptimized concentrations of HotStarTaq® *Plus* DNA Polymerase and $MgCl_2$, as well as dNTPs and an innovative PCR buffer specially developed for multiplex PCR. The new PCR buffer contains a balanced combination of salts and additives such as Factor MP, which enables comparable efficiencies for annealing and extension of all primers in the reaction (Figure 4). Nonspecific annealing is minimized and parallel amplification of all targets is successful — even with very low template amounts (Figure 2). The stringent hot-start mechanism provided by HotStarTaq *Plus* DNA Polymerase increases multiplex reaction specificity by preventing extension of nonspecifically annealed primers and primer–dimers. Optimization of reaction conditions is not required. Multiplex assays can also be easily set up at room temperature. The kit also includes Q-Solution®, a unique additive that promotes amplification of difficult-to-amplify targets such as GC-rich regions or templates with a complex secondary structure. Highly versatile CoralLoad® Dye — also provided with the kit — further increases handling convenience by improving pipetting visibility and subsequent gel loading and visualization of DNA migration (Figure 7). All of the kit components are specially developed to ensure maximum ease of use and speed, delivering consistently reliable

Table 1. Applications of multiplex PCR

Source of DNA or cDNA	Application
Plants, animals/ human	Analysis of satellite DNA (e.g., STR or VNTR analysis) Typing of transgenic plants/animals Lineage analysis (e.g., of farm animals) GMO analysis Detection of pathogens Food analysis Sex determination Detection of mutations Amplification of SNP loci Qualitative and semiquantitative gene analysis Splicing isoform identification
Bacteria/ viruses	Hygiene analysis Detection of pathogens Microbial genotyping
Environmental samples	Study of metagenomes
Other	Pooling of singleplex assays (time and cost savings) Target enrichment for high-throughput sequencing of the ancient DNA (aDNA)

results (Table 2). In contrast to alternative approaches — which fail even after lengthy optimization — the QIAGEN Multiplex PCR *Plus* Kit enables success in multiplex PCR at the first attempt using a single protocol (Figure 6).

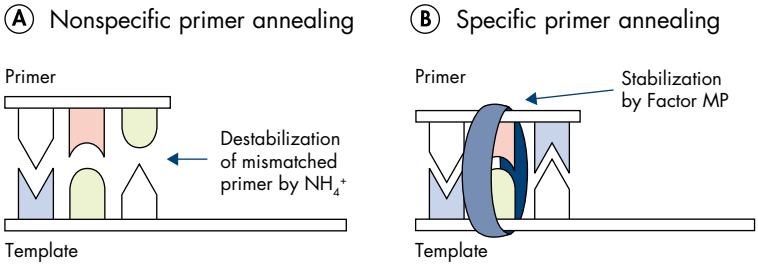

Figure 4. Unique buffer composition. The Multiplex PCR *Plus* Buffer consists of two performance enhancing components: **A** NH_4^+ ions prevent nonspecific primers from annealing to the template. **B** Synthetic Factor MP, an innovative PCR additive, increases the local concentration of primers at the template. Together with K^+ and other cations, Factor MP stabilizes only specifically bound primers, allowing efficient primer extension by HotStarTaq *Plus* DNA Polymerase.

Straightforward and fast downstream analysis

Downstream analysis of multiplex PCR products obtained with the QIAGEN Multiplex PCR *Plus* Kit is straightforward and fast using a variety of methods. Whether analysis is performed using agarose gels, capillary sequencers, or using the QIAxcel® Advanced System (Figure 5), all amplicons can be easily visualized and individual fragments can be reliably distinguished (Figure 6).

Time and cost savings for advanced applications

The QIAGEN Multiplex PCR *Plus* Kit is highly suited for a wide variety of advanced applications. The simple reaction setup, fast procedure, and ease of use provided by the kit ensure reproducible results faster, without the need for optimization of PCR parameters. Multiplex assay development is straightforward and easy, ensuring significant time and cost savings in routine research.

Figure 5. The QIAxcel Advanced System.

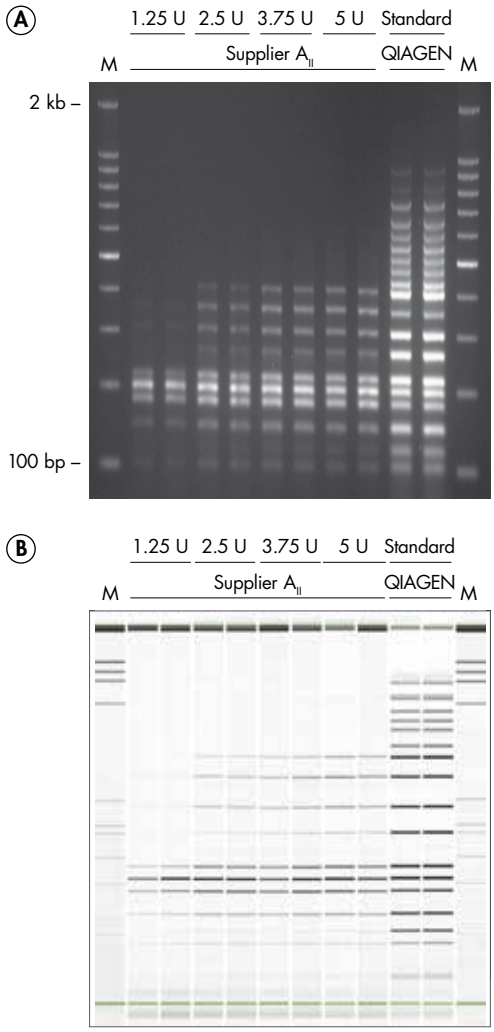

Figure 6. Efficient 19-plex PCR using the QIAGEN Multiplex PCR *Plus* Kit. Multiplex PCR of 19 targets (99–955 bp) was performed using standard conditions for the QIAGEN Multiplex PCR *Plus* Kit, without further optimization (QIAGEN) or using a variety of concentrations of a hot-start DNA polymerase from Supplier A_{II}. **A** Analysis using an agarose gel. **B** Analysis using the QIAxcel Advanced System. The QIAGEN Multiplex PCR *Plus* Kit resulted in specific amplification of all targets without the need for optimization. Despite lengthy optimization using different enzyme concentrations, multiplex PCR using the kit from Supper A_{II} resulted in missing fragments, even when using higher concentrations. **M**: GelPilot 100 bp Plus Ladder.

Table 2. Advantages of the QIAGEN Multiplex PCR *Plus* Kit

Kit component	Advantages
HotStarTaq *Plus* DNA Polymerase	Highly specific and sensitive amplification Room-temperature setup 5-minute activation time
Multiplex PCR *Plus* Buffer with Factor MP	Amplification of all targets in parallel No optimization of PCR parameters needed One single protocol for all multiplex assays Fast procedure
CoralLoad Dye	Easy visualization during pipetting Immediate gel loading Visualization of DNA migration
Q-Solution	Amplification of difficult targets

Figure 7. CoralLoad Dye for easy PCR setup and convenient DNA visualization.

Ordering Information

Product	Contents	Cat. no.
QIAGEN Multiplex PCR *Plus* Kit (30)	For 30 x 50 µl multiplex PCR reactions: 2x Multiplex PCR Master Mix (1 x 0.85 ml), 5x Q-Solution (1 x 2 ml), RNase-Free Water (1 x 1.9 ml), 10x CoralLoad Dye (1 x 1.2 ml)	206151
QIAGEN Multiplex PCR *Plus* Kit (100)	For 100 x 50 µl multiplex PCR reactions: 2x Multiplex PCR Master Mix (3 x 0.85 ml), 5x Q-Solution (1 x 2 ml), RNase-Free Water (2 x 1.9 ml), 10x CoralLoad Dye (1 x 1.2 ml)	206152

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Visit **www.qiagen.com** for more information!