# New concepts for accelerated real-time PCR analysis

Thorsten Traeger,[1] Kirsten Hildebrand,[1] Dirk Loeffert,[1] Ralf Peist,[1] Annette Tietze,[1] Andrea Stutte-Rabe,[1] Ulla Deutsch,[1] Dile Holton,[2] and Andreas Missel[1]

[1] QIAGEN GmbH, Hilden, Germany; [2] QIAGEN Sciences, Germantown, MD, USA

## Introduction

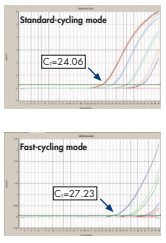
For researchers needing to increase their throughput or share a cycler with other users, there is a strong demand for faster, real-time PCR.

**Fast, real-time PCR can be achieved by:**

- Reduced DNA polymerase activation time
- Shortened amplification cycles
- Combined annealing and extension steps
- Use of a dedicated fast-cycling instrument
- Shortened RT step in one-step RT-PCR

Until now, fast, real-time PCR using standard reaction chemistry has been hampered by reduced sensitivity and increased variability of quantification data (1). We demonstrate how the combination of a newly developed fast-cycling PCR buffer with a rapid-activating hot-start DNA polymerase allow significant reduction of PCR cycling times without sacrificing specificity and sensitivity.

**Loss of sensitivity in fast cycling with standard reaction chemistry**

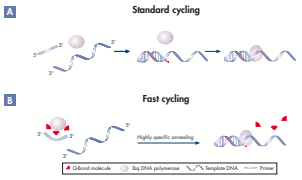



Expression of MYC in human leukocytes was analyzed by real-time, two-step RT-PCR on the Applied Biosystems® 7500 Fast System. A kit for standard cycling from Supplier A was run **A** in recommended standard-cycling mode (reduced ramping rates, 15-second denaturation, 60-second annealing/extension) and **B** in fast-cycling mode (rapid ramping rates, 10-second denaturation, 30-second annealing/extension).

## New chemistries for fast-cycling PCR

We have developed a fast-cycling PCR buffer that significantly reduces denaturation, annealing, and extension times. A novel additive, Q-Bond, dramatically increases the binding affinity of DNA polymerase to single-stranded DNA. This turns the 3-step process of template denaturation, primer annealing, and DNA polymerase binding in standard-cycling PCR (A) into a faster 2-step process (B).

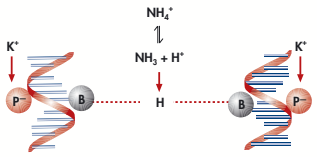
**Fast primer annealing**




**A** Without Q-Bond, the primer and DNA polymerase bind sequentially to the template, increasing primer annealing time. **B** Q-Bond increases the affinity of DNA polymerase for short single-stranded DNA, reducing primer annealing time to a few seconds.

High annealing specificity is maintained by a balanced combination of KCl and $NH_4Cl$ in the buffer. The binding of primers to imperfectly matching sequences on the template is suppressed.

**Specific primer annealing**




The fast-cycling PCR buffer contains $K^+$ and $NH_4^+$ ions to ensure specific primer annealing and efficient extension. $NH_4^+$ destabilizes the weak hydrogen bonds at mismatched bases of nonspecifically bound primers.

A novel enzyme, HotStarTaq® *Plus* DNA Polymerase, is rapidly activated in 3 or 5 minutes by a 95°C incubation at the start of PCR.

## Ultrafast cycling for end-point PCR analysis

Although fast results in PCR can be achieved on cyclers with rapid ramping rates, even faster results are possible by reducing cycling times. The QIAGEN® Fast Cycling PCR Kit, which integrates the fast-cycling PCR buffer with HotStarTaq *Plus* DNA Polymerase, provides significant time savings of up to 78% in end-point PCR. Fast results can be accomplished on all cyclers, including cyclers not capable of fast ramping rates.

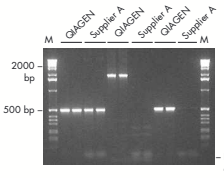
The PCR buffer minimizes amplification of nonspecific products, primer–dimer formation, and background smear in every PCR cycle. Q-Solution, an additive that enables efficient amplification of "difficult" (e.g., GC-rich) templates, is also provided with the kit.

**PCR cycling times calculated for different fragment lengths***

| Fragment length | QIAGEN Fast Cycling procedure (min) | Standard cycling procedure (min) | Time saving |
|---|---|---|---|
| **200 bp** | **15** | **68** | **78%** |
| 500 bp | 20 | 68 | 71% |
| 1000 bp | 29 | 85 | 66% |
| 3000 bp | 63 | 155 | 59% |

* Total time required for a PCR run of 35 cycles. The specified PCR cycling times do not include ramping times, which are cycler-dependent.

**Specific and reliable results**

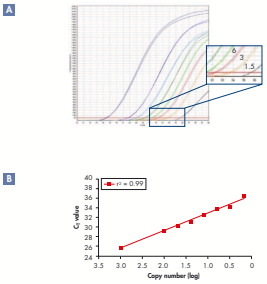



Three different PCR assays (IL9, PKC, and AGRT2) were performed using the QIAGEN Fast Cycling PCR Kit (**QIAGEN**) and a fast-cycling PCR solution from another supplier (**Supplier A**) according to the manufacturer's instructions. Reactions were performed on a fast cycler from Supplier A. The QIAGEN Fast Cycling PCR Kit provided highly specific results for each assay, whereas results using Supplier A were unpredictable with high drop-out rates and unspecific results (e.g., primer–dimers). **M**: marker.

## Fast SYBR Green quantification with high specificity

In biological systems, minute changes in transcript abundance often lead to strong biological effects. Therefore, a method for reliable and reproducible discrimination between similar copy numbers is critical. With QuantiFast™ SYBR Green Kits, even small differences in the amount of low-copy targets can be clearly distinguished.

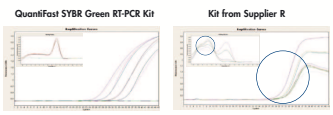
**Resolution of small differences in copy number**




The QuantiFast SYBR Green PCR Kit and Mastercycler® ep *realplex* were used to detect the single-copy gene SRY in a genomic DNA sample. **A** Curves for 1000 down to 1.5 copies can be clearly distinguished from each other. **B** A plot of copy number (log) versus $C_T$ value demonstrates high linearity

Achieving high specificity with SYBR Green based real-time PCR is important, since SYBR Green binds all double-stranded DNA. QuantiFast SYBR Green Kits promote specific primer annealing and provide a stringent hot start, preventing the formation of nonspecific PCR products that can affect SYBR Green fluorescence. Quantification is specific and sensitive, even with low template amounts.

**Specific one-step RT-PCR**

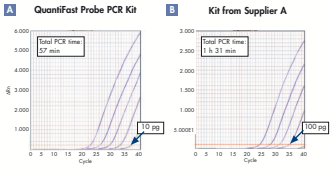



Expression of BCL2 in HeLa cells was analyzed on the LightCycler® 2.0. Unlike the instrument-dedicated kit from Supplier R (which was used according to the fast-cycling protocol), the QuantiFast SYBR Green RT-PCR Kit provided specific amplification and sensitive quantification.

## Speed and sensitivity in probe-based detection

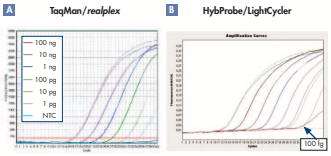
Reducing the duration of each PCR cycle leads to faster PCR, but can impair PCR performance. The use of our fast-cycling technology by QuantiFast Probe Kits enables faster results in probe-based real-time PCR without compromising sensitivity. Fast and sensitive quantification is possible on all available cyclers and with different types of sequence-specific probe, such as TaqMan® and FRET probes.

**Faster results without compromising sensitivity**




Reactions were run on the ABI PRISM® 7900 using a TaqMan gene expression assay for IL1RN (a cytokine). The QuantiFast Probe PCR Kit was 40% faster than the standard-cycling kit from Supplier A, and also more sensitive: $C_T$ values were much lower and transcript could be quantified from down to 10 pg cDNA.

**Sensitive detection independent of cycler and probe**

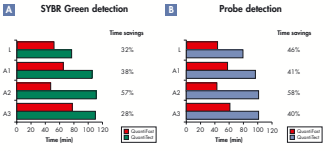



The QuantiFast Probe PCR +ROX Vial Kit provided accurate gene expression analysis from high to low amounts of human leukocyte cDNA with PCR efficiencies of greater than 90%. **A** Reactions run on the Mastercycler ep *realplex* (block cycler) using a TaqMan assay for ubiquitin. **B** Reactions run on the LightCycler 2.0 (capillary cycler) using a FRET assay for β-2 microglobulin. Both cyclers were run using maximum ramping rates and short cycling steps

## Summary

- A patent-pending, fast-cycling PCR buffer containing Q-Bond significantly reduces denaturation, annealing, and extension times.
- HotStarTaq *Plus* DNA Polymerase possesses no enzyme activity prior to PCR, and is rapidly heat-activated in 3 or 5 minutes.
- The fast-cycling PCR conditions provide significant time savings of up to 78% in end-point PCR and up to 60% in real-time PCR without compromising specificity and sensitivity.
- New QIAGEN chemistries enable fast-cycling on all cyclers, including those not capable of achieving rapid ramping rates.

**Significantly reduced PCR times**




Time savings of up to 60% were achieved in **A** SYBR Green based real-time one-step RT-PCR using the QuantiFast SYBR Green RT-PCR Kit and **B** probe-based real-time two-step RT-PCR using QuantiFast Probe PCR Kits (40 cycles without melting curve analysis; comparison with standard QIAGEN real-time PCR kits). **L**: LightCycler 2.0; **A1**: ABI PRISM 7900; **A2**: Applied Biosystems 7500 Fast System; **A3**: ABI PRISM 7000.

QuantiFast Kits and the QIAGEN Fast Cycling PCR Kit are intended for research use. No claim or representation is intended for its use to provide information for the diagnosis, prevention, or treatment of a disease.

**References**

1. Hilscher, C., Vahrson, W., and Dittmer, D.P. (2005) Faster quantitative real-time PCR protocols may lose sensitivity and show increased variability. Nucleic Acids Res. **33**:e182.



1043234 02/2007