Concepts for accelerated end-point and real-time PCR analysis

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Introduction

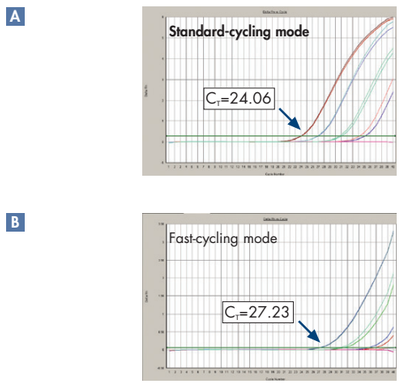
For researchers needing to increase their throughput or share a cycler with other users, there is a strong demand for faster PCR results.

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

However, fast, real-time and end-point PCR using standard reaction chemistry is often compromised by reduced sensitivity and increased variability of quantification data (1). We demonstrate how the combination of a fast-cycling PCR buffer with a rapid-activating hot-start DNA polymerase allows significant reduction of cycling times without sacrificing specificity and sensitivity.

Loss of sensitivity in fast cycling with standard reaction chemistry



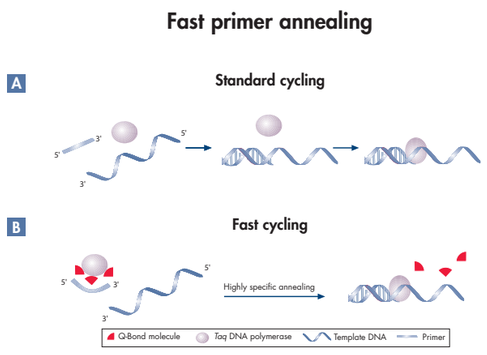
Expression of MYC in human leukocytes was analyzed by real-time, two-step RT-PCR on the Applied Biosystems® 7500 Fast System. A kit for standard cycling from Supplier A was run **A** in recommended standard-cycling mode (reduced ramping rates, 15-second denaturation, 60-second annealing/extension) and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

Reference

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.

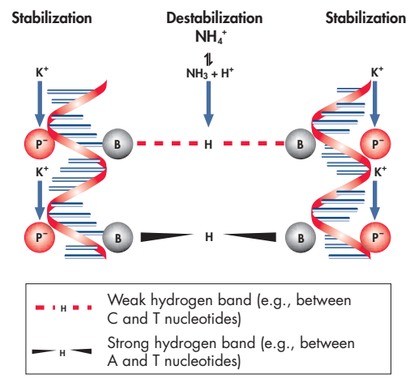
Patented and proprietary chemistry for fast-cycling PCR

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).



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 (watch an animation on QIAGEN's PCR buffer on www.qiagen.com/PCR-video).



K^+ binds to the phosphate groups (P) on the DNA backbone, stabilizing the annealing of the primers to the template. NH_4^+, which exists both as the ammonium ion and as ammonia under thermal-cycling conditions, can interact with the hydrogen bonds between the bases (B), destabilizing principally the weak hydrogen bonds at mismatched bases. The combined effect of the two cations maintains the high ratio of specific to nonspecific primer–template binding over a wide temperature range.

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.

PCR cycling times calculated for different fragment lengths*

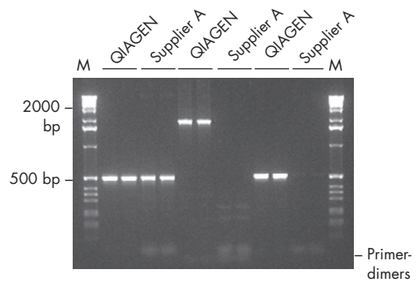
Fragment length	QIAGEN Fast Cycling procedure (min)	Standard cycling procedure (min)	Time saving
200 bp	**15**	**68**	**78%**
500 bp	20	68	71%
1000 bp	29	85	66%
3000 bp	63	155	59%

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

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.

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, along with CoralLoad® for faster and more convenient handling. CoralLoad is a colored, fast cycling buffer that enables direct loading of the reactions onto agarose gels, minimizing time and effort for end-point-PCR analysis.

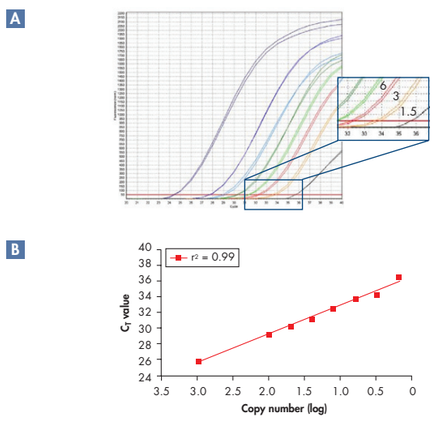
Specific and reliable results



Fast and highly sensitive SYBR® Green and probe detection

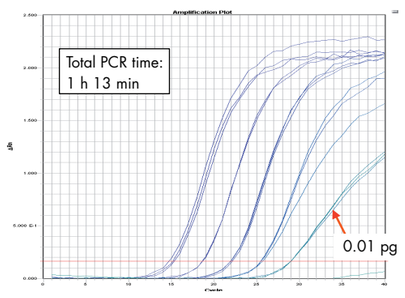
In biological systems, minute changes in transcript abundance often lead to strong biological effects. Therefore, a method for reliable and reproducible discrimination between similar copy numbers is critical. With QuantiFast® PCR Kits, even small differences in the amount of low-copy targets can be clearly distinguished without compromising sensitivity. A specially developed RT-PCR buffer enables cDNA synthesis in just 10 minutes, accelerating one-step RT-PCR experiments.

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.

QuantiFast Probe RT-PCR Kit (28S rRNA)

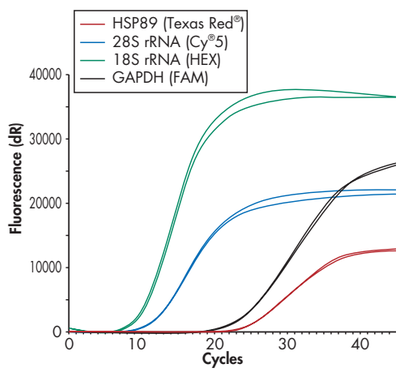


Reactions were run in triplicate using 10-fold dilutions of human leukocyte RNA (100 pg to 0.01 pg) and a TaqMan® gene expression assay for 28S rRNA. The QuantiFast Kit values and quantification from as little as 0.01 pg RNA.

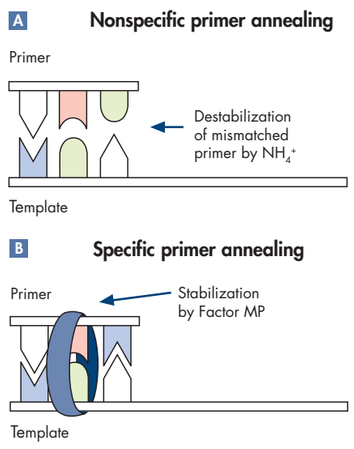
Speed and accuracy in multiplex detection

Amplification of control and target genes in the same reaction increases the reliability of gene quantification, as well as sample throughput. However, comparable efficiencies for annealing and extensions of all primers in the reaction tend to be challenging with conventional PCR chemistries.

QuantiFast Multiplex Kits, comprising the unique multiplex buffer containing Factor MP, enable rapid and accurate detection of multiple targets differing widely in abundance.



Real-time, 4-plex, one-step RT-PCR was carried out using the QuantiFast Multiplex RT-PCR +R Kit and Primer Express® designed TaqMan assays. Duplicate reactions were run using 10 ng RNA from Ramos cells as template. All 4 targets, which varied greatly in abundance, were reproducibly detected.



A NH_4^+ ions prevent nonspecific primers from annealing to the template. Synthetic Factor MP, an innovative PCR additive, increases the local concentration of primers at the template. **B** Together with K^+ and other cations, synthetic Factor MP stabilizes specifically bound primers, allowing efficient primer extension by HotStarTaq *Plus* DNA Polymerase.

Summary

- The patented and proprietary fast-cycling PCR buffer containing Q-Bond significantly reduces denaturation, annealing, and extension times.
- HotStarTaq *Plus* DNA polymerase 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.
- A shortened RT step accelerates one-step RT-PCR experiments.
- The unique Factor MP enables rapid and accurate quantification of multiple targets of different abundance in the same reaction.
- QIAGEN´s fast cycling technology enable fast cycling on all cyclers.
- Patented fast cycling chemistry has been applied to a variety of QIAGEN PCR solutions, see www.qiagen.com for more information.

The applications presented here are for research use only. Not for use in diagnostic procedures.

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