LightCycler® 480 System: A New Standard for Accurate High-Throughput qPCR

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Introduction

The LightCycler® 480 System is a novel multiwell plate-based real-time PCR platform for qualitative and quantitative detection of nucleic acids and genotyping, especially designed for automated workflows with high throughput. Similar to the capillary-based LightCycler® 1.5 and 2.0 Systems, the LightCycler® 480 System is a versatile platform, providing precise and reproducible results, maximum flexibility, and outstanding speed, thus combining features that were thought to be hard to reconcile.

Achieving qPCR Accuracy

To achieve rapid and reproducible results from a multiwell PCR plate, precise target temperatures are required during cycling. Each sample position of the plate should reach target temperature quickly and simultaneously. In the LightCycler® 480 Instrument, this has been achieved by the inclusion of a so-called Therma-Base within the thermal block cycler. The Therma-Base is a heat distribution and temperature equilibration device whose working principle is based on the evaporation and condensation of a working fluid in a thin vacuum cavity (Figure 1). Tailor-made 96- and 384-multiwell plates are designed for a perfect fit to the respective thermal blocks, a prerequisite for fast energy transfer to and from the reaction mix. Inserted plates are held down by a heated lid that prevents condensation in the upper part of the reaction vessel.

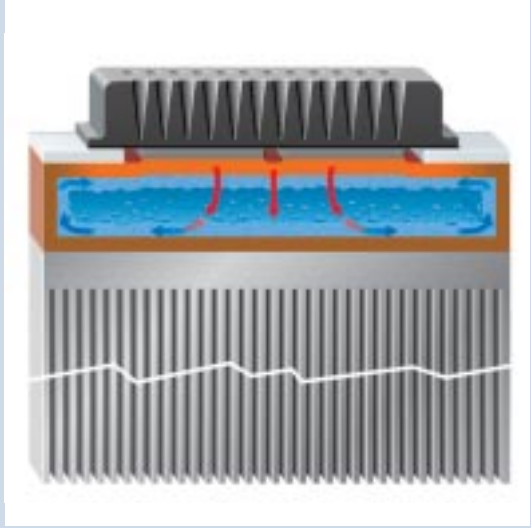

Figure 1: Cross-section of the LightCycler® Thermal Block Cycler. Upper layer: mulitwell plate mount; middle layer: Therma-Base; lower layer: cooling element

The optics consist of an special arrangement of an elaborate large field lens and a cooled CCD camera that measures fluorescence uniformly and with high sensitivity over the complete plate. Two freely combinable filter sets allow specific excitation and detection with fluorescent dyes detectable from 500–670 nm. A very compact instrument size was achieved by folding the optical path using mirrors.

One-component master reagents containing hot-start enzymes are another key element contributing to the accuracy of the LightCycler® 480 System. For each of the main real-time PCR applications, the enzyme variants and buffer conditions were carefully chosen and optimized to yield robust and highly sensitive results even when high-speed protocols are applied.

For data analysis, the LightCycler® 480 System includes highly accurate and flexible software modules. Based on bias-free second derivative Cp determination and non-linear fit algorithms for standard curves, the LightCycler® 480 Basic Software provides unmatched quantification opportunities. The separately available LightCycler® 480 Relative Quantification Software goes one step further by additionally offering PCR efficiency consideration and a calibrator-based method (*e.g.*, comparing target and reference genes).

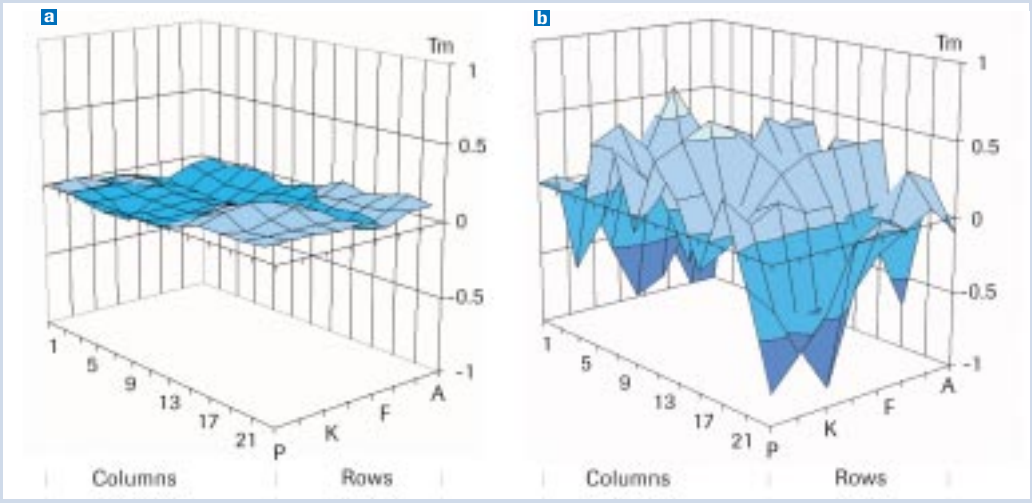

Figure 2: Monitoring thermal block cycler accuracy by melting temperature determination. (*a*) LightCycler® 480 Thermal Block Cycler with Therma-Base (*b*) other real-time PCR System without Therma-Base. The melting point temperature (T_m) of a given oligonucloetide/SimpleProbe probe duplex was used as a sensitive detection parameter to demonstrate temperature homogeneity across a multiwell plate. The variation of the measured T_m from the expected T_m of the oligonucleotide is shown using the expected T_m (50°) as zero.

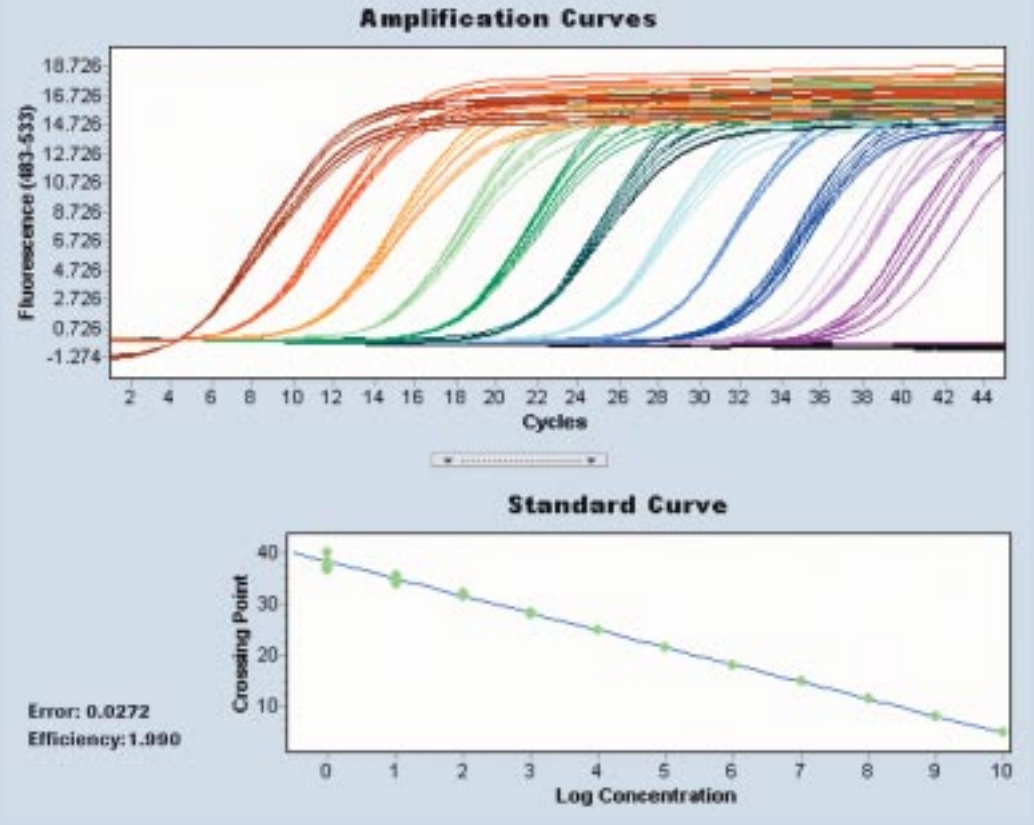

Figure 3: Linear range of the LightCycler® 480 Instrument. Serial 1:10 dilutions (10^{10} to 10^0 copies) with nine replicates of a plasmid DNA sequence were amplified with the LightCycler® 480 Probes Master and detected with a Universal ProbeLibrary probe. The PCR result shows a log-linear relationship over a broad dynamic range (10 log intervals) and high reproducibility for the replicates (Cps) from the different dilution steps.

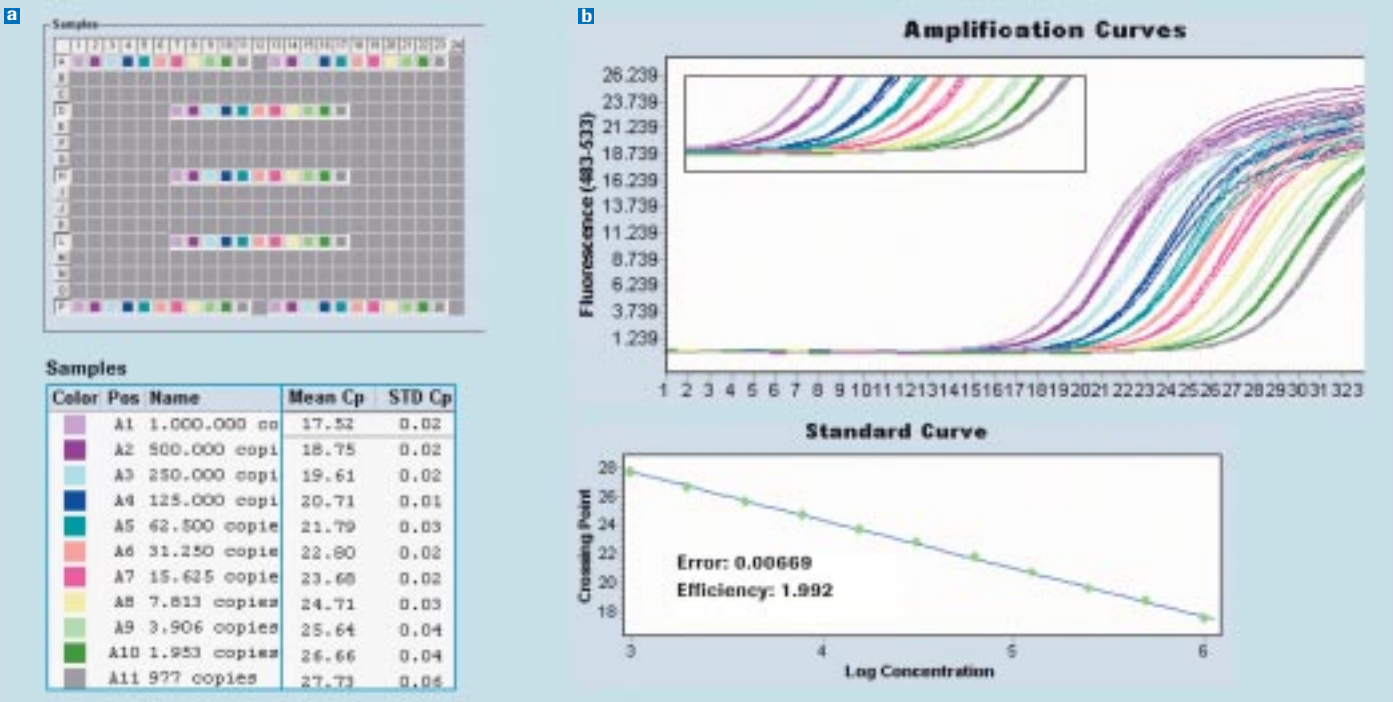

Figure 4: Two-fold resolution of the LightCycler® 480 Instrument. Serial 1:2 dilutions with seven replicates of a viral target sequence were assayed with the LightCycler® SYBR Green I Master reagent. (*a*) Copy numbers (below) and pipetting scheme (above) (*b*) Amplification curves (color match those used in *(a)*, *i.e., grey*: 977 copies, *green*: 1953 copies, *ect.*) and standard curve.

Demonstrating qPCR Accuracy

As outlined above, temperature homogeneity is a major prerequisite for qPCR reproducibility across a multiwell plate. Melting temperature (T_m) analysis provides a powerful and highly sensitive tool for monitoring (Figure 2). In a corresponding experimental setup, the same reaction mix (an oligonucleotide bound to a complementary SimpleProbe probe) is added to wells at many different positions on the plate and fluorescence is monitored while the temperature is raised slowly at a constant rate. At the specific melting temperature of the reaction mix, the decrease of fluorescence reaches a maximum. Highly reproducible T_m values (Figure 2a) are only observed if – during the melting process – the temperature reaches the same value simultaneously everywhere on the plate. On the other hand, inhomogeneous heating leads to melting temperatures that appear higher or lower than the expected value (Figure 2b).

Profiting from qPCR Accuracy

Designed for high reproducibility, the LightCycler® 480 System makes it possible to perform qPCR assays with very low inter- and intra-assay variance. When dilution replicates are positioned on different areas of the plate ("walk-around-the-block" experiment), it can be shown that even low concentrations of target give highly reproducible amplification curves. As illustrated in Figure 3, this finally results in a high dynamic range of the system, allowing the reliable detection and quantification of 10^{10} to 10^0 copies of a target gene. Within that dynamic range, concentrations differing only by a factor of two can be distinguished reproducibly as well (Figure 4).

In both types of experiments, the LightCycler® 480 System shows outstanding resolution, sensitivity, and reproducibility, as well as high homogeneity of PCR data (Cps) over the entire plate. The reproducibility is reflected by high crossing-point uniformity within replicate groups and low standard variation.

As fluorescence detection is largely independent of a sample's position on the plate, there is no need for normalization to a reference dye's signal (*e.g.*, ROX) in the reaction mixture.

Summary

As illustrated by the features of its individual components (hardware, software, and reagents) and the experimental data presented, the LightCycler® 480 System as a whole provides optimal conditions for highly accurate and reproducible real-time PCR applications.

With the LightCycler® family of PCR systems, Roche Applied Science has redefined real-time PCR speed and accuracy a number of years ago. For the first time, a real-time PCR platform now offers the LightCycler® System's unique combination of accuracy and speed also for multi-well plate-based assays, with comparable performance on the level of 96 or 384 sample throughputs and for all relevant qPCR applications. ■

Order INFO

Product	Pack Size	Cat. No.
LightCycler® 480 Instrument	96-well or 384-well instrument	04 640 268 001 04 545 885 001
LightCycler® 480 SYBR Green I Master	5 x 1 ml (2x conc.)	04 707 516 001
LightCycler® 480 Probes Master	5 x 1 ml (2x conc.)	04 707 494 001
LightCycler® 480 Genotyping Master	4 x 384 µl (5x conc.)	04 707 524 001
LightCycler® 480 Multiwell Plate	96-well plate 384-well plate	04 729 692 001 04 729 749 001
LightCycler® 480 LIMS/Bar-Code Module	1 software package	04 727 886 001
LightCycler® 480 Genotyping Software	1 software package	04 727 860 001
LightCycler® 480 Relative Quantification Software	1 software package	04 727 851 001