High-Throughput Analysis Using the Novel LightCycler® 1536 Real-Time PCR System

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Introduction
An important trend in biological and biochemical analysis over the past 15 years has been miniaturization and parallelization of analytical procedures. Large-scale high-throughput analysis of gene expression or genetic polymorphisms is an essential element of modern functional genomics, where individual samples are screened against many thousands of target genes. According to its superior sensitivity and accuracy, qPCR is a well-established gold standard for precise profiling of gene expression levels or genotyping of known SNPs.

The LightCycler® 1536 System is a novel platform, capable of performing qPCR-based DNA/RNA analysis in an array-like format at high-speed, in a proprietary multiwell plate in 1536-format. Scaling qPCR to a higher throughput, with concurrent miniaturization of individual reactions, provides a new generation of an enabling nucleic acid analysis system, combining the strengths of qPCR with the parallelism and throughput capability of low-density microarrays.

LightCycler® 1536 System:
General design and system components
Large-scale PCR-based gene analysis can be performed only if signals are generated, captured and analyzed in a highly reproducible manner. More precisely, thermal control, optical readout, and the algorithms used to characterize genes qualitatively and quantitatively have to be highly precise and reliable. The LightCycler® 1536 System meets these requirements at an excellent level, with hardware, software, consumables, and reagents all contributing to its superb performance in a high-throughput environment.

LightCycler® 1536 Instrument
The LightCycler® 1536 Instrument (Figure 1) is based on the well-established LightCycler® 480 platform architecture. It comes with a novel thermal cycler module tailored for heating and cooling of miniaturized qPCRs in a multiwell plate with 1536 individual reaction vessels. To perform optical readout of chemical detection formats as specific as possible while reducing the overall complexity of experimental layouts in a high-throughput scenario, the LightCycler® 1536 Instrument supports the combination of two excitation wavelengths (465nm; 530nm) with two detection filters (510nm; 580nm). These filters are optimized for detecting green intercalating dyes as well as mono- and dual-color hydrolysis probes (e.g., FAM, Yellow 555).

LightCycler® 1536 Software
The LightCycler® 1536 System provides a basic software module enabling users to easily set up and run reaction protocols. The analysis module is based on highly robust algorithms providing Cp-values and endpoint fluorescence data. Compared with classical LightCycler® Systems, the LightCycler® 1536 Software particularly provides enhanced compatibility to automated high-throughput data analysis workflows. Straightforward features for sample list import and result export, either in common XML or text formats, as well as storage of results in a clearly arranged file-based architecture, strongly support data handling in a high-throughput environment.

LightCycler® 1536 Multiwell Plate
The LightCycler® 1536 Multiwell Plate is the first high-density qPCR plate for real-time PCR analyses enabling unsurpassed thermal performance in miniaturized reaction volumes of only 0.5–2 µl. The plate consists of two components (Thermaxis® technology): a thermally con-
ductive part containing well-like structures for the reaction liquid and an insulating top-layer which prevents the impact derived from the heated cover of the instrument. Continuous V-shaped grooves at the bottom of the multiwell plate support a perfect fit to the thermal cycler mount and optimal thermal coupling (Figure 2).

**LightCycler® 1536 reagents**
The LightCycler® 1536 System includes generic master mixes, RealTime ready Master, optimized for low-volume qPCR, in particular in combination with an automated reaction setup (e.g., performed by a liquid dispenser).

Main applications such as qualitative and quantitative gene expression as well as endpoint genotyping assays are supported. In modern high-throughput facilities, where tens of thousands of individual data points are generated per working day, all elements of an analytical workflow have to be highly reliable, robust and traceable. The latest generation of RealTime ready reagents provide an advanced surveillance concept suitable for proper workflow process quality control as well as for tracking and visualization of pipetting errors. Furthermore, these novel reagents – which are developed for both plate-based LightCycler® platforms (LightCycler® 480 / 1536 Systems) – are compatible with fast hot-start protocols, thereby ensuring maximum speed during thermal cycling.

**Performance Data**
As illustrated by the features of its individual components (hardware, software, consumables, reagents) and by the following experimental examples, the LightCycler® 1536 System as a whole provides optimal conditions for highly accurate and reproducible real-time PCR applications in a high-throughput scenario. Reproducibility and resolution power of the system can be demonstrated using, e.g., a 1:2 serial dilution over 16 different concentration steps (Figure 3).

Learn more about the novel LightCycler® 1536 System at www.lightcycler1536.com

**Figure 2:** Schematic of the LightCycler® 1536 Multiwell Plate.

**Figure 3:** Sensitivity and reproducibility of the LightCycler® 1536 System. Sixteen concentration steps of a 1:2 series dilution from $10^6$ to $3 \times 10^1$ copies/sample (96 replicates each) distributed in a checkerboard scheme across the entire multiwell plate as depicted in the Cp heatmap chart (6 times 4 x 4 replicates).

**Product**

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**Coming Soon**

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