

LightCycler® 480 Real-Time PCR System: Innovative Solutions for High Throughput PCR

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Introduction

Since their introduction in 1998, the LightCycler® Real-Time PCR Systems from Roche Applied Science have stood for maximum flexibility, speed, and data accuracy. The latest instrument, the LightCycler® 480 Real-Time PCR System, continues this tradition and extends it to higher throughputs by permitting plate-based analysis.

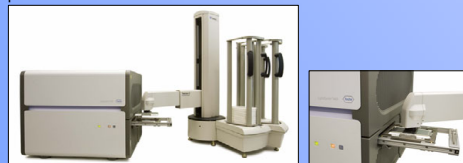
The plate-based LightCycler® 480 System is a highly versatile real-time PCR system for analysis of gene expression and genetic variation. The modern instrument design, outstanding technical and software features, as well as the advanced reagents and disposables that are part of the LightCycler® 480 System, are equal to the demands of even the most challenging real-time PCR applications.

In addition, the LightCycler® 480 System offers a new mutation scanning technique based on high-resolution melting (HRM). This highly sensitive post-PCR method enables high-throughput SNP screening at much lower cost.

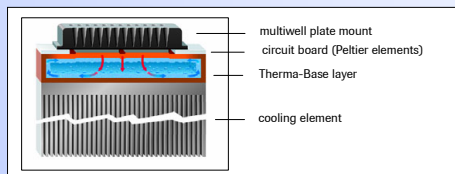


Innovative Solutions for High throughput PCR

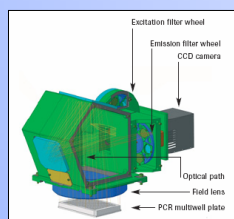
Since the software controls system loading procedures, the PCR run, and data analysis, the LightCycler® 480 System can easily be integrated into a computer-controlled environment or a completely automated workflow. The system's components (block cycler unit, optical system and software) ensure highly robust and stable performance.



The LightCycler® 480 System has revolutionized block cycler temperature control by introducing a highly efficient heat-equalizing layer (Therma-Base) between the heat block and the cooling element. The Therma-Base layer is a thin cavity lined with a wick structure and filled with fluid. By using condensation and evaporation to dissipate heat, the Therma-Base can rapidly adjust to temperature changes.

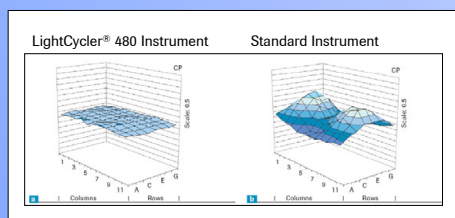


The excellent performance of the optical system in the LightCycler® 480 Instrument is due to a high intensity xenon lamp, which emits light across a broad spectrum. Flexible, built-in filters can be adjusted to produce many excitation and emission wavelengths, thus allowing a variety of fluorescent dyes and detection methods to be used. The special arrangement of the optical components and the optimum focal length in the LightCycler® 480 Instrument ensure specific signal excitation and uniform data capture across the entire multiwell plate, independent of sample position.



Demonstrable Performance

By removing the effects of spreading resistance, the LightCycler® 480 thermal block cycler provides well-to-well temperature homogeneity. The lack of inter-well temperature variability across the entire multiwell plate (shown below) confirms this homogeneity. Due to this high temperature homogeneity, the crossing point (CP) homogeneity across all samples is exceptionally high compared to that of other real-time PCR instruments; this is especially evident when a low concentration (100 copies) of a given target sequence (442 bp) is amplified via a fast PCR protocol (20 µl reaction volume, hydrolysis probe format).



Applications Supported by the Software

The innovative LightCycler® 480 software combines state-of-the-art design and unique LightCycler® 480 algorithms. These ensure fast, accurate data generation without sacrificing versatility. The software can handle all the most common real-time PCR applications like gene detection, gene expression, genotyping analysis, and gene scanning.

Gene Detection: Benefit from an advanced optical system and enhanced multiplexing capabilities for multitarget analysis.

Gene Quantification: Easily customize data analysis using either basic or advanced quantification methods. Utilize sophisticated software and unique algorithms to generate highly accurate gene quantification data (e.g., E-Method).

Genotyping: Achieve reliable genotyping results via superior post-PCR melting curve analysis. Save costs by covering all alleles of a given SNP with a single probe.

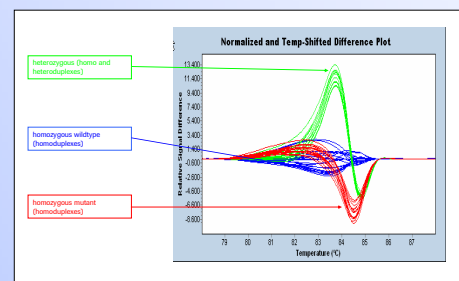
Gene Scanning: Employ the innovative high-resolution melting (HRM) method to scan genes for unknown variations.

Gene Scanning

This novel technique can scan for unknown mutations in a given sequence as shown in the example below.

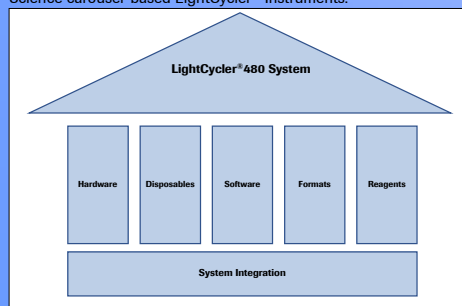
This new application requires only PCR reagents, a simple primer pair for the gene of interest, and a special fluorescent dye (available in the LightCycler® High Resolution Melting Master). After PCR, the amplicons are analyzed by high-resolution melting. Amplicons with sequence variations can be differentiated due to the different shapes of their melting curves.

For this technique, an instrument must have precise temperature control, high-resolution fluorescence acquisition and appropriate Data Analysis Software; all are available on the LightCycler® 480 System.



Systems Technical Background

Innovative technological enhancements allow the LightCycler® 480 Instrument to set new benchmarks for rapid data generation and accurate real-time PCR analysis. In particular, the sophisticated design of the thermal block cycler unit, the optical system, and the software deliver the unparalleled sensitivity, accuracy, and reproducibility one has come to expect from Roche Applied Science carousel-based LightCycler® Instruments.



Conclusion

The LightCycler® 480 System is a powerful combination of proven high performance technology and innovative instrument design. It delivers accurate real-time PCR data quickly, and permits you to automate your PCR workflow using multiple robotic interfaces, so you can generate data around-the-clock. With support for multiple RT-PCR applications, including high-resolution melting, the LightCycler® 480 Real-Time PCR System supports a broader range of research applications than is currently possible with other plate-based real-time PCR systems.

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