



Diagnosics

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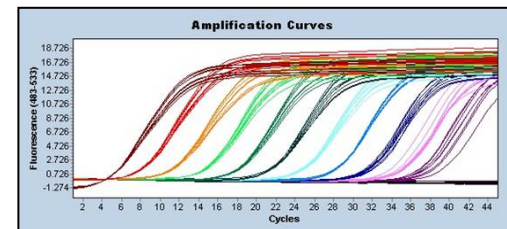
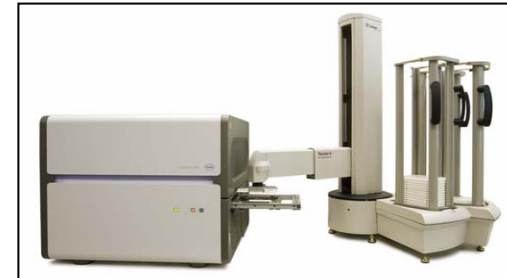
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# LightCycler<sup>®</sup> 480 Real-Time PCR System: Innovative Solutions for High-Throughput PCR

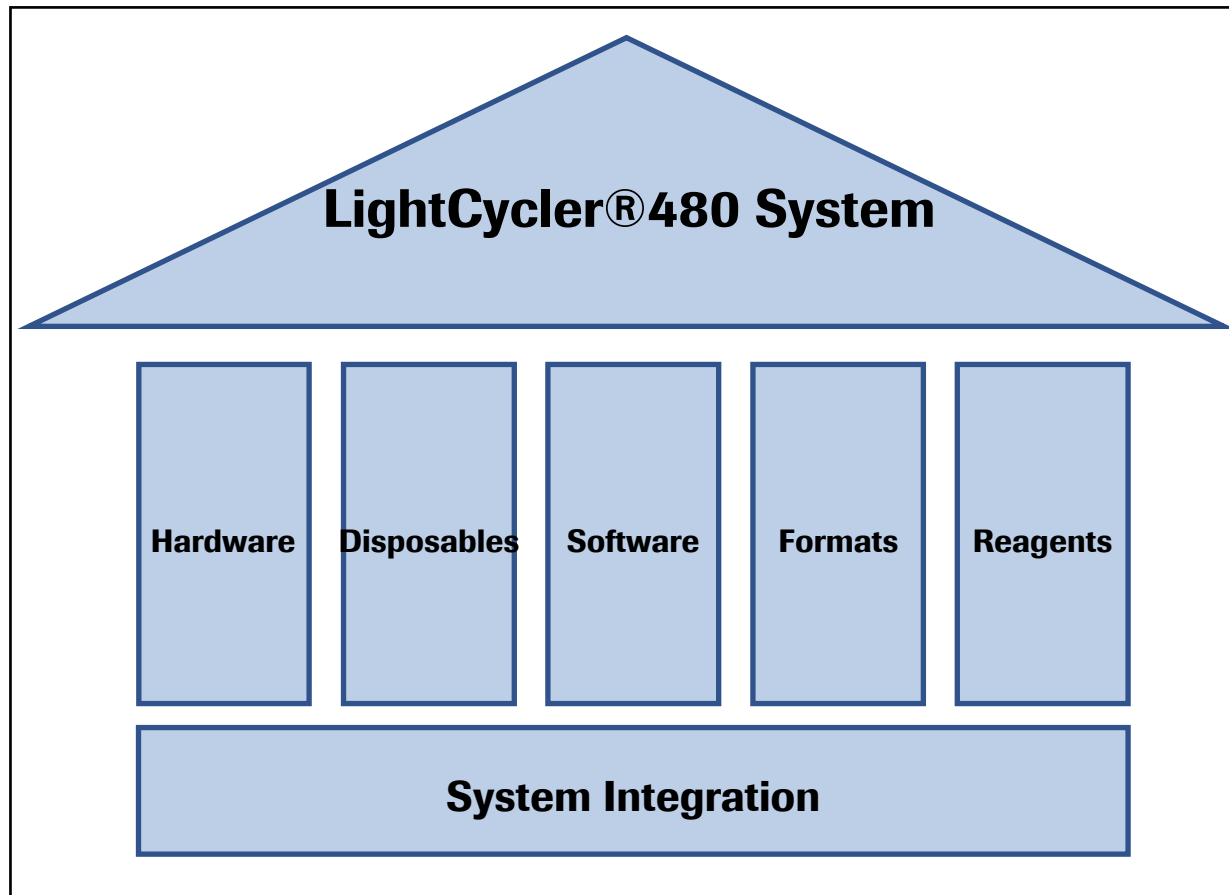


# Options for High-Throughput PCR

- Robotics
  - Automated plate loading
  - Ready-to-use and stable reagents
  
- Increasing throughput by mean of sample throughput:
  - 96 and/or 384 well plates
  - Rapid real-time PCR
  - Monoplex vs. multiplex PCR
  
- Software Solutions
  - Template function
  - Automated data analysis
  - Macro (automated experimental setup plus analysis)
  - Export/Import of data or LIMS connectivity



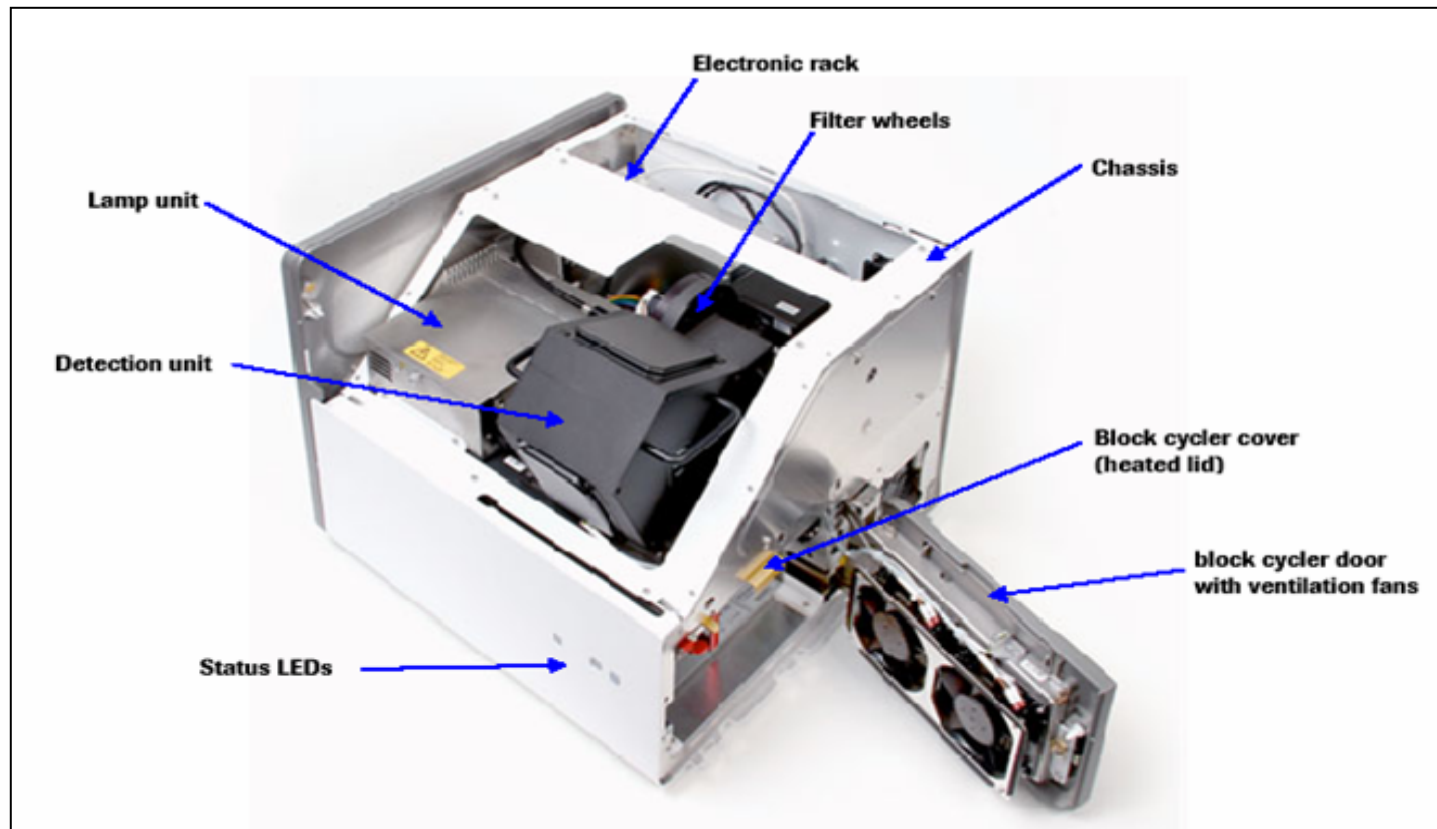
# Prerequisite for High-Throughput PCR



- Real-time PCR is simultaneous amplification and detection of nucleic acids within on device
- As the real-time PCR System is assembled out of subcomponents (e.g., block cycler, optical unit, software), the quality depends on all parts of the system
- If automation is required, the proper interaction of all subcomponents must be given

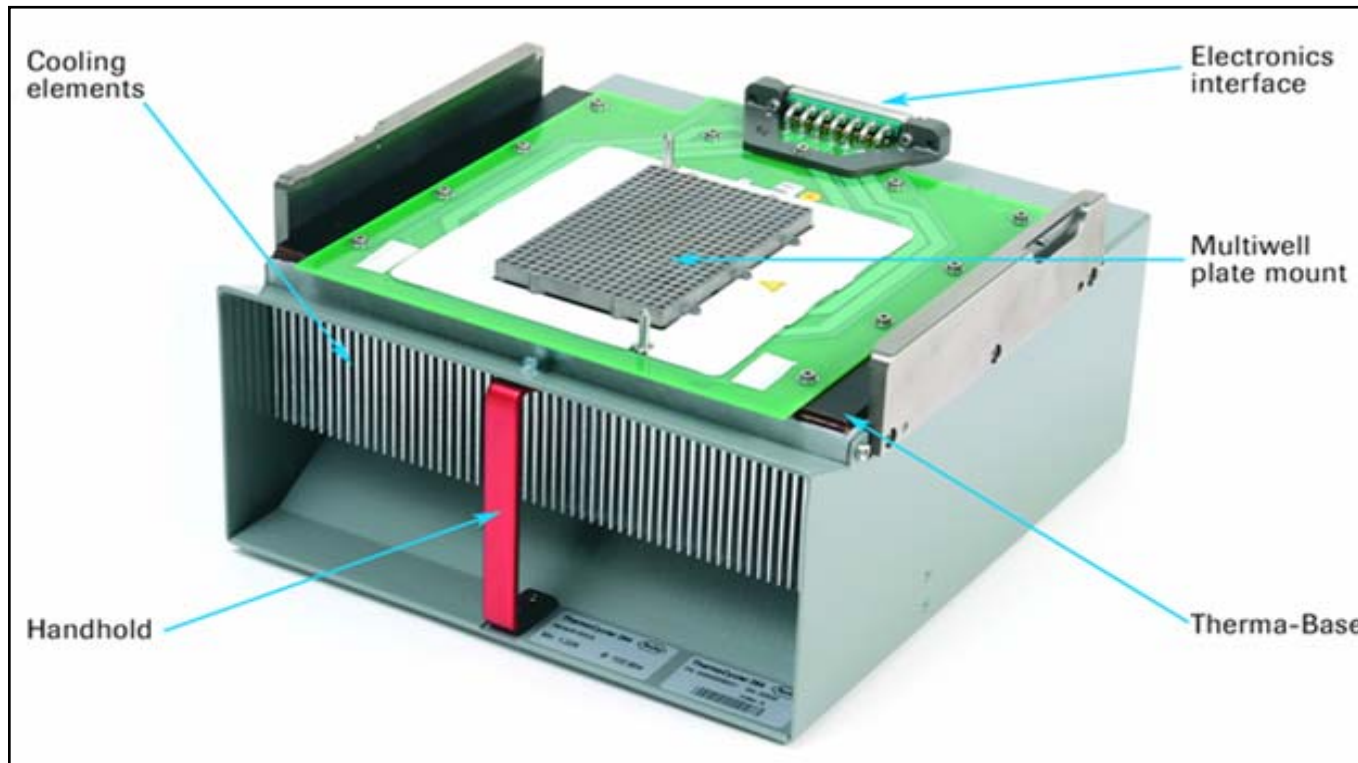
# LightCycler<sup>®</sup> 480 Instrument

## *General Architecture*



# LightCycler<sup>®</sup> 480 Thermal Block Cycler

## *Speed and Accuracy*

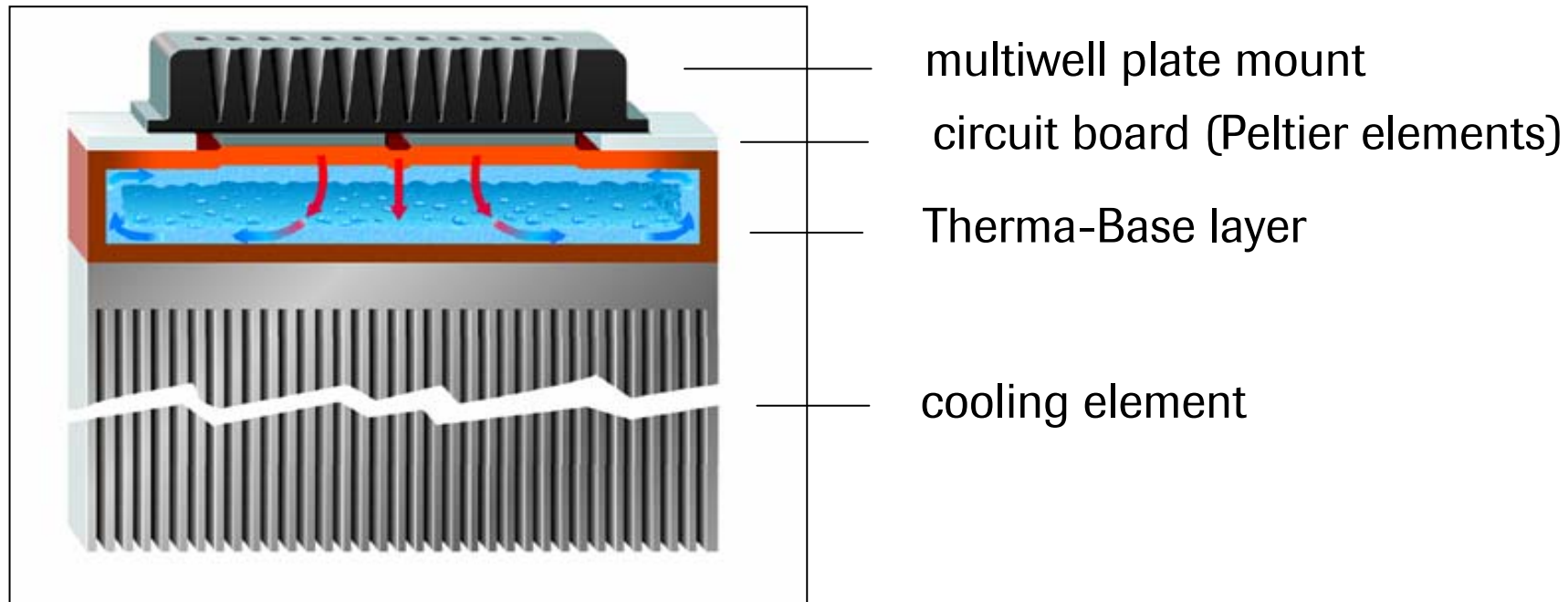


Therma-Base  
for optimized heat  
equalization

- **Homogenous temperature distribution over the plate**
- **Fast PCR runs:**  
**96 wells in < 1 hour**  
**384 wells in < 40 min**

# LightCycler<sup>®</sup> 480 Instrument Heat Sink

## *Therma-Base*



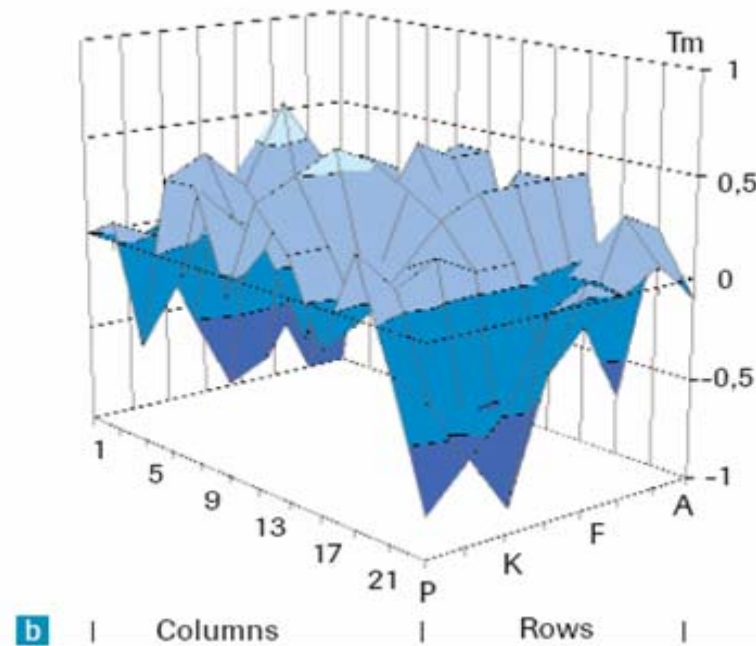
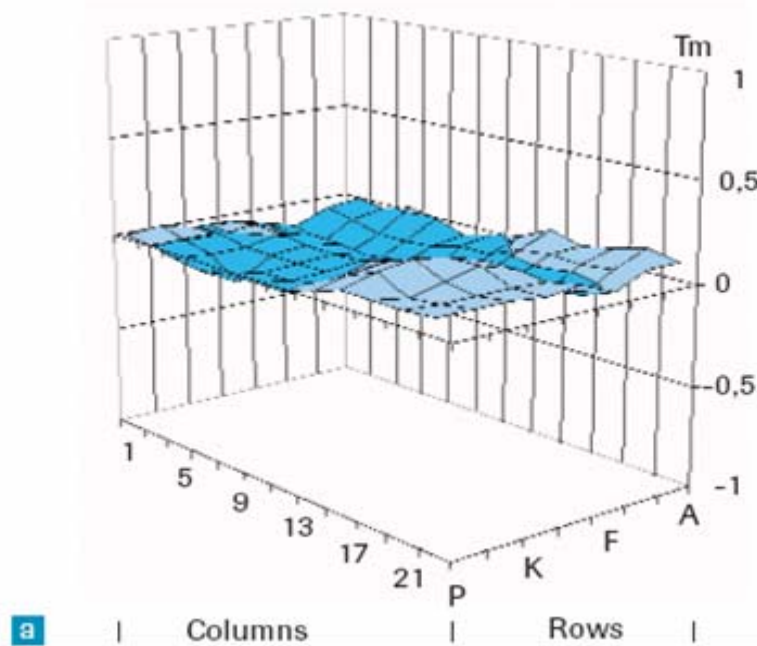
- **Thin sealed vacuum vessel with working fluid in a wick structure**
- **Rapidly transfers heat by evaporation and condensation**
  - **enables both rapid and accurate cycling!**

# Thermal Uniformity *Instrument Comparison*



## LightCycler<sup>®</sup> 480 Instrument

## Standard Instrument

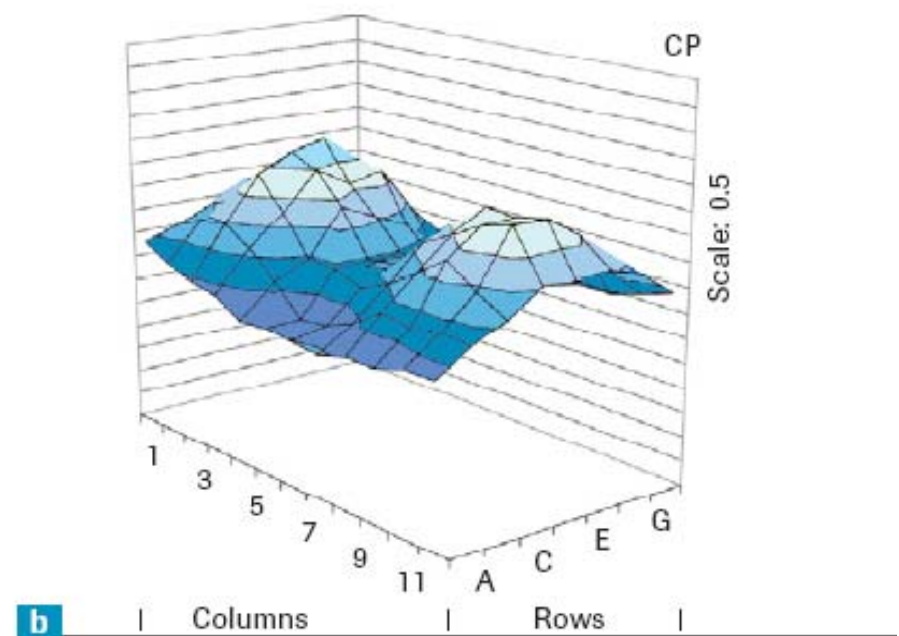
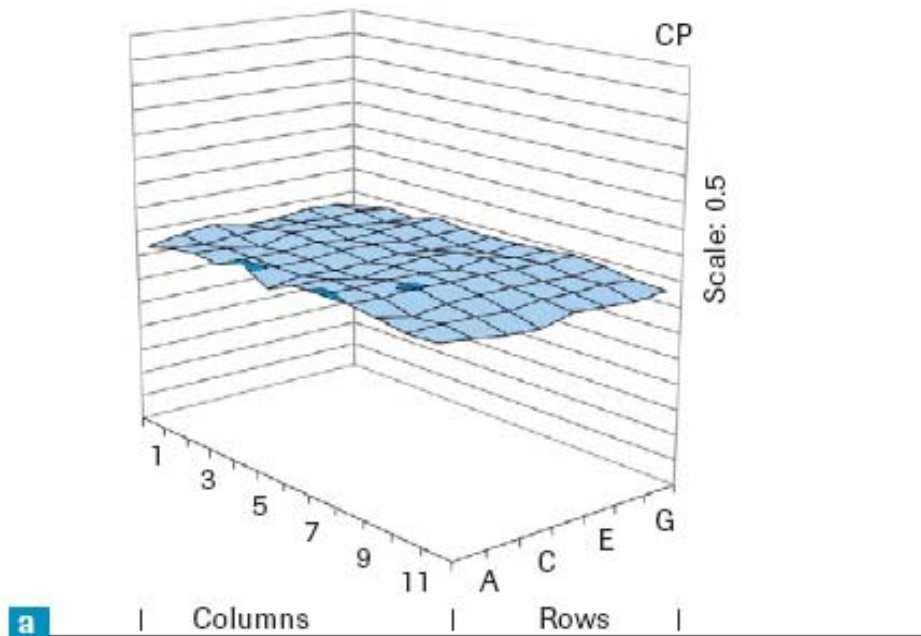


# Thermal Uniformity *Instrument Comparison*



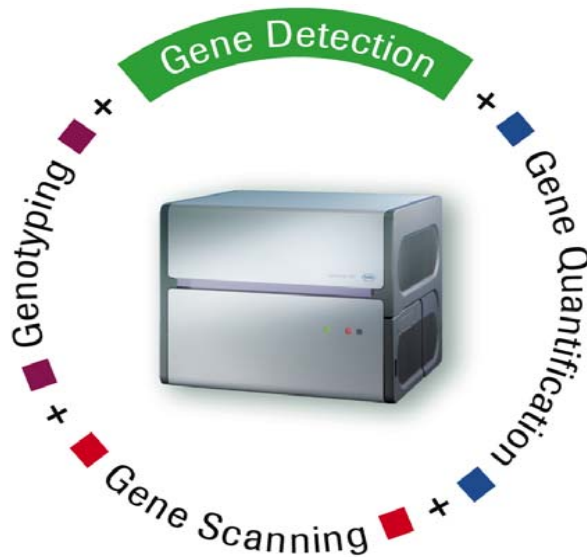
## LightCycler<sup>®</sup> 480 Instrument

## Standard Instrument



# Versatility of the LightCycler<sup>®</sup> 480 System

## *Application Gene Detection*



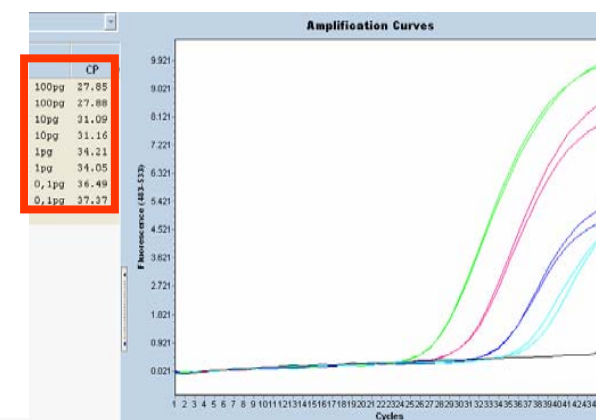
- Detecting e.g., bacteria in sample material
  - Rapid real-time PCR
  - Multicolor options

# LightCycler<sup>®</sup> 480 RNA Master Hydrolysis Probes

## *Speed and Reagents*

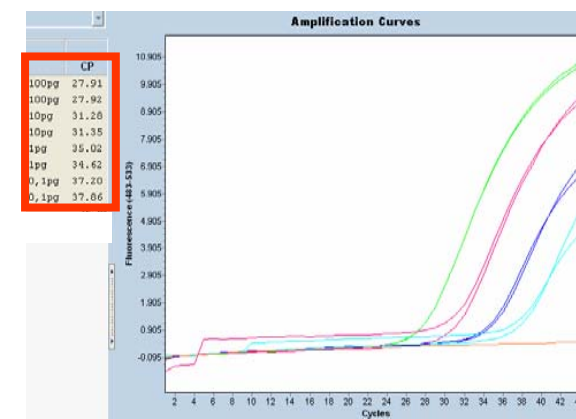
96 well format &  
conventional cycling protocol.  
Total time for 45 cycles:  
**1h 6 min.**

Temp (°C)	Time	Cycles
63	<b>3 min</b>	1
95	30 sec	1
92	10 sec	45
60	30 sec	
72	1 sec	



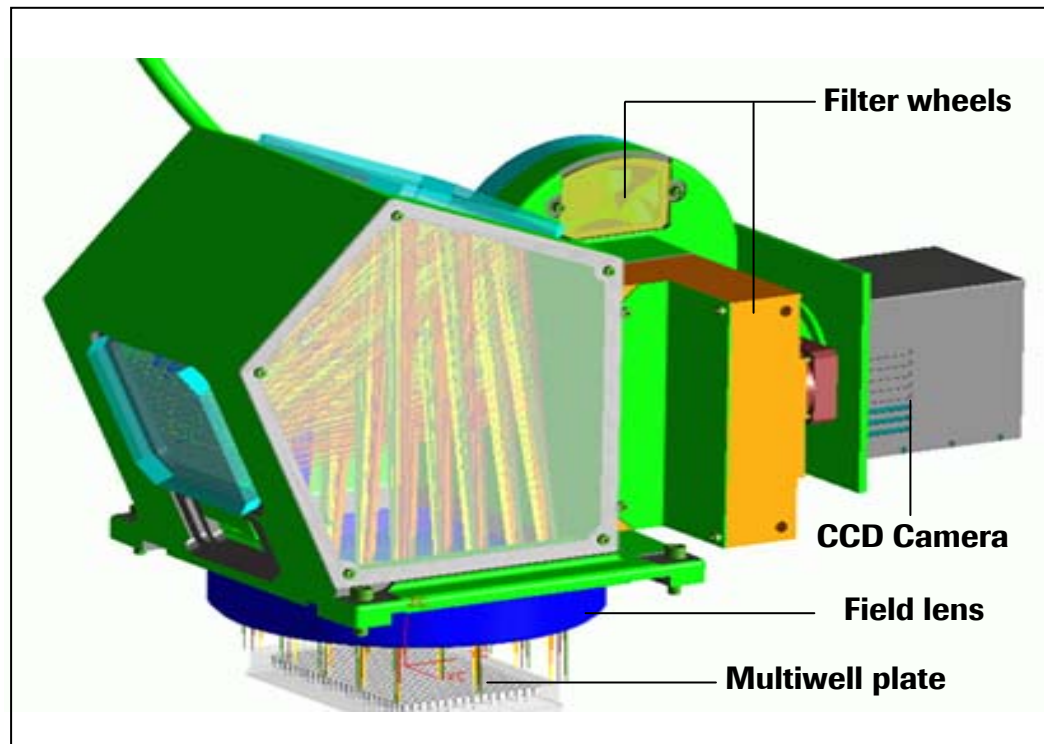
96 well format &  
fast cycling protocol.  
Total time for 45 cycles:  
**42min:31sec.**

Temp (°C)	Time	Cycles
63	<b>3 min</b>	1
95	30 sec	1
92	<b>1 sec</b>	45
60	<b>10 sec</b>	
72	<b>1 sec</b>	



# LightCycler<sup>®</sup> 480 Optical System

## *Sensitivity and Homogeneity*



- **Xenon lamp**
- **CCD camera**
- **Five excitation filters**
- **Six detection filters**
  
- **Optimized arrangement of optical components**
- **Homogeneous excitation and fluorescence detection**



# LightCycler® 480 System

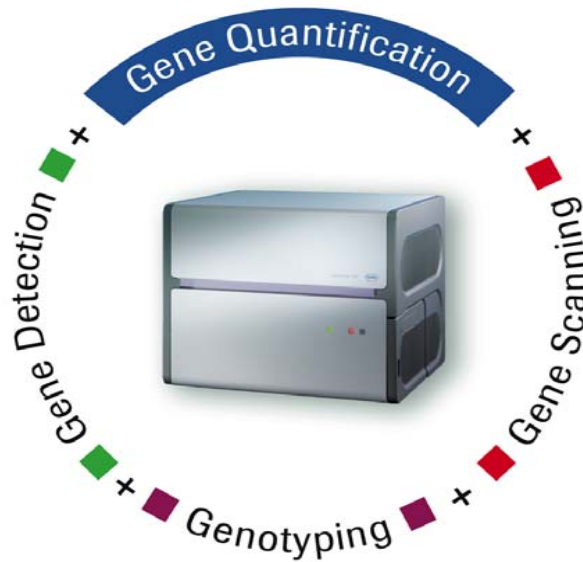
## Assay Formats and Dyes

<b>Xenon lamp</b>							
<b>Excitation filters</b>	450	483	483	523	558		615
<b>Emission filters</b>	500	533	533	568	610	640	670
<b>Dyes (Examples)</b>	LightCycler® Cyan 500	SYBR Green I	Fluorescein (Fluos / FAM)	HEX (VIC)	LightCycler® Red 610	LightCycler® Red 640	Cy5
<b>Detection formats</b>	Hydrolysis probes (R), HybProbe probes (D)	SYBR Green I	Hydrolysis probes (R), HybProbe probes (D), SimpleProbe probes (R)	Hydrolysis probes (R), HybProbe probes (A)			

Legend: Reporter (R), Donor (D), Acceptor (A).

# Versatility of the LightCycler<sup>®</sup> 480 System

## *Application Gene Quantification*



- Analyzing expression level of gene of interest
  - $\Delta\Delta\text{ct}$  if basic method for analysis is required
  - The  $\epsilon$ -method to use individual PCR efficiency for calculation for advanced analysis options

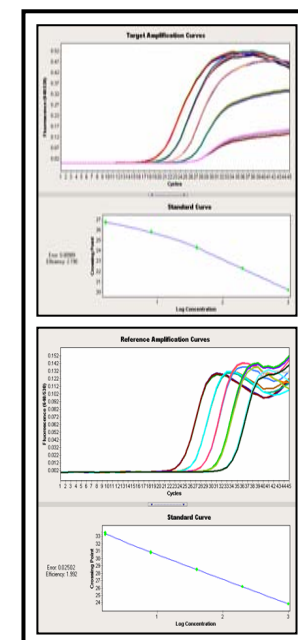
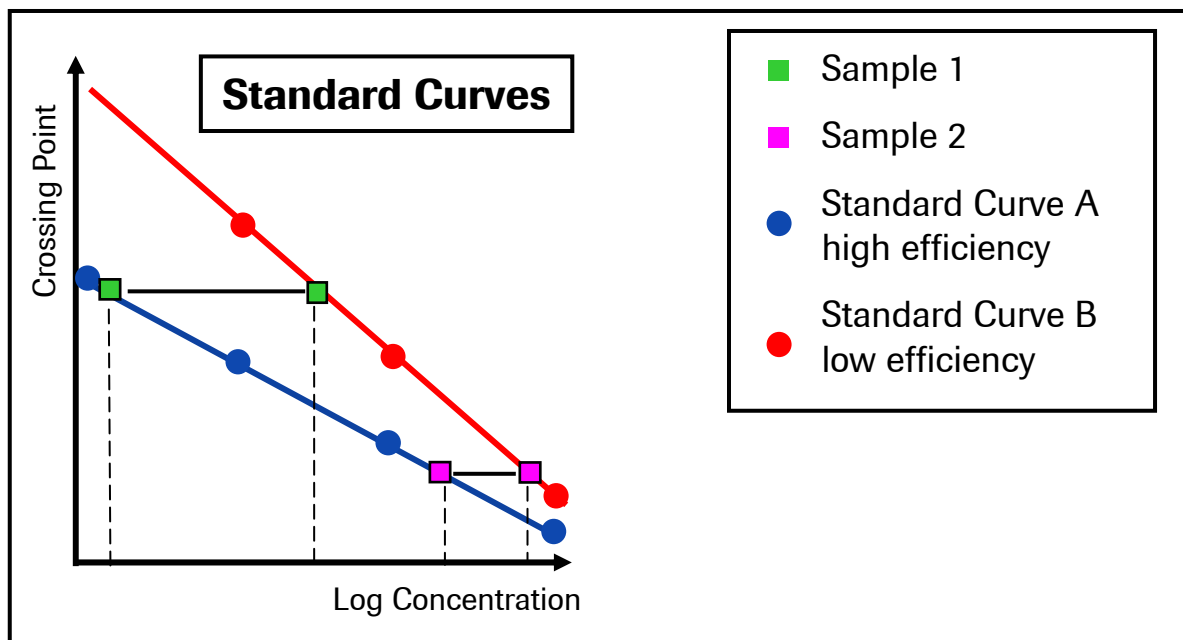
# Calibrator normalized Relative Quantification

## *Target Normalized to a Reference Gene*

<p><b><u>Approximative</u></b>                  = without efficiency correction  <math>\Delta\Delta C_T</math> method</p>
<p>no PCR efficiency correction: <math>E = 2</math></p>
<p><b>relative amount = <math>2^{-\Delta\Delta C_T}</math></b></p>

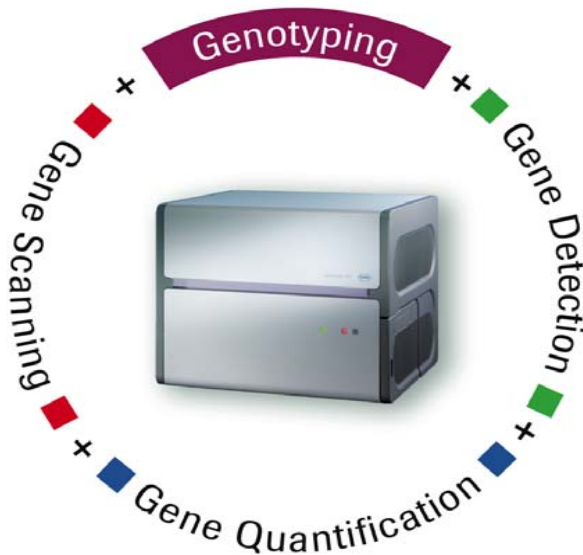
<p><b><u>Exact</u></b>                  = with efficiency correction  <math>E</math>-method</p>
<p>correction for PCR efficiency differences between target and reference</p>
<p><b>relative amount =</b>  <math>E_T^{CpT(C) - CpT(S)} \times E_R^{CpR(S) - CpR(C)}</math></p>

# Effect of PCR Efficiency Differences



# Versatility of the LightCycler<sup>®</sup> 480 System

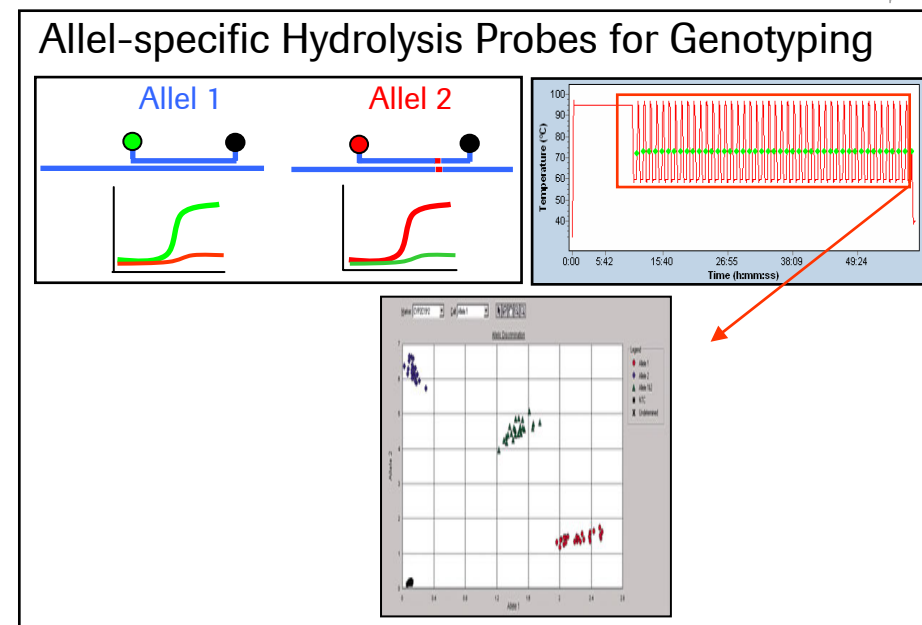
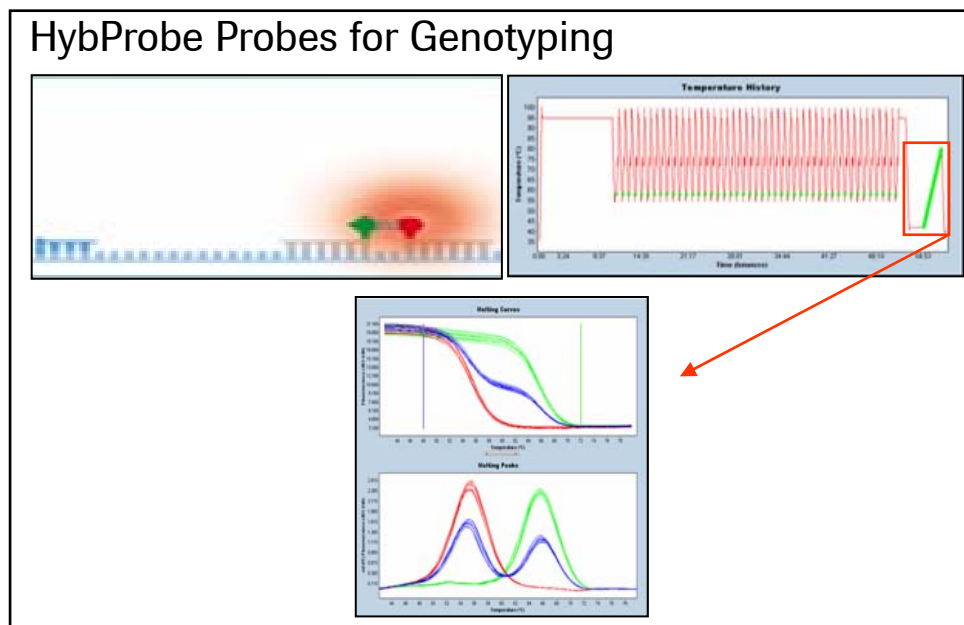
## *Application Genotyping*



- Detecting **known** variants
  - Based on labeled Probes

# Strategies for Genotyping

## *Melting Curve Analysis & Allel-specific PCR*

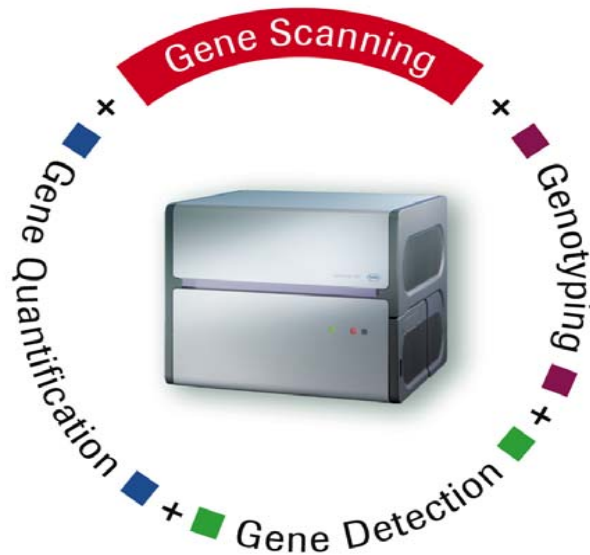


Provide the most versatile, robust and cost-effective high-throughput PCR-based mutation detection solution available (formats, algorithms, applications).

- Melting curve analysis with hybridization or SimpleProbe probes
- End-point analysis with hydrolysis probes

# Versatility of the LightCycler<sup>®</sup> 480 System

## *New Application Gene Scanning*



- Find **new** variants in gene of interest

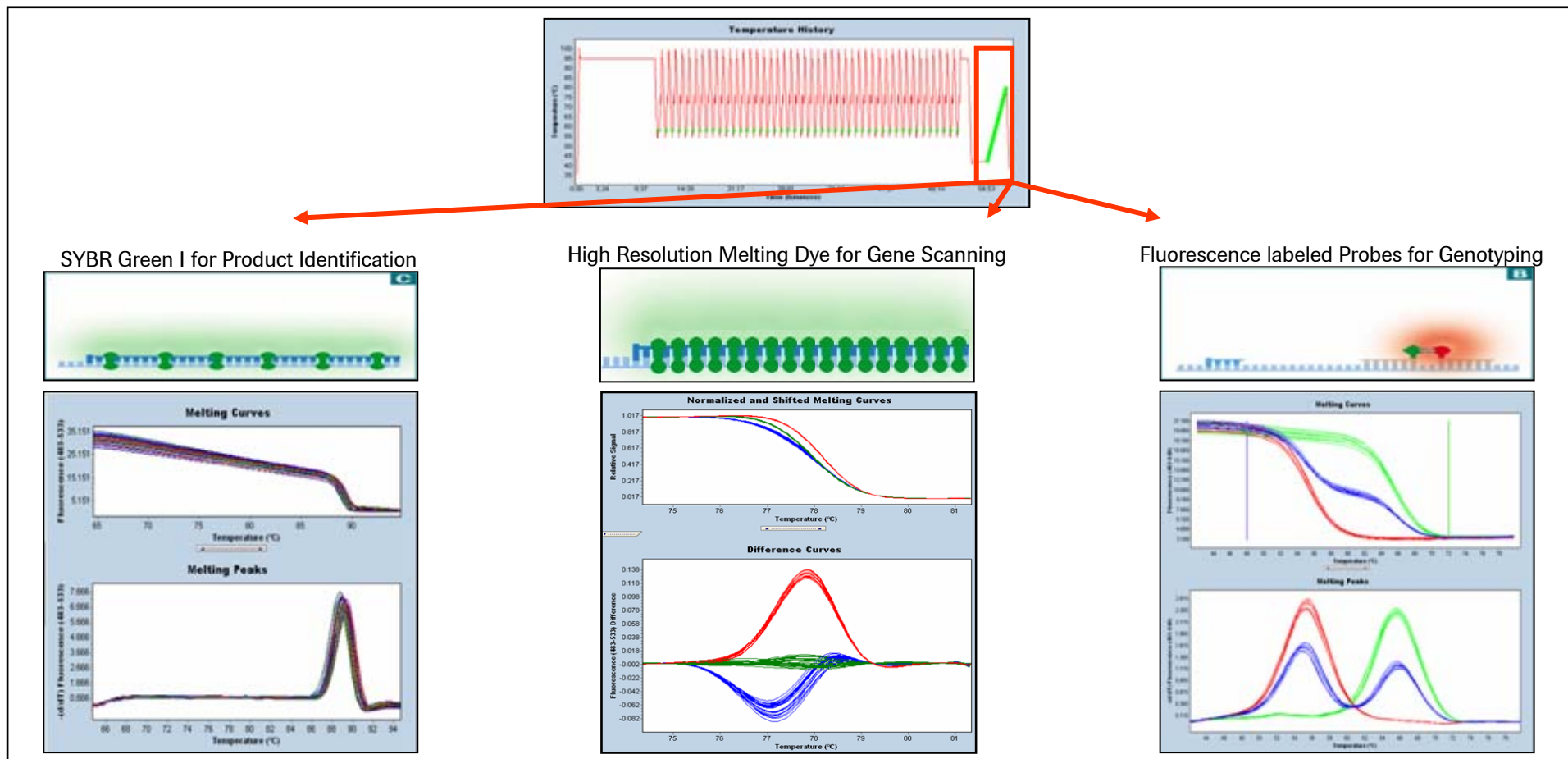
## Prerequisites and Innovations

### *What Is Needed to Perform HRM?*

- **Precise Instrument** to allow genotyping and/or mutation scanning of whole PCR products.
  - homogenous temperature profile and temperature control
  - high sensitivity optical system (light source, filters and detection system)
- **Novel intercalating dye** to identify heteroduplex DNA
  - saturating, non-inhibitory dsDNA binding without redistribution during melting
- **Analysis software** generating normalized and temperature shifted difference plot (instead of melting curves derivatives used with probes)

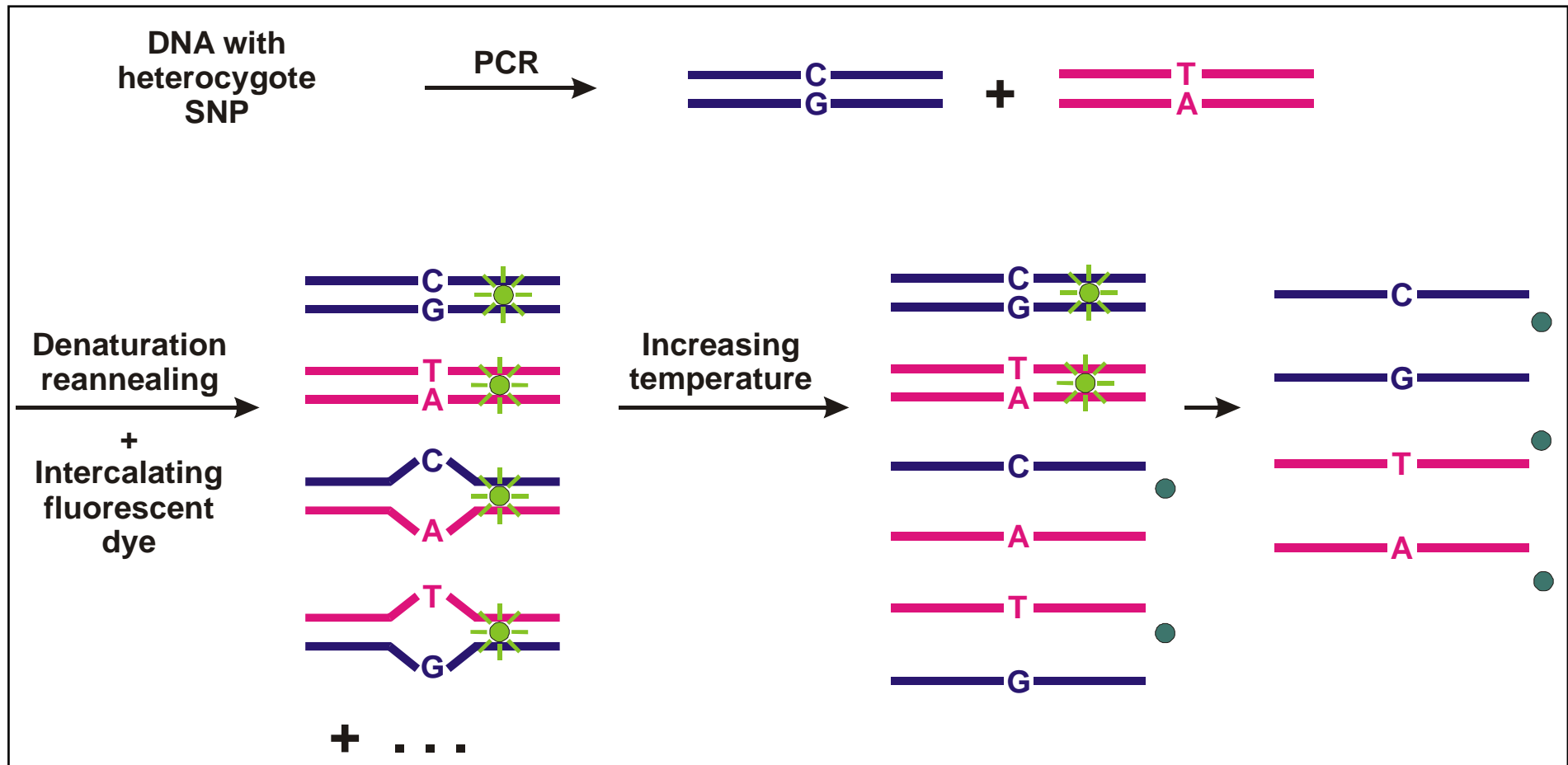
# Melting Curve Analysis

## *Established & New Applications*



# Amplicon Melting

## *Principle of Gene Scanning by HRM*



## Variation in $T_m$

- The  $T_m$  of an amplicon depends mainly on GC content. Alterations in the amplicon may influence the  $T_m$

Highest Stability

Lowest Stability

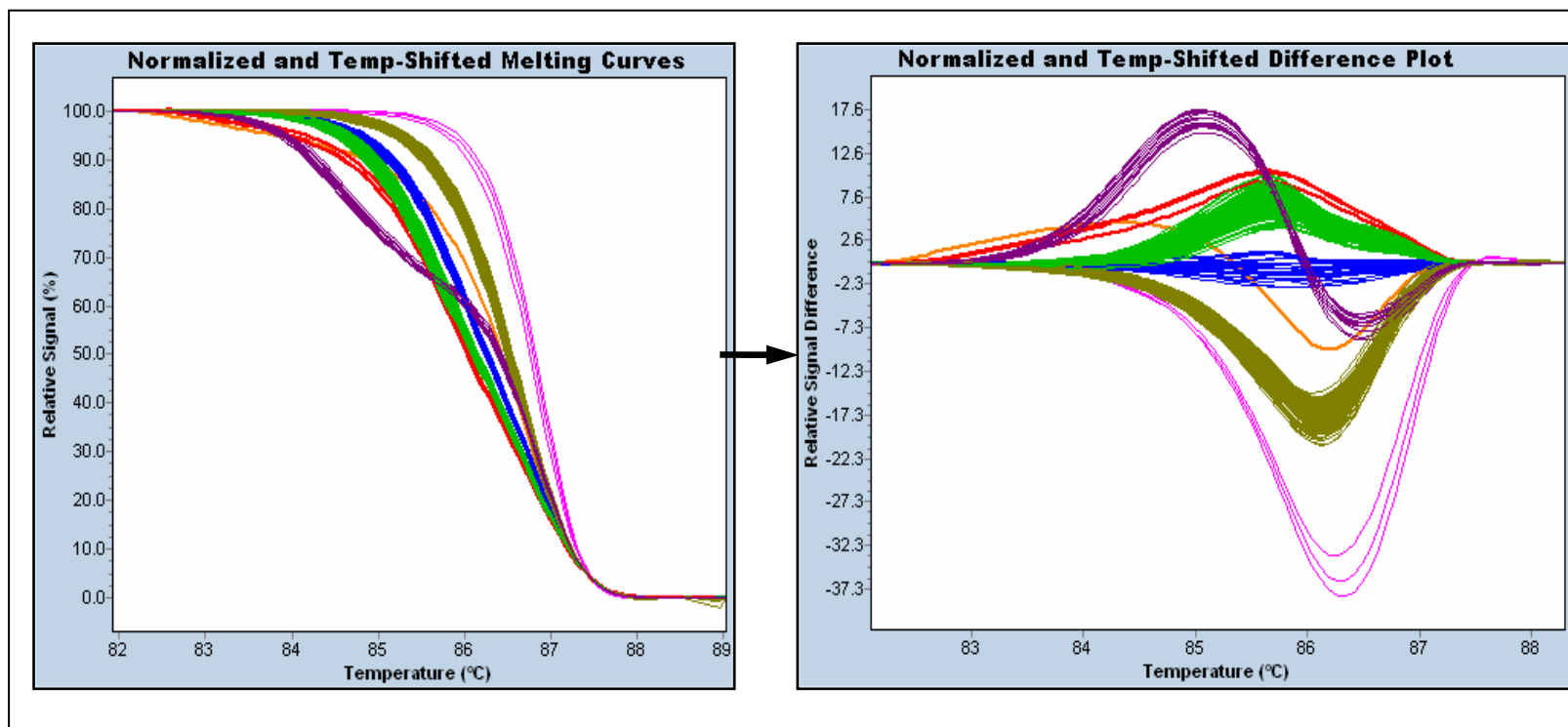
$G:C > A:T > G:G > G:T = G:A > T:T = A:A > T:C > A:C > C:C$

- Amplicon Melting of homozygote samples (containing homoduplexes wt or mut) give very similar curve shapes.
- Amplicon Melting of heterozygote samples (containing homo and heteroduplexes) give curve shapes which are highly distinct.

# Gene Scanning

## *Example: Target MBL2*

- Sequence variations in the MBL2 gene (384 samples, 219 bp amplicon): Differentiation of the four most frequent genotypes into four main groups; a few samples show three additional genetic variants.



# High-Resolution Melting

## *Novel Method With Many Potential Applications*

- Gene Scanning to discover SNPs and/or somatic mutations
- Genotyping of known SNPs
- Characterization of haplotype blocks
- Screening for loss of heterozygosity
- Allelic prevalence in a population
- Species identification/taxonomy
- DNA mapping (find individuals with many highly variable, informative loci)
- Association (case/control) studies
- Identification of candidate predisposition genes
- DNA methylation analysis



## Summary

- Real-time PCR depends on quality and proper interaction of all subcomponents of a real-time PCR System including reagents, disposables and detection formats
- The LightCycler® 480 System is a powerful combination of proven high performance technology and innovative instrument design.
- The LightCycler® 480 System supports a broader range of research applications than is currently possible with other plate-based real-time PCR systems.

