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LightCycler® 480

Real-Time PCR and LightCycler480 instrument

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Jesús Lascorz, PhD
EMEA Roche Life Science Customer Support Center
Mannheim - Germany

Support

Roche Customer Support Center - San Cugat

 sant_cugat.rde-call_center@roche.com

 **900 300 705**

Real-Time PCR

Detection Formats

LightCycler 480 Instrument

Real-Time PCR

Detection Formats

LightCycler 480 Instrument

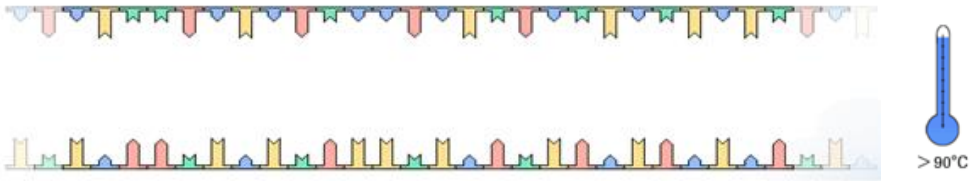
Polymerase Chain Reaction (PCR)

How does PCR work?

A typical PCR cycle consists of the following three steps:

1. **Denaturation (e.g. 95°C)**

   Separates double stranded DNA into single stranded DNA

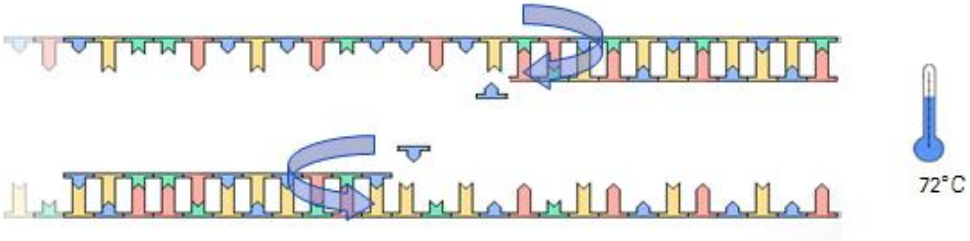


2. **Annealing (e.g. 60°C)**

   Targeting the sequence. Primers hybridize to the target DNA strand

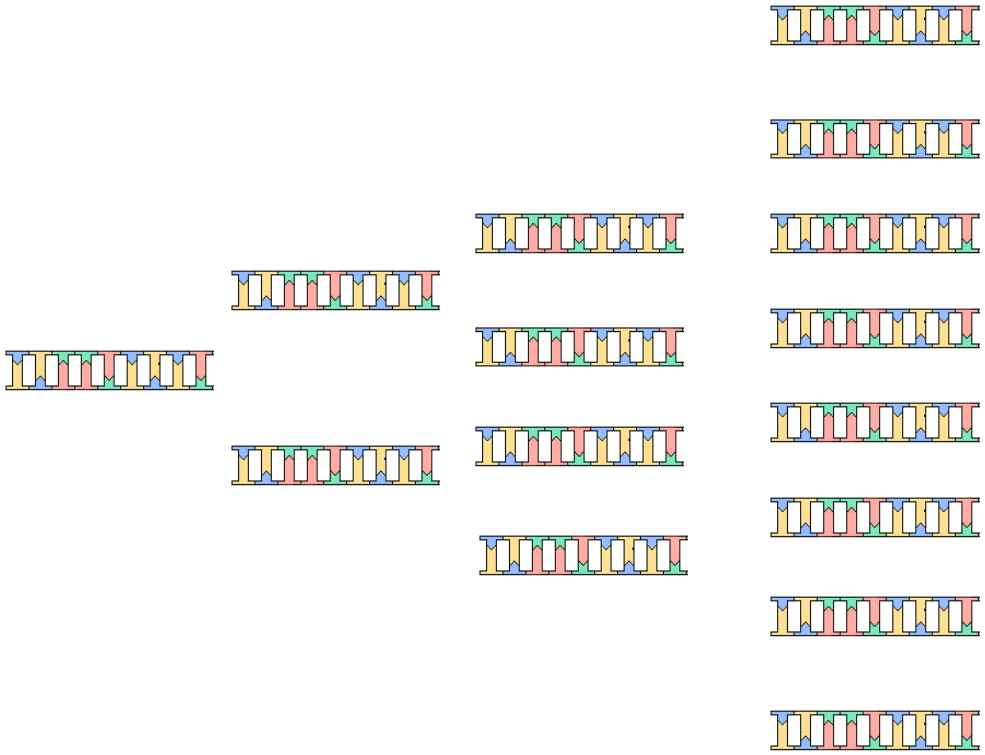
3. **Extension (e.g. 72°C)**

   DNA polymerase synthesizes new double-stranded DNA



Polymerase Chain Reaction (PCR)

How does PCR work?



Fragments: 1 2 4 8 ..... 2^{cycles}

Polymerase Chain Reaction (PCR)

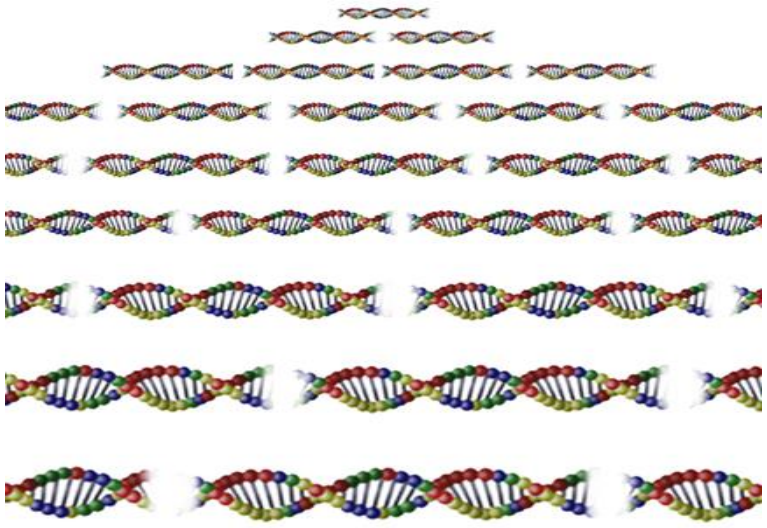
How does PCR work?

$$N = N_0 \times 2^n$$

N: Number of amplified molecules

N_0: Initial number of molecules

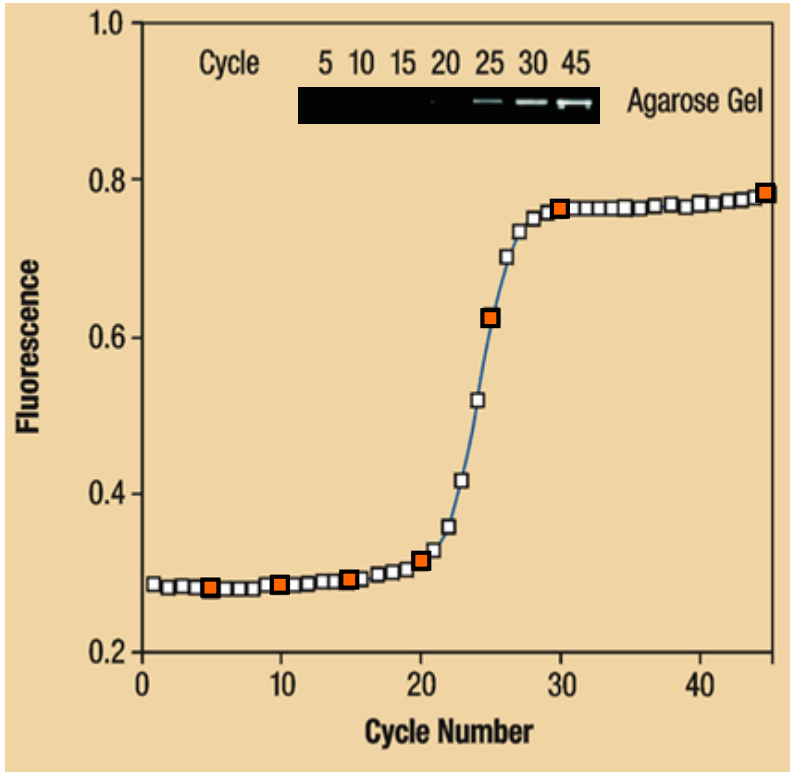
n: Number of amplification cycles



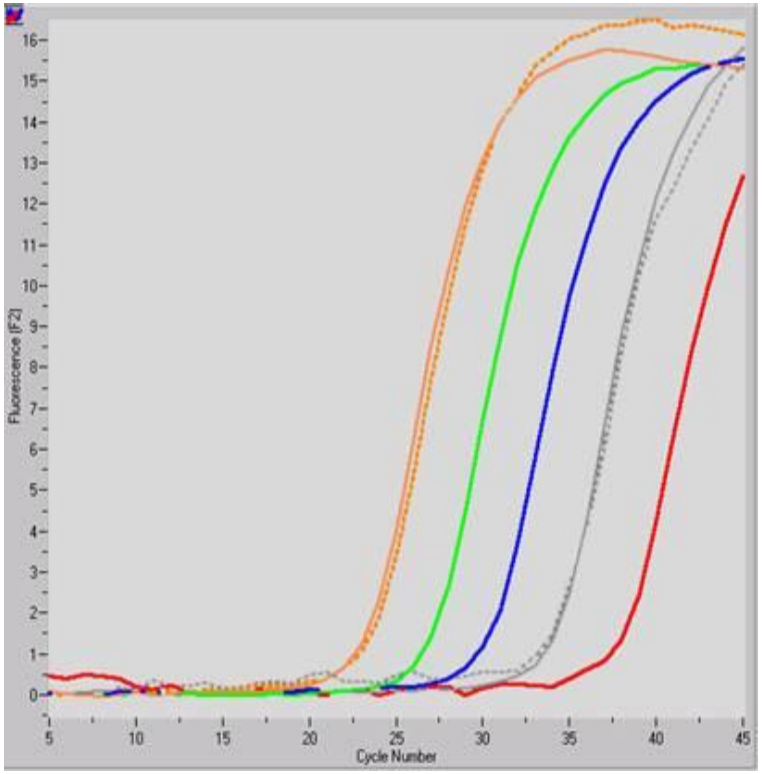
No. of Cycles	No. of Target Amplicons
1	2
2	4
3	8
4	16
5	32
10	1024
20	1,048,576 (1.0 E6)
30	1,073,741,824 (1.0 E9)
37	137, 438, 953, 472 (1.3 E11)
40	1,099,511,627,776 (1.1 E12)

Polymerase Chain Reaction (PCR)

Block Cycler PCR vs. Real-Time PCR



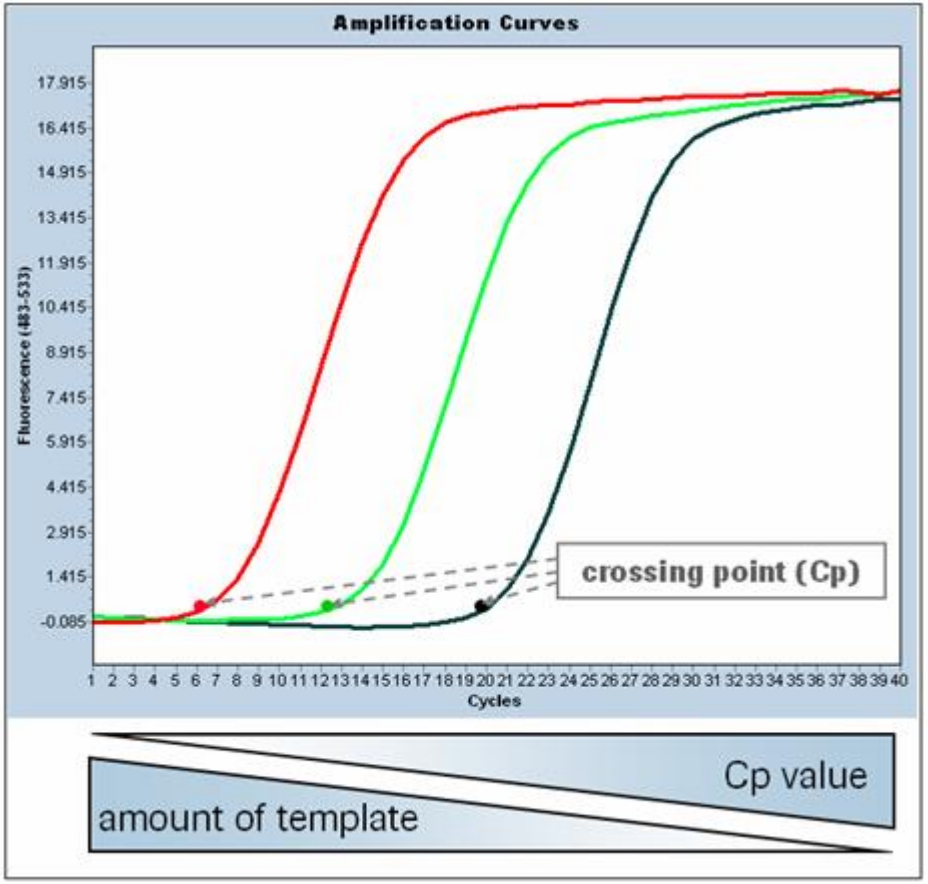
Agarosegel Blotting



LightCycler® Instrument

Real-Time PCR

Correlation concentration – crossing point (Cp)



high target concentration

↓

early increase in fluorescence

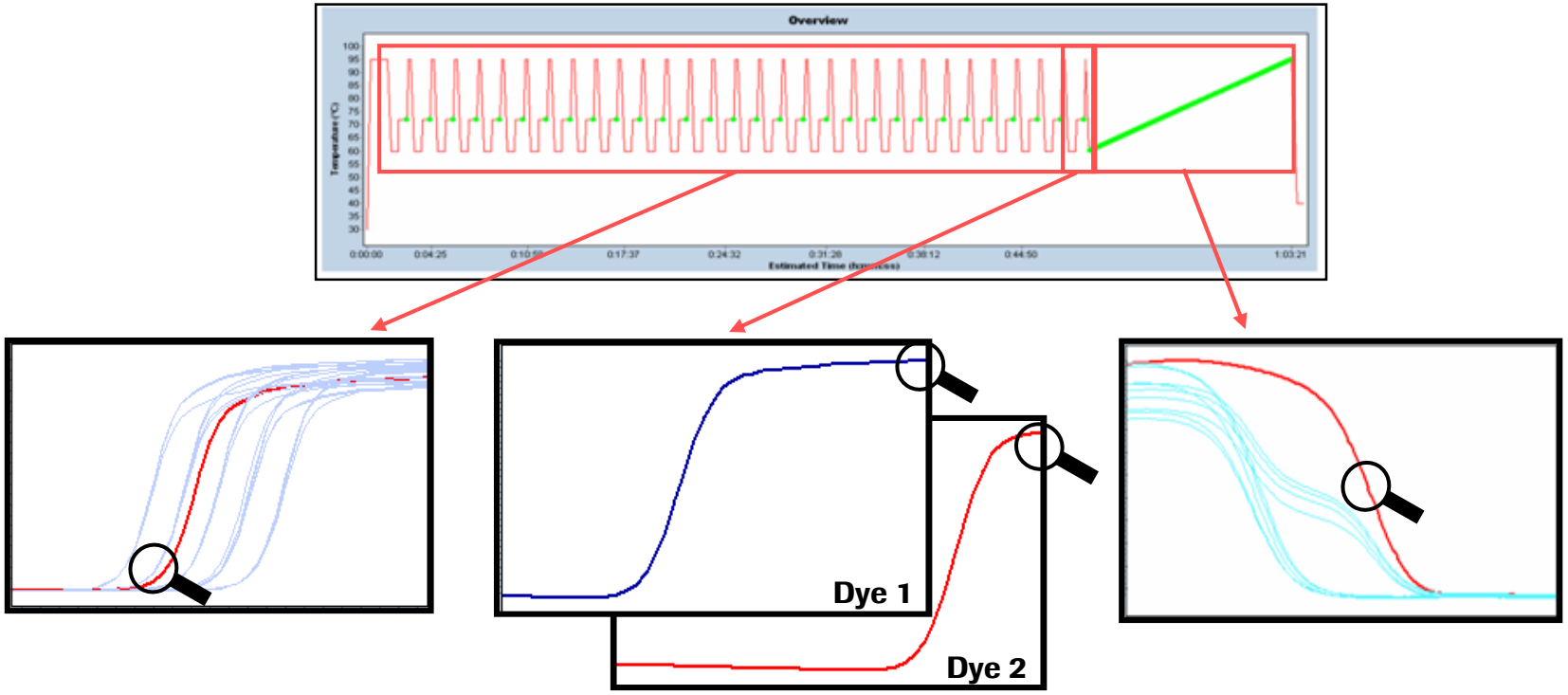
↓

early Cp/Cq

(cycle at which the fluorescence of a sample rises above the background fluorescence)

Cp/Cq‘s are not comparable between different systems

Real-Time PCR
Applications



Quantification

Absolute Quantification

Relative Quantification

Qualitative Detection

Endpoint Analysis

Endpoint Genotyping

Melting Curve

Product Identification (Tm)

Melting Curve Genotyping

High Resolution Melting (HRM)

Real-Time PCR

Detection Formats

LightCycler 480 Instrument

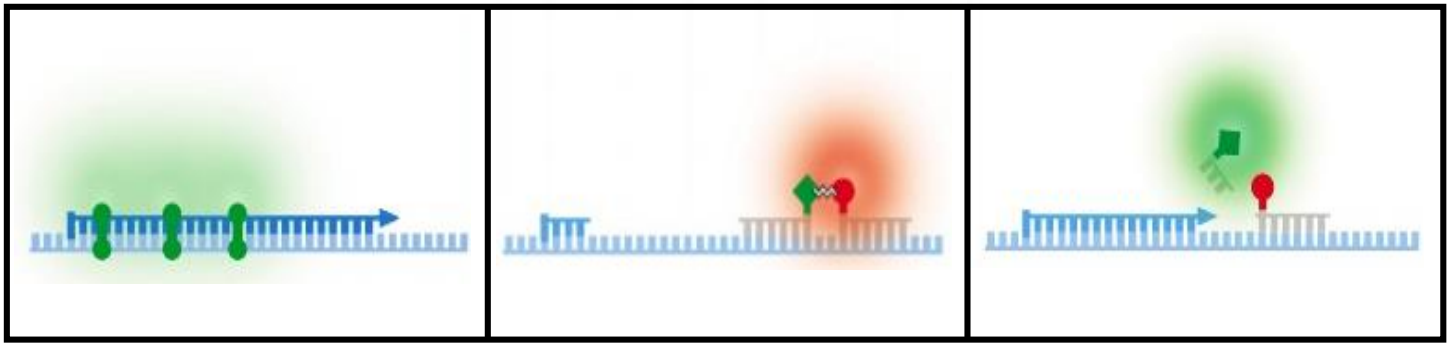
LightCycler® 480

Detection Formats

SYBR Green I
ResoLight

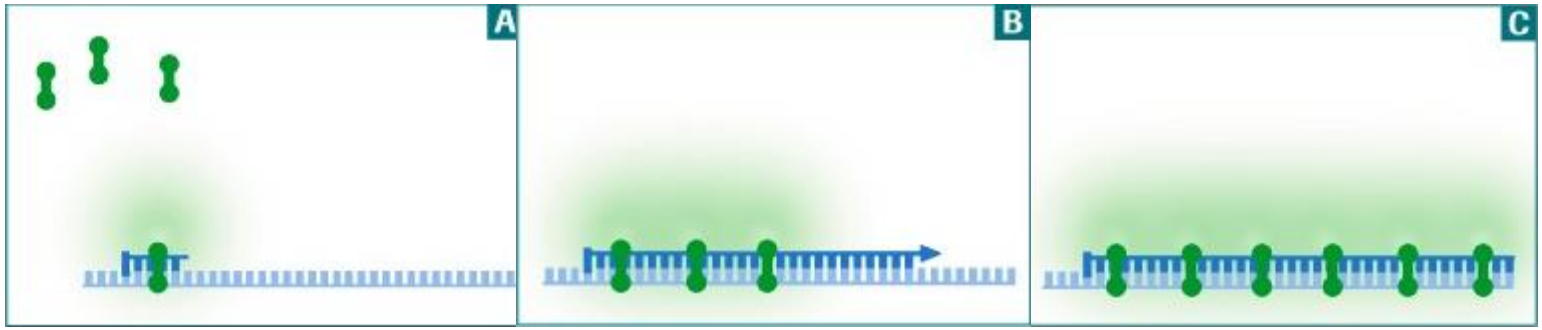
Hybridization Probe

Hydrolysis Probe



Detection formats

SYBR Green I



Advantages:

- Sequence independent, universal dsDNA dye
- Amplicons 100–1000bp possible

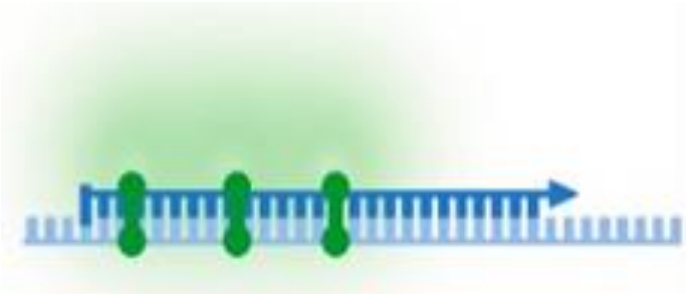
Disadvantages:

- Not sequence specific, melting curve necessary for product indentification
- One target per well, multiplexing not possible

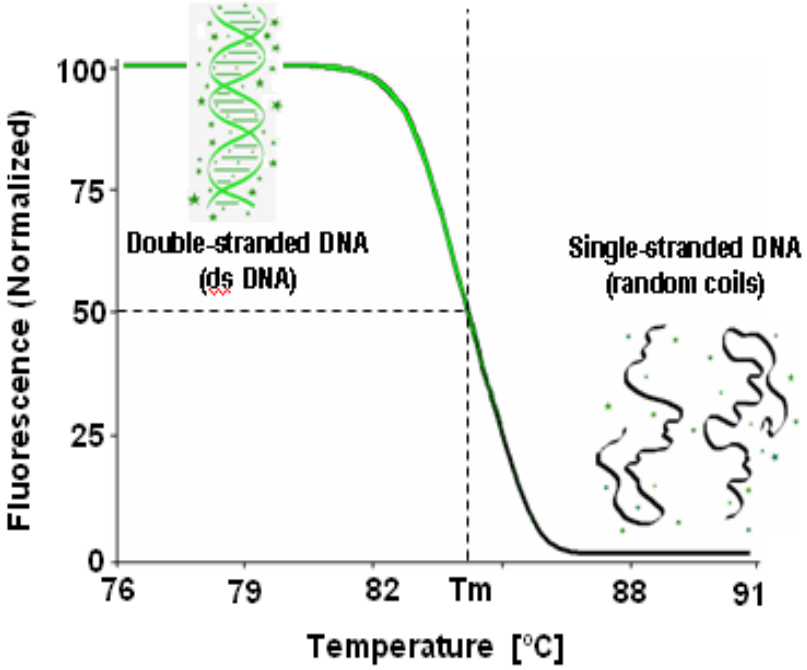
➲ **Recommendation**: For the quantification of many targets in short time, or when targets are frequently changed.

SYBR Green

Tm calling and product identification



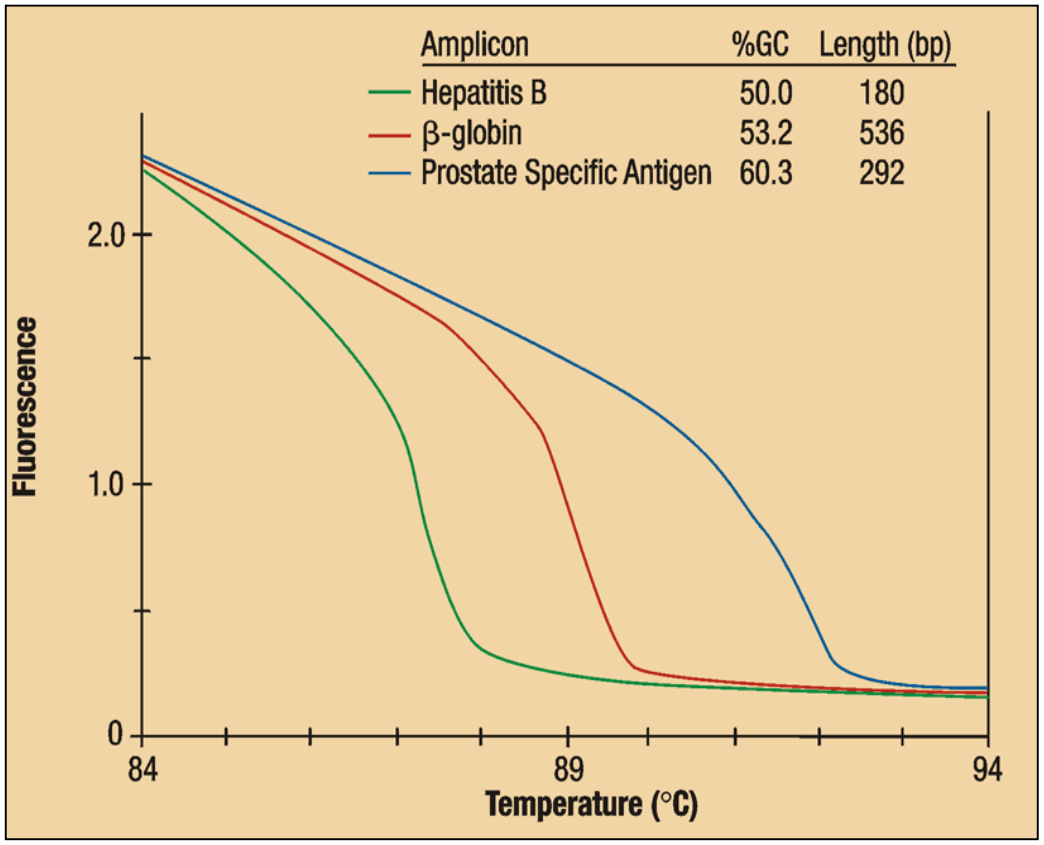
SYBR Green I dye
bound to double-stranded DNA



Melting curve

SYBR Green

Factors influencing melting behavior



Tm varies by:

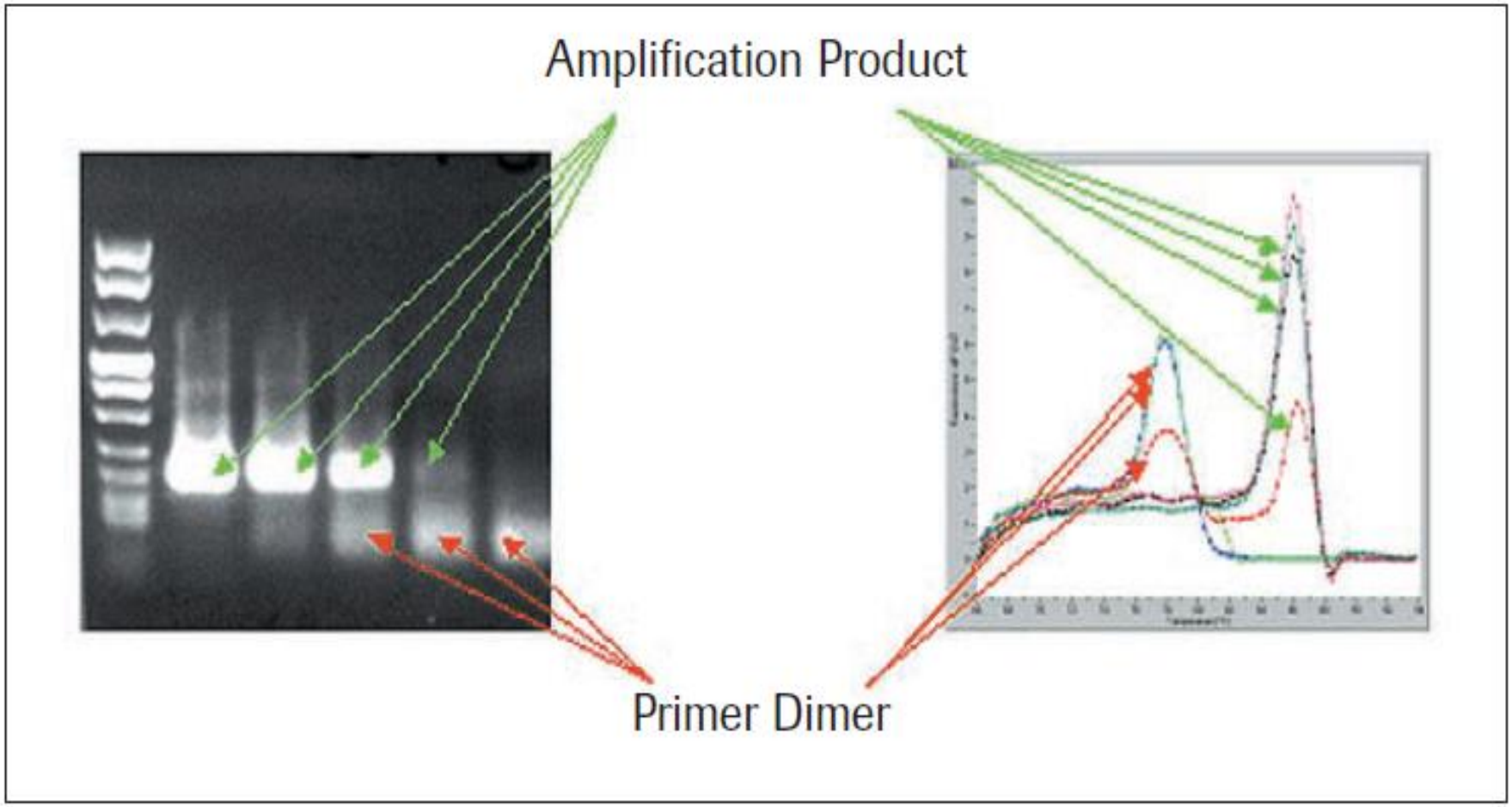
- **GC-content**
- **amplicon length**

Tm is also influenced by:

- Salt concentration
- $MgCl_2$ concentration
- SYBR Green I concentration

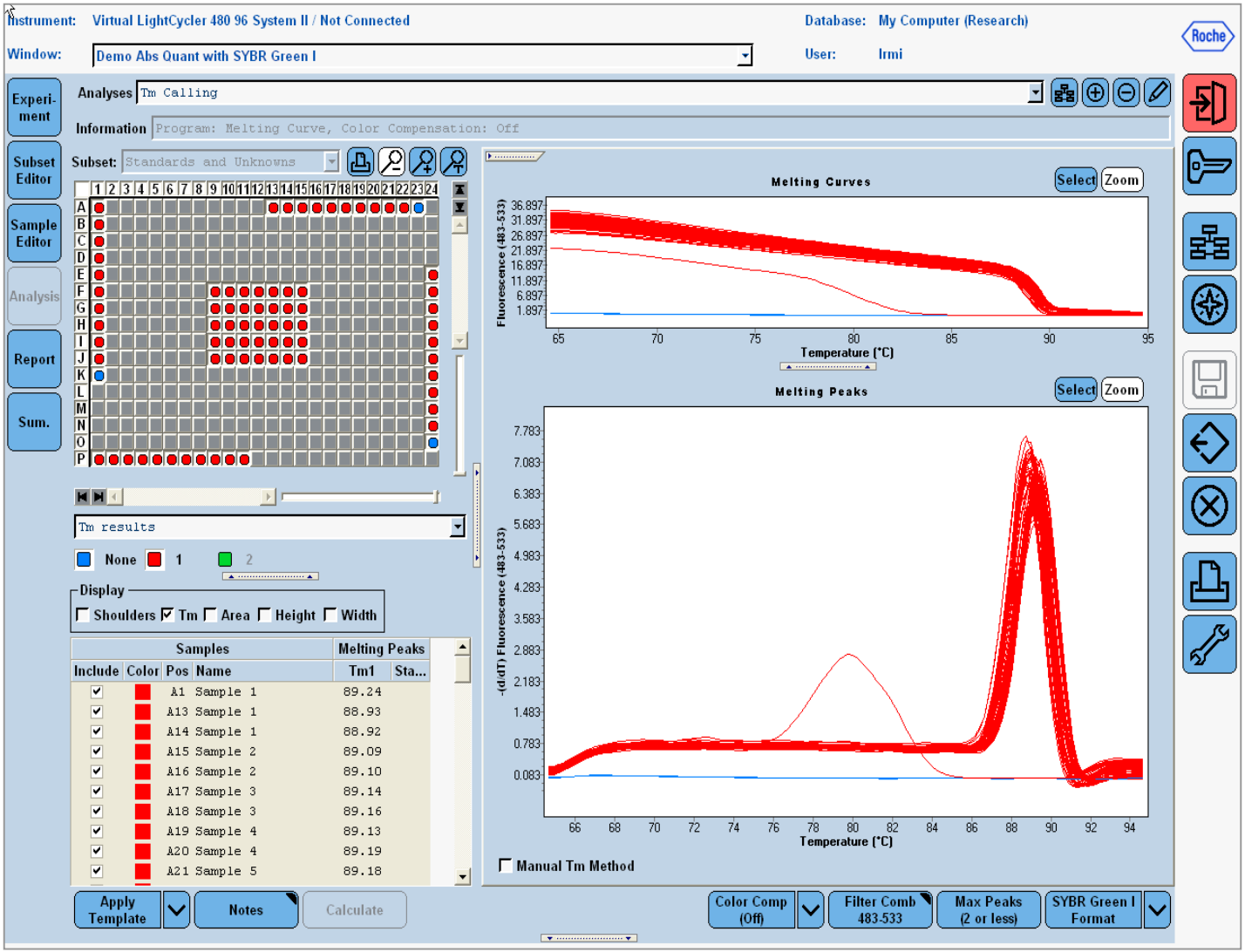
SYBR Green

Primer dimers identification



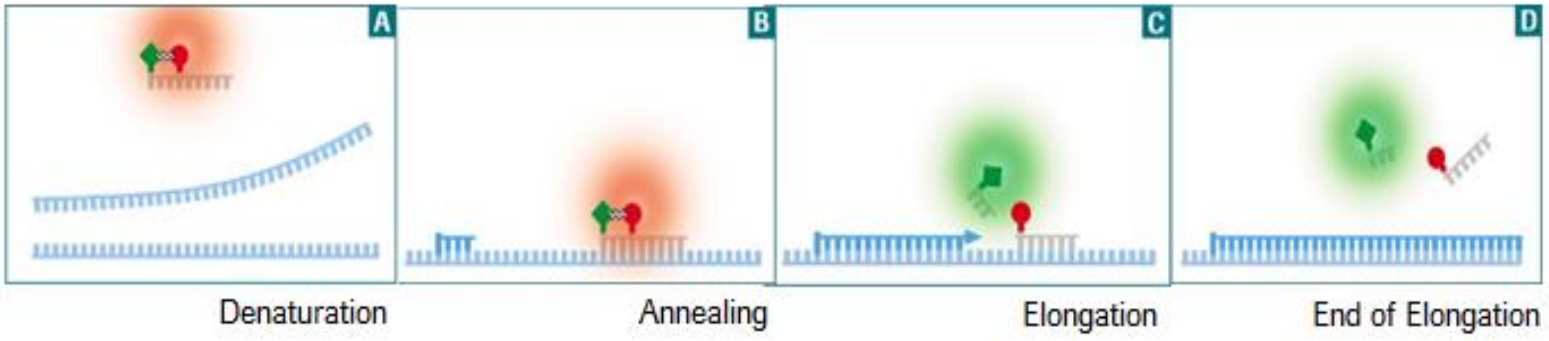
LightCycler® 480 Tm Calling software

Analysis



Detection formats

Hydrolysis probes



Fluorescence is quenched

FRET quenching
(reporter dye and quencher)

Fluorescence is not quenched

Probe hydrolysed
(5´ exonuclease activity Taq polymerase)

↑ Measurement

Advantages:

- Sequence specific
- Multiplex is possible
- Most assays without optimization

Disadvantages:

- Need of specific probe
- No melting curve possible

➲ **Recommendation:** For all targets possible, specially if more than one target is done in a single run.

Quenchers

General recommendations (Operators Manual)

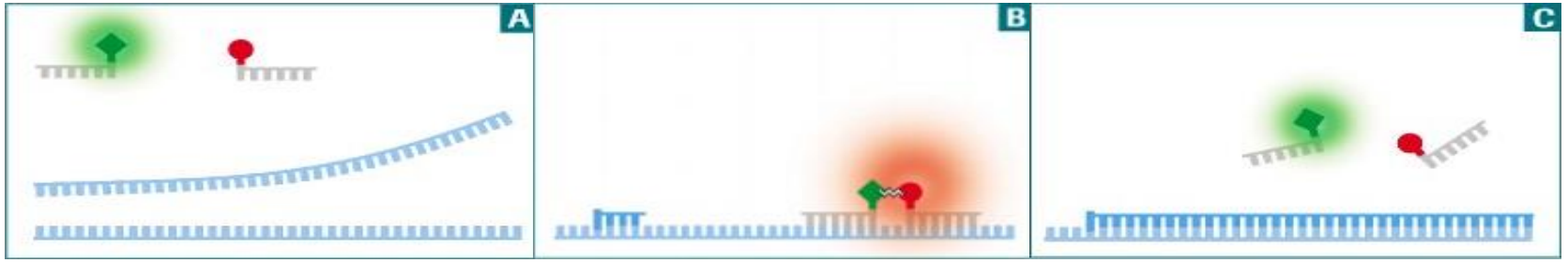
- For multicolor hydrolysis probe assays, it is strongly recommended to use **dark quencher** dyes (i.e., dye molecules which efficiently quench the fluorescence of a FRET reporter dye without emitting fluorescence themselves).

- Roche Diagnostics recommends the use of **BHQ-2** (Black Hole Quencher, quenching range 550 – 650 nm) for the hydrolysis probe reporter dyes Cyan 500, FAM, HEX, LightCycler Red 610, LightCycler Red 640, or Cy5.

- Alternatively, **DABCYL** (quenching range 380 – 530 nm) can be used for quenching Cyan 500, FAM, or HEX with a little lower quenching efficiency.

Detection formats

HybProbes (Hybridization Probes)



FRET reporting
(donor probe and acceptor probe)

Advantages:

- Very specific
- Multiplex is possible
- Optimal for Melting Curve Genotyping

Disadvantages:

- Optimization sometimes needed
- Higher cost

➲**Recommendation:** For quantification of few targets in long-time studies, for Virology/Microbiology (very specific), for multiplex PCR

Real-Time PCR

Detection Formats

LightCycler 480 Instrument

Roche LightCycler® history

20 years qPCR innovation

LightCycler® Carousel
32 samples

1998

LightCycler® 480
96 or 384 samples

2005

LightCycler® 1536
1,536 samples

2009

LightCycler® Nano
32 samples

2011

LightCycler® 96
96 samples

2012

LightCycler® 480 System

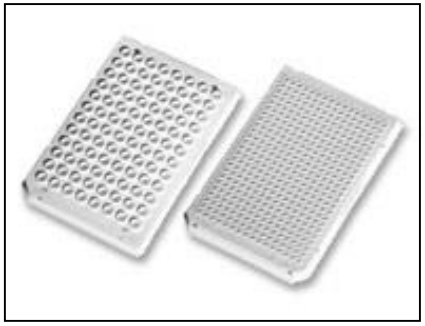
Components

Instrument

Block Kit

Software Modules

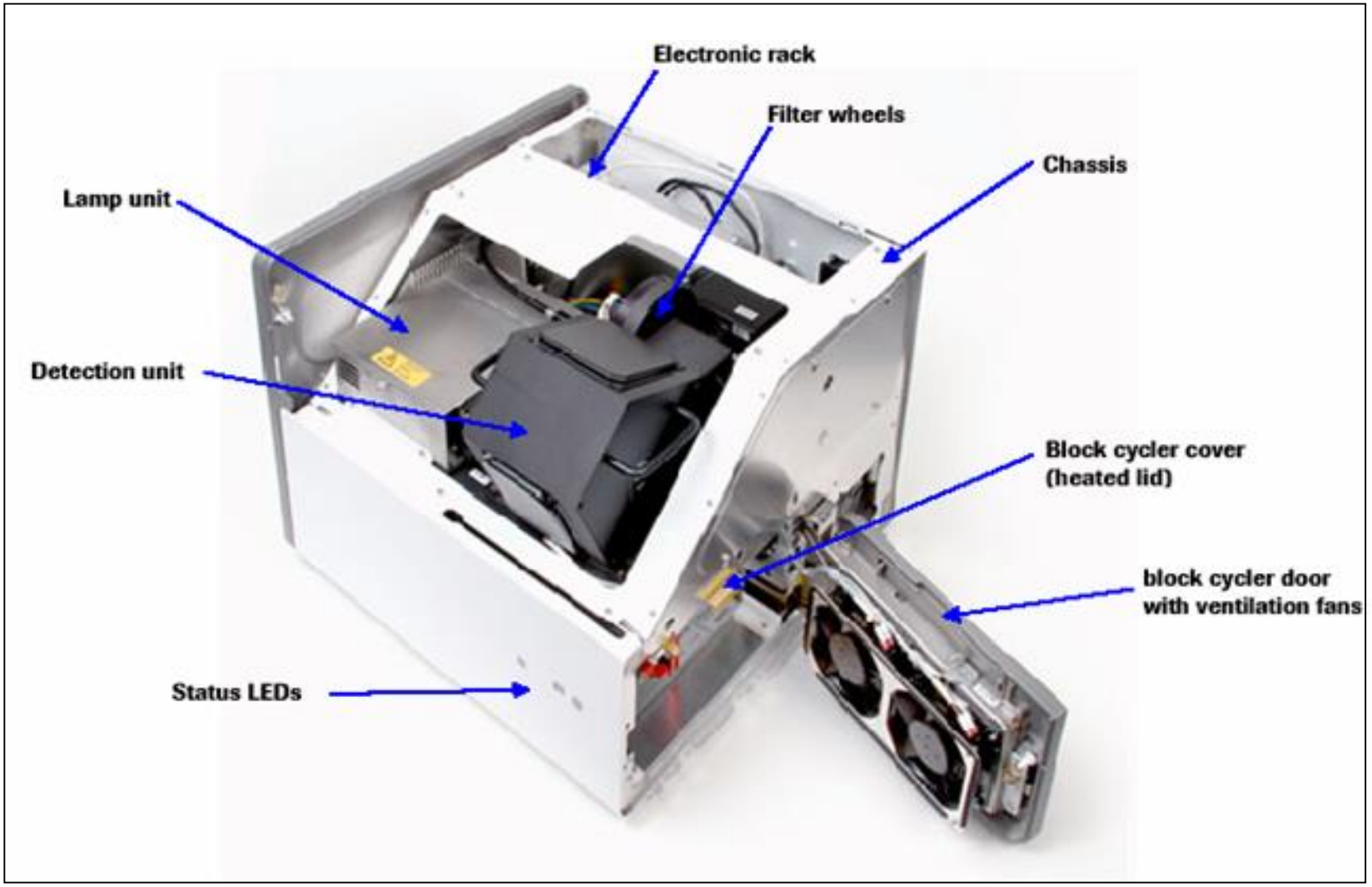
Reagent Kits



Multiwell Plates

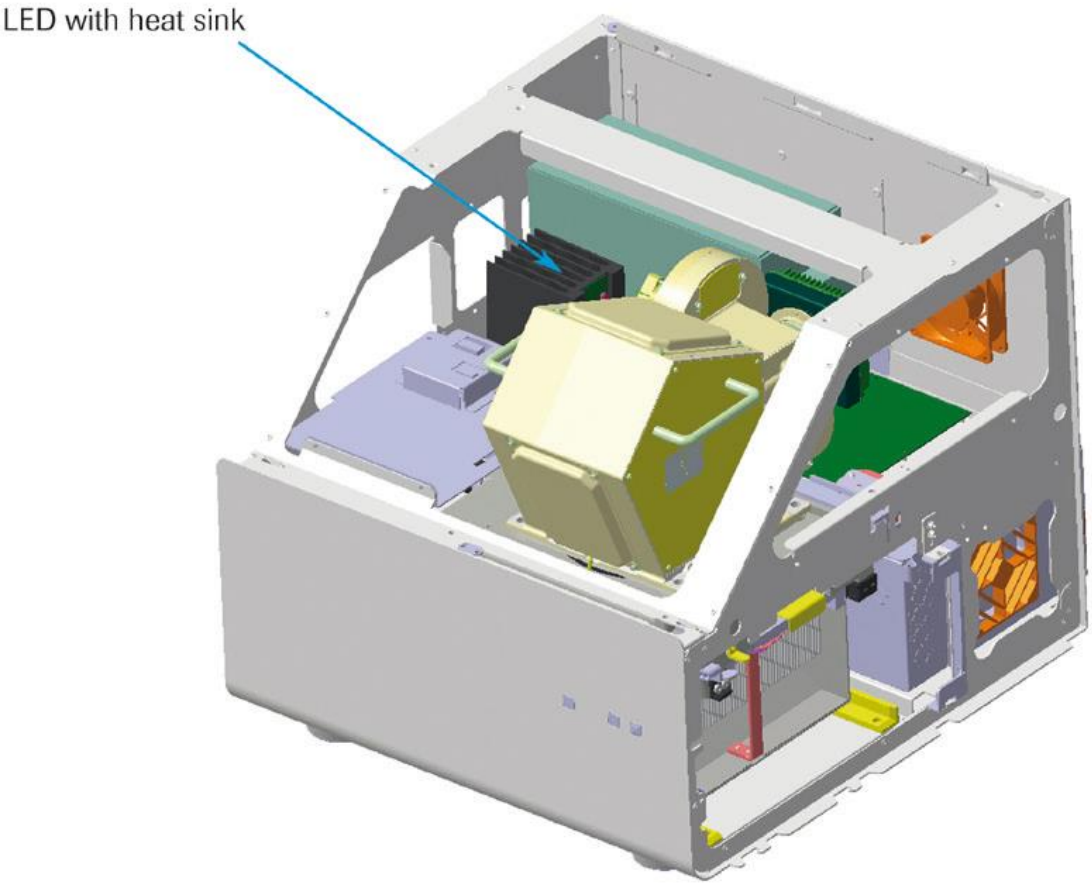
LightCycler® 480 Instrument

General architecture – Xenon lamp



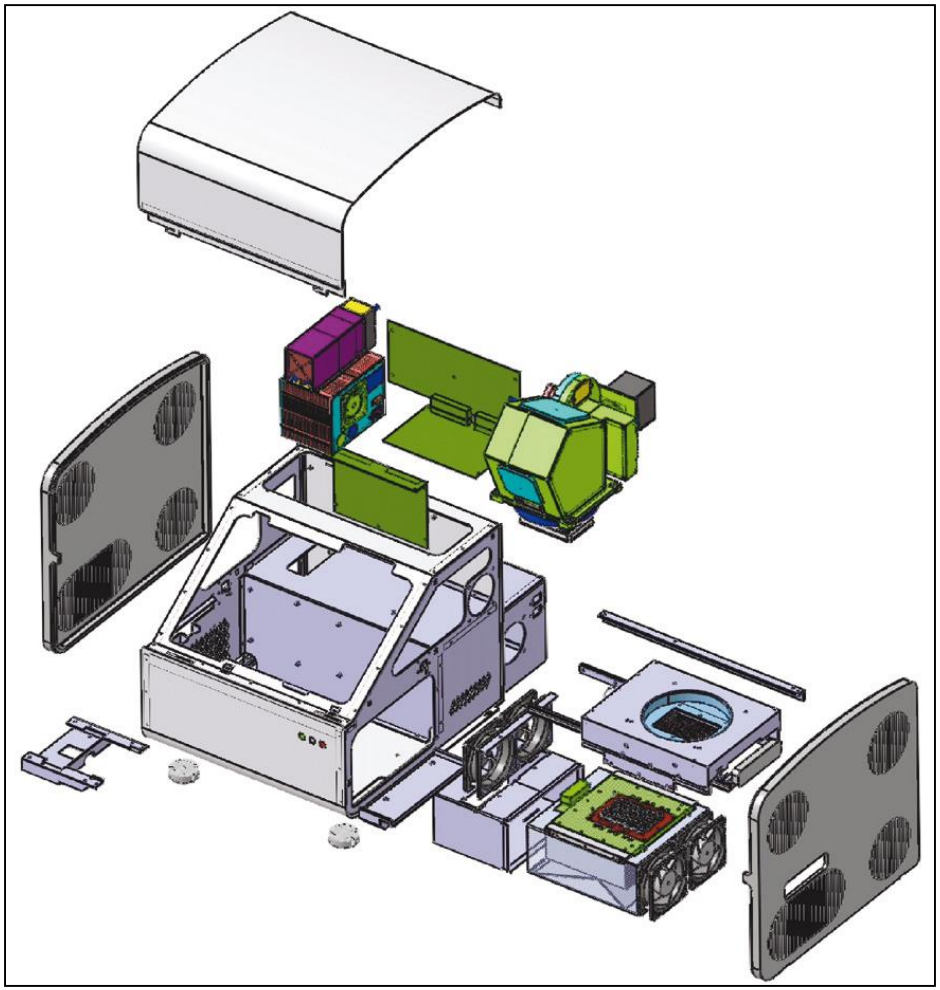
LightCycler® 480 Instrument

General architecture – LED lamp



LightCycler® 480 Instrument

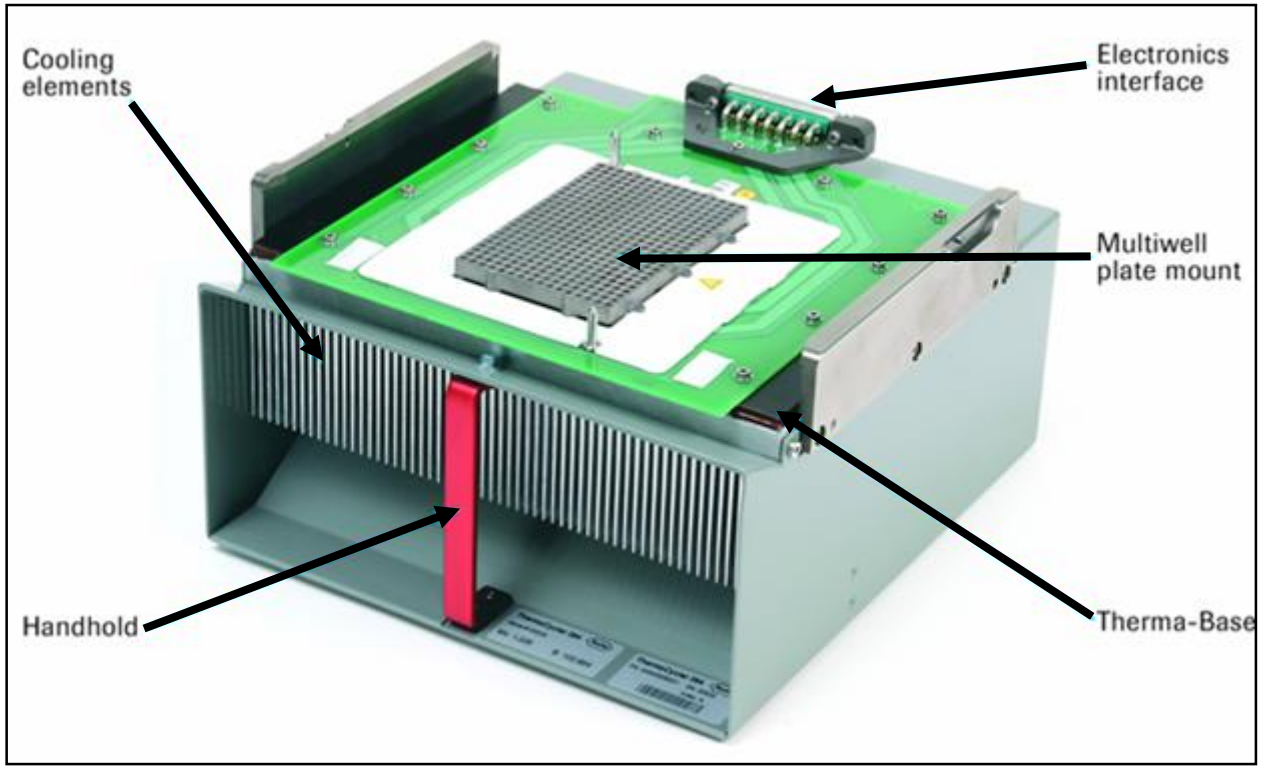
Modular design and maintenance-free



- Modular design facilitates easy maintenance and optimal service ability.
- No routine maintenance is required (e.g., instrument calibration runs).

LightCycler® 480 Thermal Block Cycler

Speed and accuracy



Therma-Base for optimized heat equalization

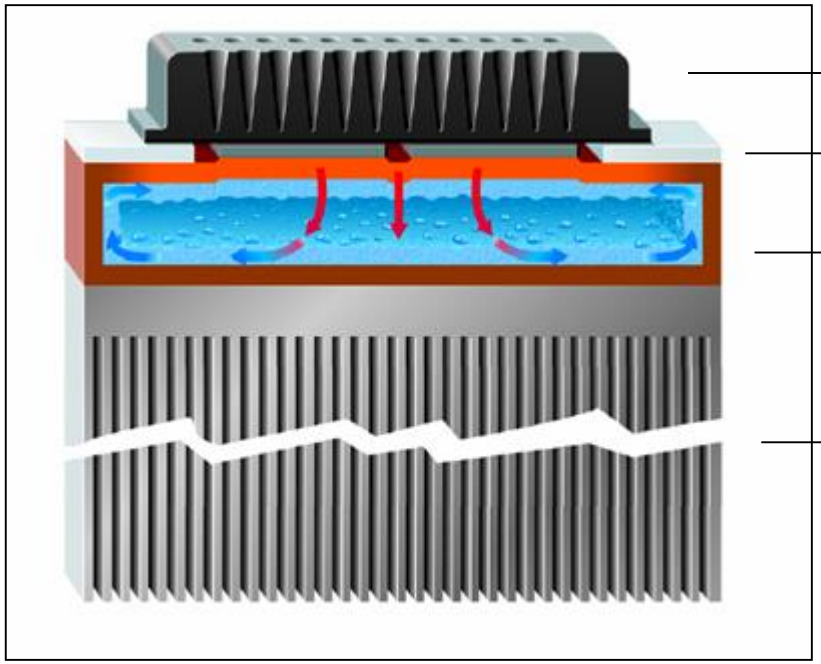
- Homogenous temperature distribution over the plate

- Fast PCR runs:

96 wells in < 1 hour
384 wells in < 40 min

LightCycler® 480 Instrument Heat Sink

Therma-base

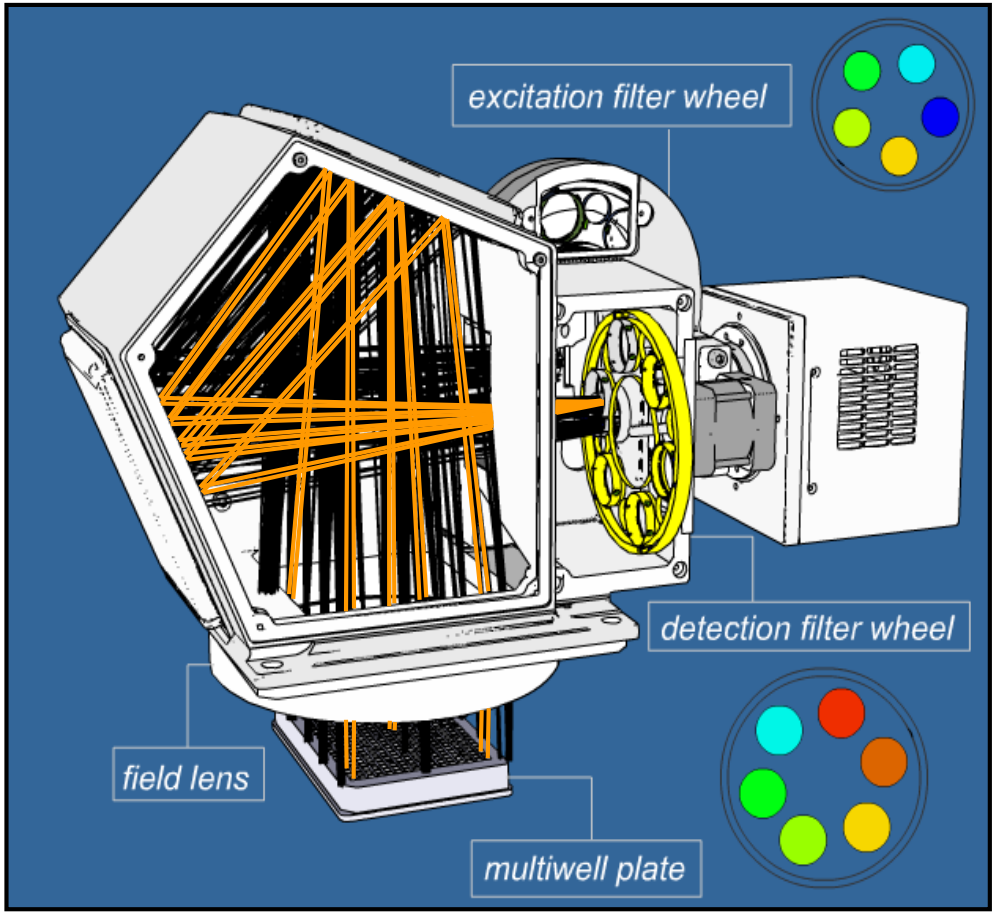


- Thin sealed vacuum vessel with working fluid in a wick structure
- Rapidly transfers heat by evaporation and condensation

→ enables both rapid and accurate cycling!

LightCycler® 480 Optical System

Sensitivity and homogeneity



- (Xenon) **LED lamp**
  high intensity
  broad dynamic range
  lifetime for LED: approx. 10,000 hrs
- **CCD camera**
- **Five excitation filters**
- **Six detection filters**
- Optimized arrangement of optical components
- **Homogeneous excitation and fluorescence detection**
- **No ROX reference dye required**

LightCycler® 480 Instrument II

Filter combinations for fluorophores

Fluorophore	Excitation Filter	Emission Filter	Detection Format
LightCycler® Cyan 500	440	488	Hydrolysis Probes (Reporter)
SYBR Green I	465	510	SYBR Green I
Fluorescein (Fluos / FAM)	465 498	510 580	Hydrolysis Probes (Reporter) HybProbe Probes (Donor) SimpleProbe Probes Hydrolysis Probes (Reporter, only in combination with Cyan 500)
VIC / HEX / Yellow555 / Joe	533	580	Hydrolysis Probes (Reporter)
LightCycler® Red 610	533 498	610 610	Hydrolysis Probes (Reporter) HybProbe Probes (Acceptor)
LightCycler® Red 640	498	640	HybProbe Probes (Acceptor)
Cy5 / Cy 5.5 / LightCycler® Red 705	618 498	660 660	Hydrolysis Probes (Reporter) HybProbe Probes (Acceptor)

LightCycler® 480 Instrument II

Optical unit (filter set)

LED (390 – 710 nm)								
Excitation filters		440	465		498	533		618
Emission filters		488	510		580	610	640	660
Dye		LightCycler® Cyan 500	SYBR Green I ResoLight	Fluorescein FAM		HEX (VIC)	LightCycler® Red 610	LightCycler® Red 640	Cy5
Detection formats	Melting Curve		•						
	HRM		•						
	SimpleProbe probes			•					
	HybProbe probes				*		•	•	•
	Hydrolysis probes 1–3 colors			•		•			•
	Hydrolysis probes 4 colors	•			•		•		•

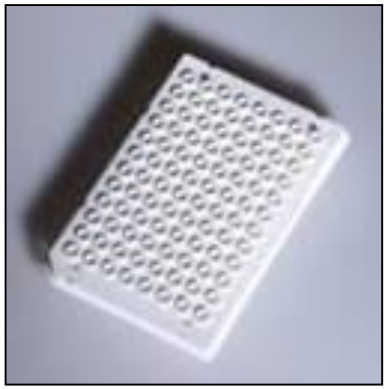
*FRET Donor

LightCycler® 480 System

Disposables

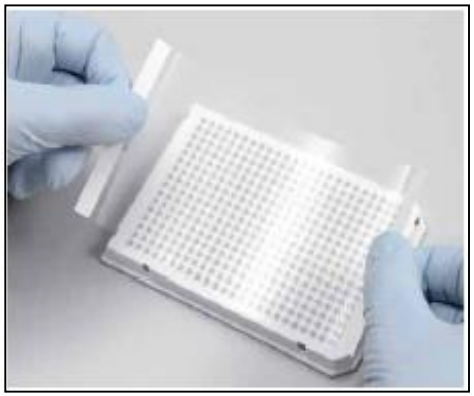
- **Optimal fit for good temperature transfer**, results in
  - fast cycling times (384-wells in < 40 min, 96-wells in < 1h)
  - homogenous temperature distribution in each well

- **Sealing foil** to prevent of evaporation and contamination



96-well plate
for 10–100 µl

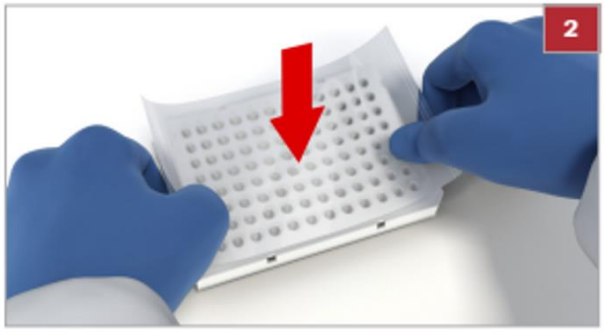
384-well plate
for 3–20 µl

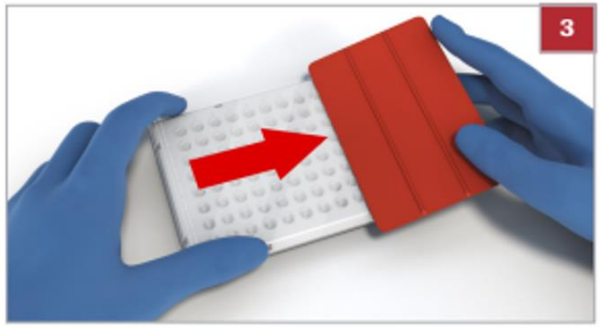


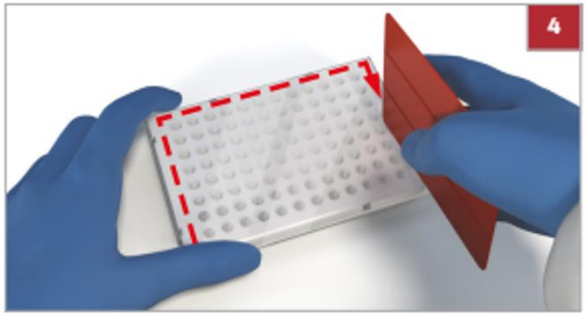
Sealing foil

LightCycler® 480 System

Sealing the plate







Doing now what patients need next