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The LightCycler® 480 Real-Time PCR System

Unleash the Power of Real-Time PCR

Steven van Konynenburg
Field Applications Specialist

LightCycler® 480 System
Overview

1. General Description

2. LightCycler® 480 Instrument and Specifications

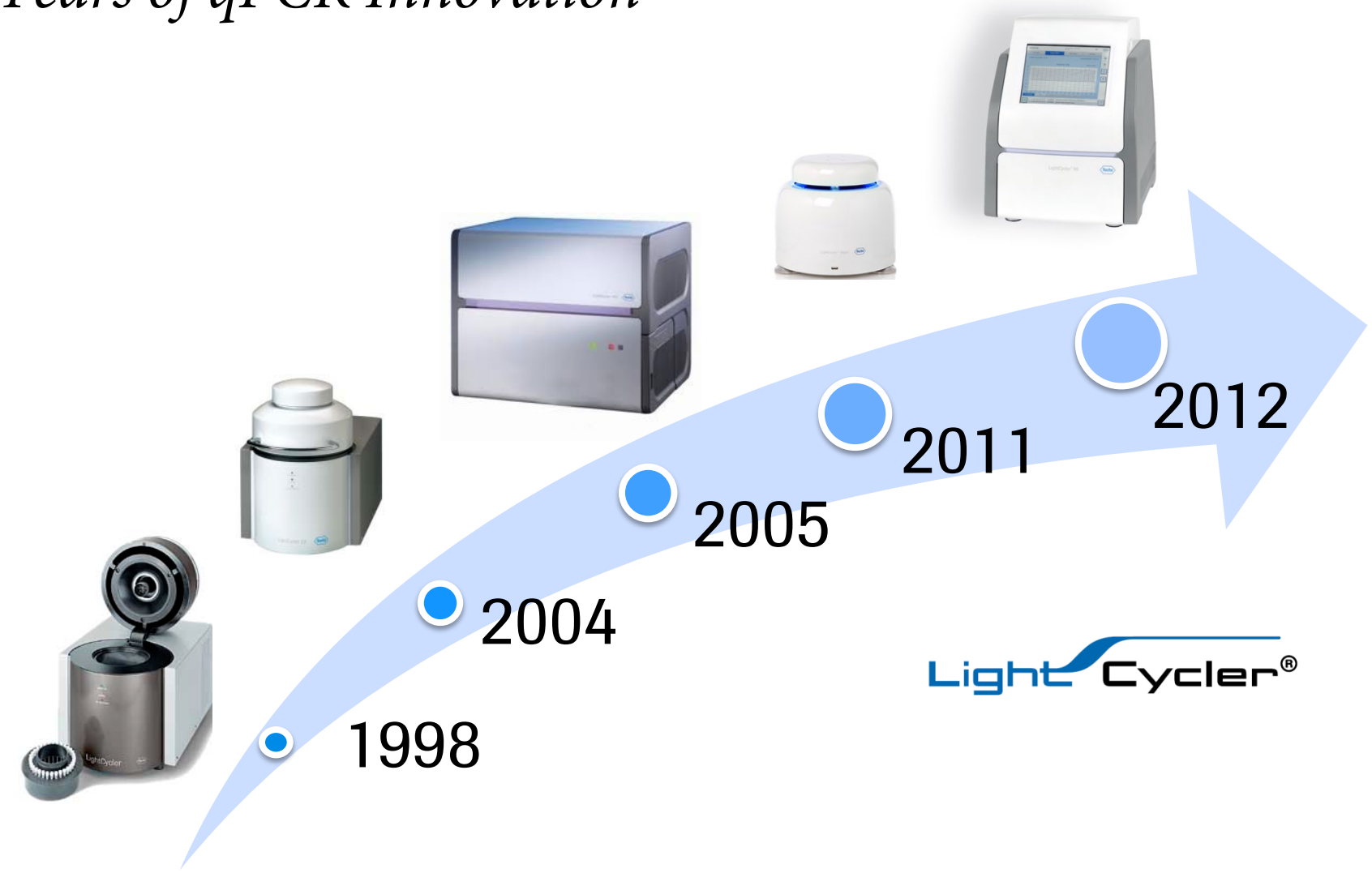
3. Assay Formats, Dyes, and Applications

4. LightCycler® 480 Performance

5. LightCycler® 480 Software

The Roche LightCycler® Story

17 Years of qPCR Innovation



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LightCycler® Systems
Technological (R)Evolutions

- Accuracy
- Speed
- Versatility
- Sensitivity
- Throughput
- Automation

Carousel-Based LightCycler® System

LightCycler® 480 System

LightCycler® 480 System
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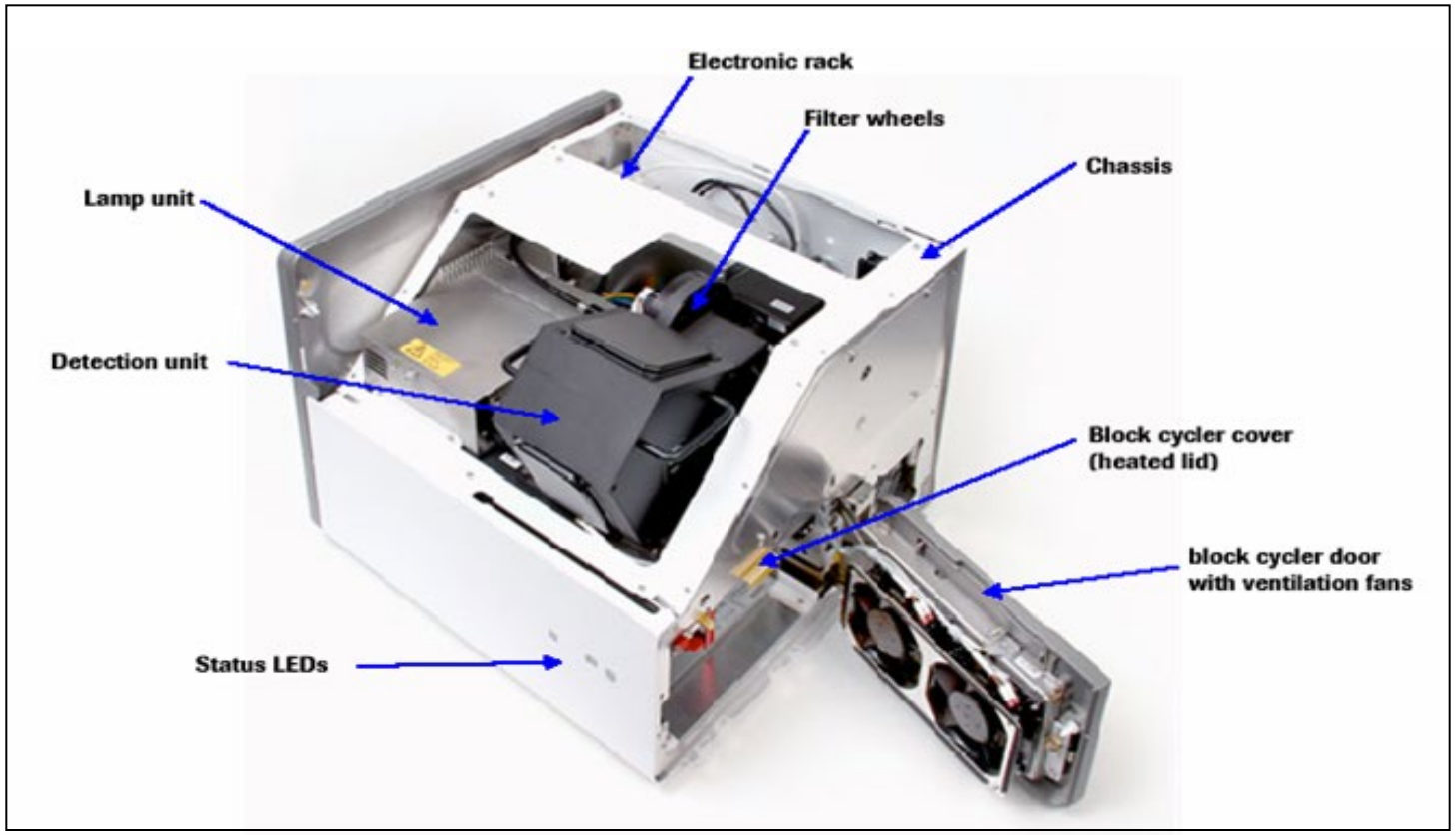
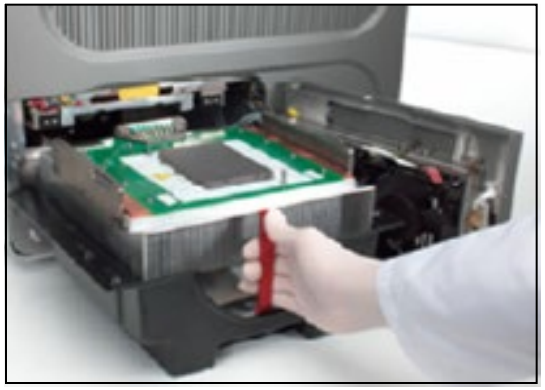
LightCycler® 480 Instrument
General Architecture



LightCycler® 480 System

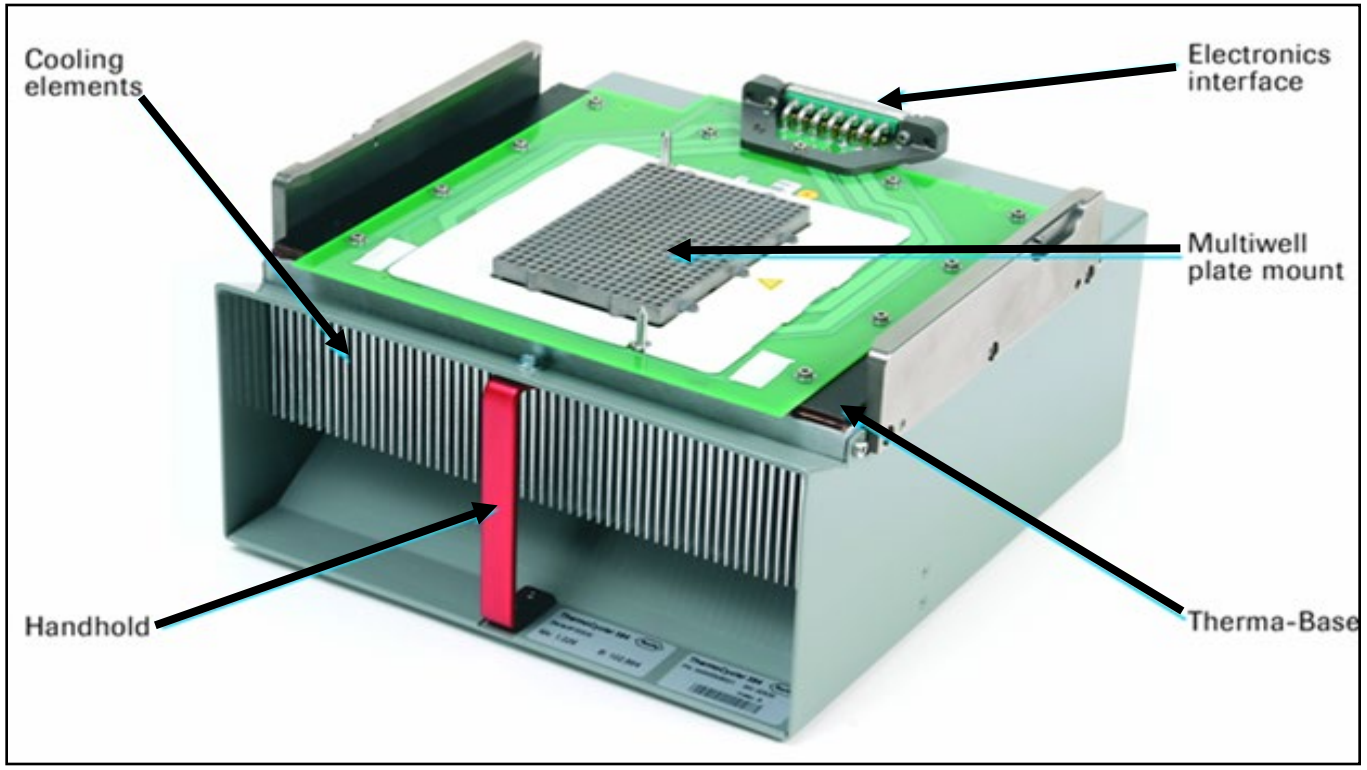
96 ⟷ 384 Switch



- Interchangeable thermal block cycler
- Do-it-yourself, fast
- Loading help for convenience
- No service engineer required
- No re-calibration necessary
- Instrument automatically detects and identifies block

LightCycler® 480 Thermal Block Cycler

Speed and Accuracy

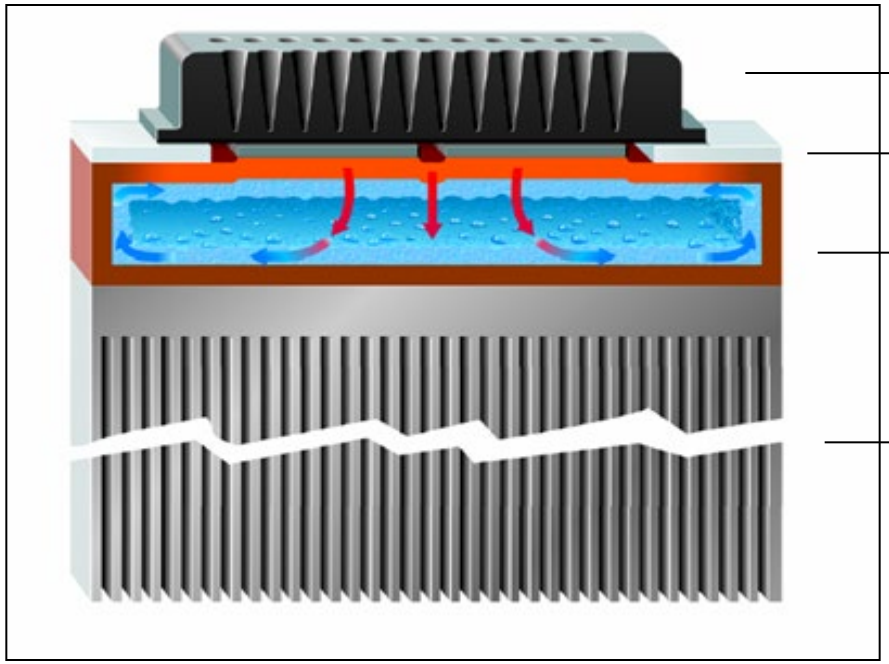


Therma-Base for optimized heat equalization

- 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

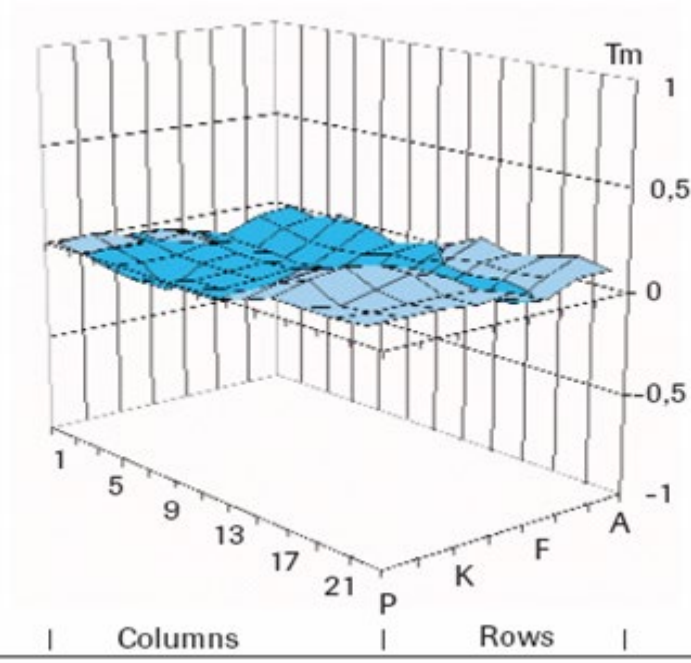


- Thin sealed vacuum vessel with working fluid in a wick structure
- Rapidly transfers heat by evaporation and condensation

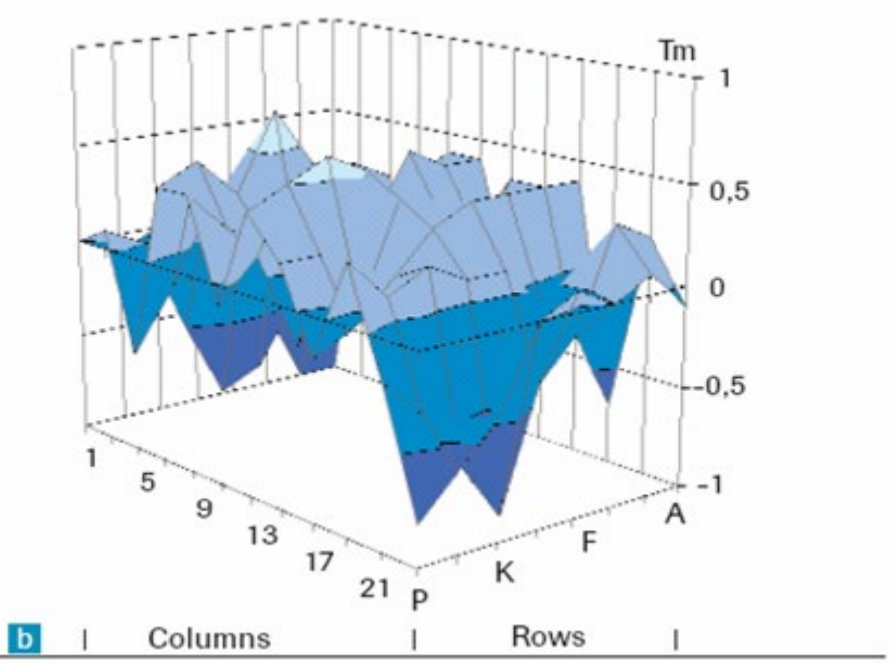
→ **Enables both rapid and accurate cycling!**

Thermal Uniformity
Instrument Comparison

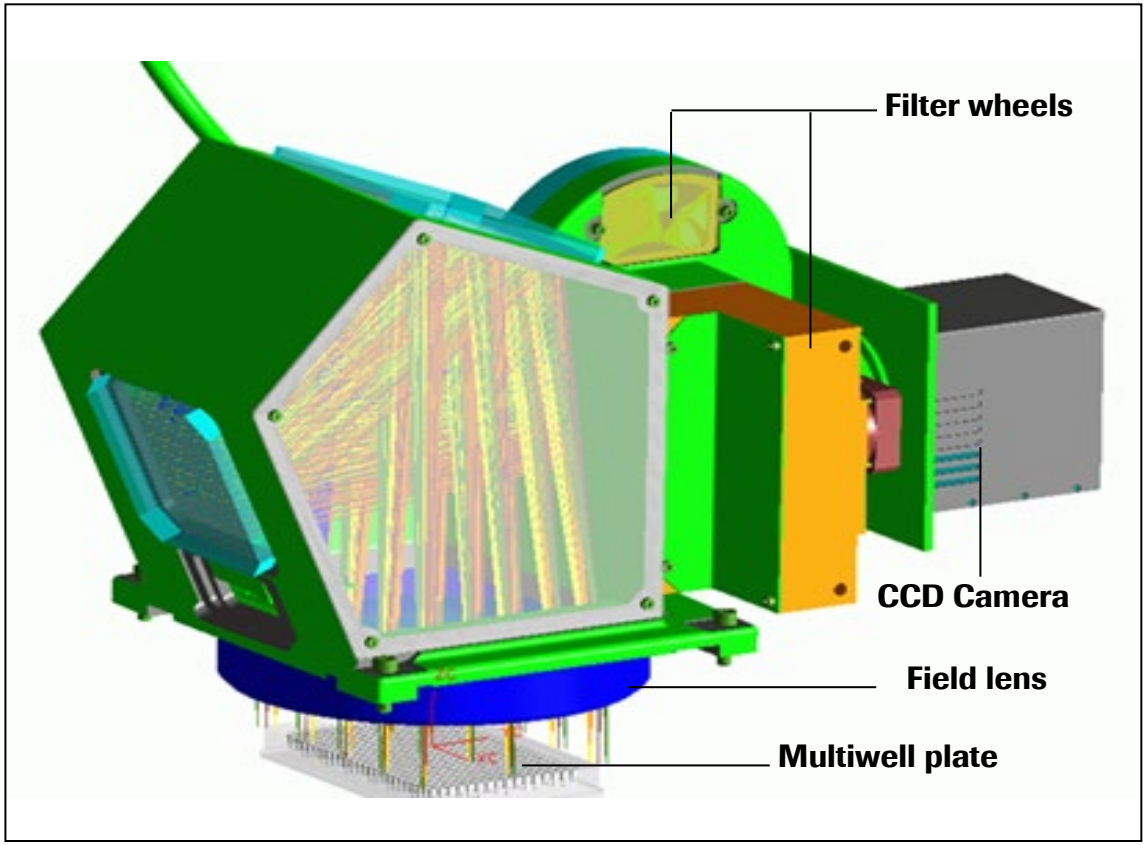
LightCycler® 480 Instrument



Standard Instrument



LightCycler® 480 Optical System
Sensitivity and Homogeneity



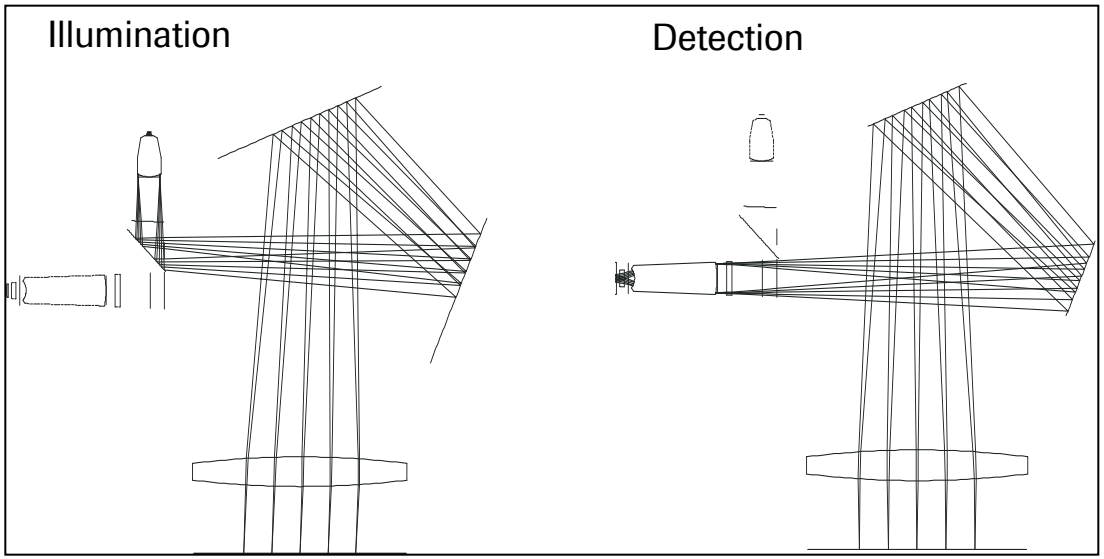
- LED
  - high intensity
  - broad dynamic range
  - lifetime: 10 000 hrs
- CCD camera
- Five excitation filters
- Six detection filters

- Optimized arrangement of optical components
- Homogeneous excitation and fluorescence detection

LightCycler® 480 Optical System

Path of Rays

- Homogeneous illumination and imaging due to long object-image distance.
- Pinhole to mask lateral portions of emitted light and focus on central, perpendicular portions.
- Large field lense to efficiently collect rays also from lateral wells.



LightCycler® 480 Optical Unit
Characteristics

5 excitation – and 6 emission filters for

- Easy duplexing
  - Easy and unbiased performance of dual color Hydrolysis Probes assays using universal color compensation objects for FAM/HEX (VIC) or FAM/Yellow 555 combinations

- Unmatched multiplexing
  - 4 color Hydrolysis probes
  - 4 color HybProbe probes

- The long-pass filter grants access to dyes of higher wavelength

	Instrument II Bandpass (Band Width)
Excitation	▪ 440 nm (35) ▪ 465 nm (25) ▪ 498 nm (40) ▪ 533 nm (25) ▪ 618 nm (35)
Detection	▪ 488 nm (20) ▪ 510 nm (20) ▪ 580 nm (20) ▪ 610 nm (20) ▪ 640 nm (20) ▪ 660 nm (95) long pass

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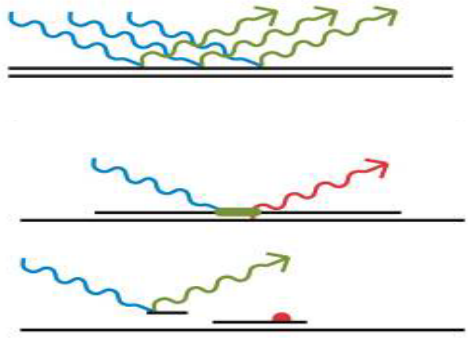
LightCycler® Assay Formats
... Most Commonly Used



Sequence-independent formats like **SYBR Green I**

Sequence specific detection formats: **HybProbe Probes**

Hydrolysis Probes or Universal ProbeLibrary Probes

Channels, Dyes, and Detection Formats

Xenon lamp (430–630)								
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	•			•		•		•

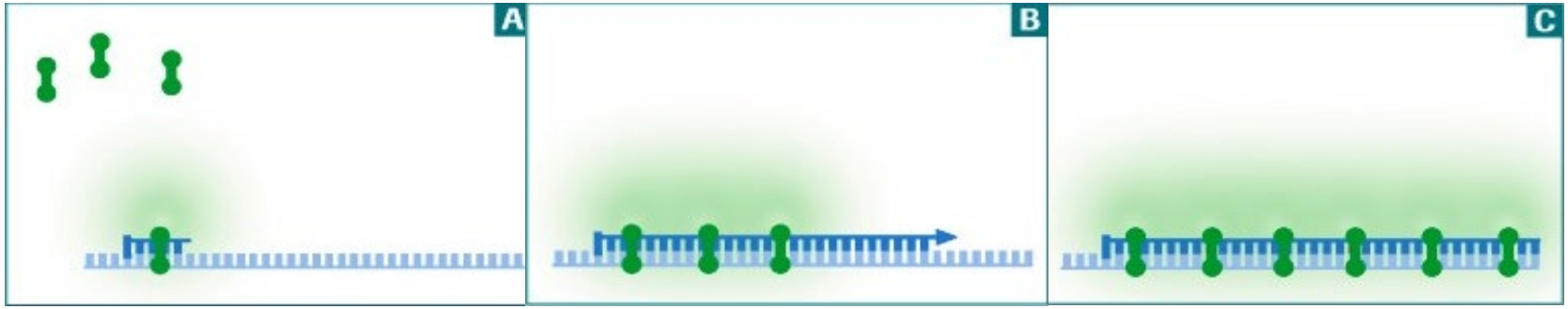
LightCycler® 480 System

Allows Use of All Most Currently Used Detection Formats

Detection format	Excitation (nm)	Detection (nm)	Dye	Applications
SYBR Green I	465	510	SYBR Green I	Qualitative and quantitative PCR, Product verification
HybProbe probes	498	610 640 660	LightCycler® RED 610 LightCycler® RED 640 Cy5	Quantification, SNP/mutation analysis via melting curves
Hydrolysis probes	440 465 533 533 618	488 510 580 610 660	LightCycler® CYAN 500 FAM VIC, HEX, Joe, Yellow555 LightCycler® RED 610 Cy5, Cy 5.5	Quantification, Endpoint genotyping
SimpleProbe probes	465	510	Fluorescein	SNP analysis
High Resolution Melting	465	510	LightCycler® 480 ResoLight Dye	Gene/mutation scanning, SNP analysis

Sequence Independent Assay Format

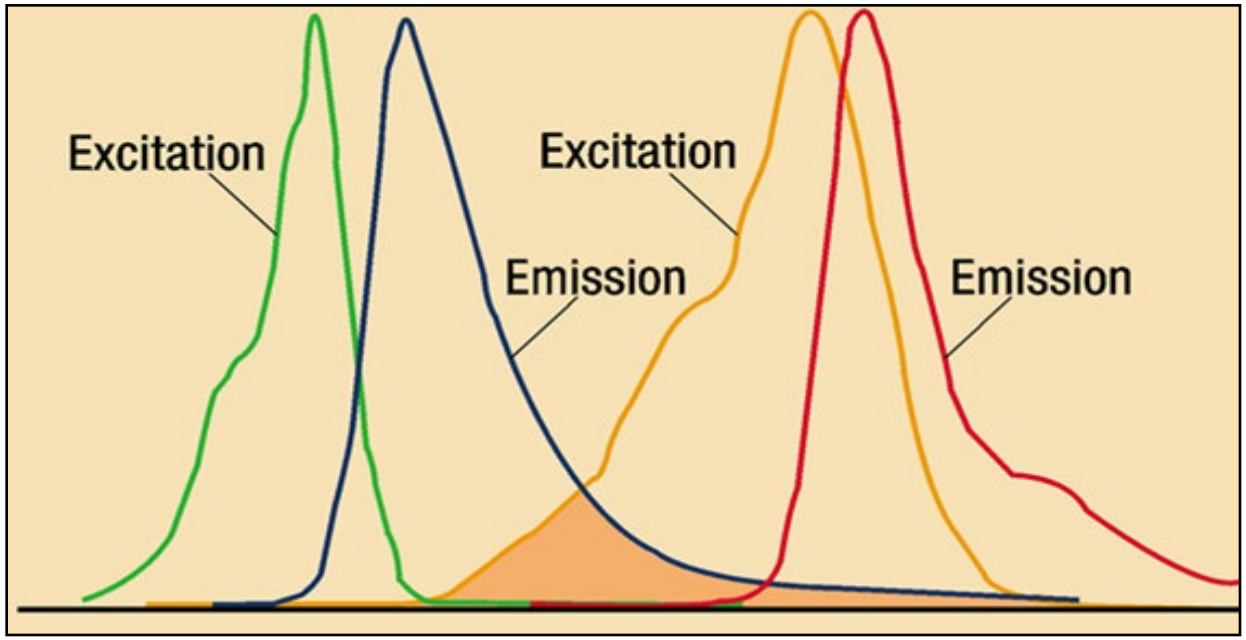
SYBR Green I



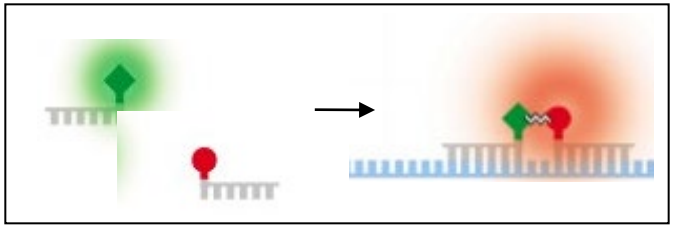
- When SYBR Green I dye **intercalates** into **dsDNA**, its fluorescence greatly increases
- All SYBR Green I assays have to be verified by a **Melting Curve Analysis** for side products other than the specific amplicon
- Dyes like the **Roche Dye ResoLight** completely **saturate** the double stranded nucleic acid and can be used to detect unknown variations in a process called "***high resolution melting***"

Detection of Sequence-Specific Probe Binding

Fluorescence Resonance Energy Transfer (FRET)

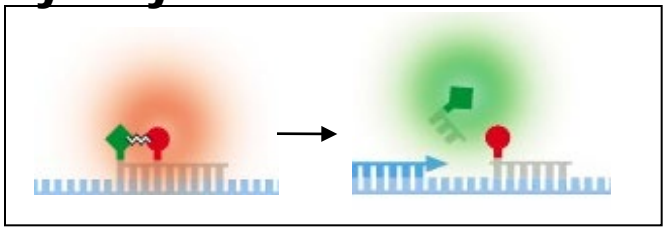


HybProbe Probe



FRET *reporting*

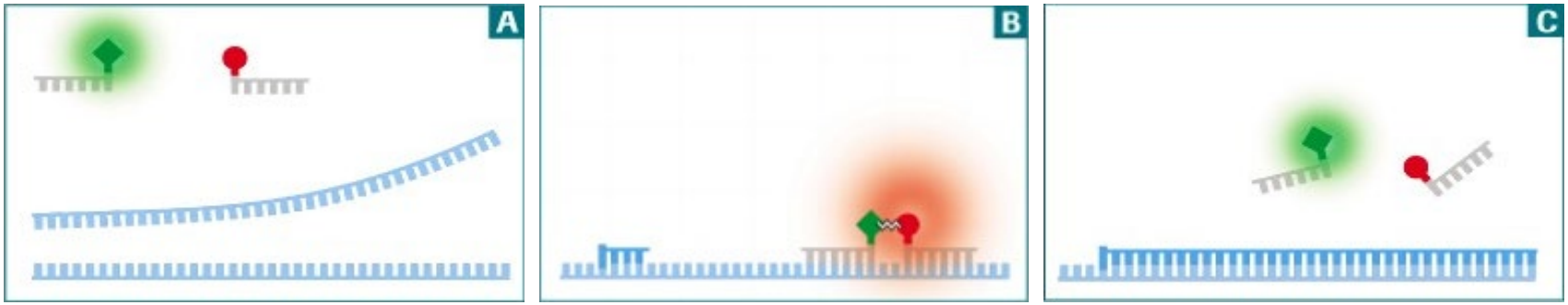
Hydrolysis Probes



FRET *quenching*

Probe Based Assay Format

HybProbe *Format*

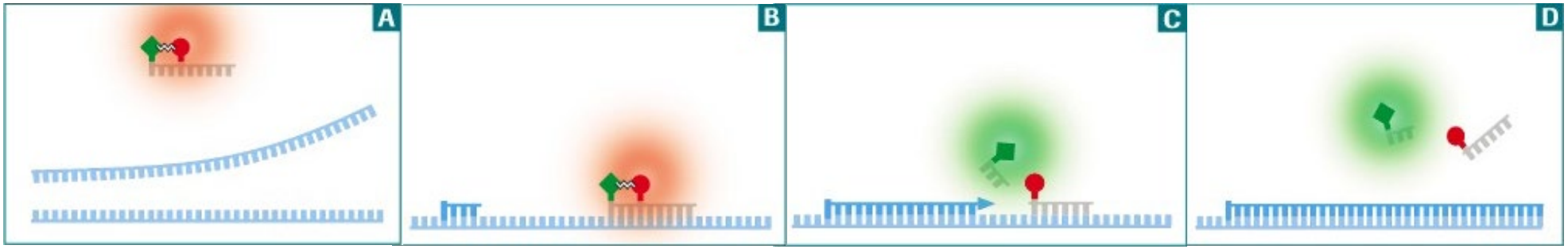


Denaturation Annealing Elongation

- The unique LightCycler® **HybProbe probes** are 2 sequence specific, non-extendable oligonucleotide probes labeled with different dyes close emitting light when coming in close proximity
- As both probes stay intact after amplification, they may be used for a subsequent melting curve analysis (e.g., SNP detection)
- The LightCycler® 480 optical system allows the usage of the HybProbe Format and allow *quadruplex* multiplexing of HybProbe Probes

Probe Based Assay Format (II)

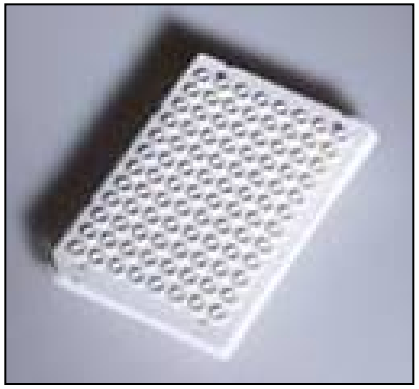
Hydrolysis Probe Format



- **Hydrolysis probes** emit fluorescence when the **5´ 3´ exonuclease activity of Taq polymerase** hydrolyzes them
- Universal ProbeLibrary Probes are similar to Hydrolysis Probes
- The LightCycler® 480 optical system allows the usage of the Hydrolysis Probe Format and allow *quadruplex* multiplexing. For unbiased fluorescence detection of duplex assays using dye combinations like **FAM/(VIC) HEX** or **FAM/Yellow 555** are supported by universal **Color Compensation Objects**.

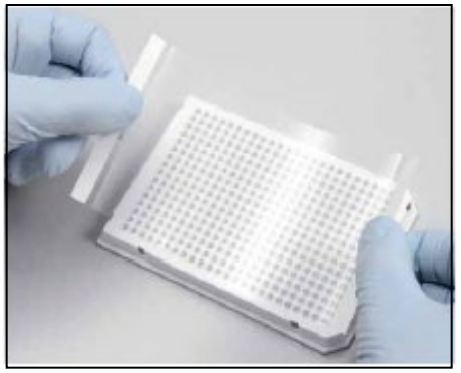
LightCycler® 480 Multiwell Plates

Disposables



96-well plate for 10–100 µl

384-well plate for 5–20 µl



Sealing foil

- **Barcode labeled multiwell plates**
  - LightCycler® 480 Multiwell Plates clear, (96 and 384 well)
  - LightCycler® 480 Multiwell Plates **white,** (96 and 384 well)
- **Best fit for optimized heat transfer**
  - 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**

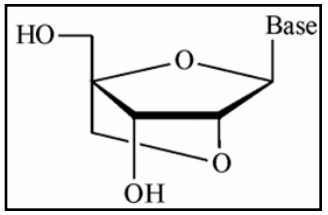
LightCycler® 480 Reagents

Optimized for Each Application

- LightCycler® 480 SYBR Green I Master
- LightCycler® 480 Probes Master
- LightCycler® 480 Genotyping Master
- LightCycler® 480 High Resolution Melting Master
- LightCycler® 480 ResoLight Dye
- LightCycler® 480 RNA Master Hydrolysis Probes
- RealTime ready RNA Virus Master
- LightCycler® Multiplex RNA Virus Master
- LightCycler® 480 Control Kit
- LightCycler® 480 QC Kit

Universal ProbeLibrary (UPL)
Overview

- Universal ProbeLibrary (UPL) Assays
  - for gene expression quantification of a large number of organisms
  - UPL comprises 165 short hydrolysis probes
    - 8-9 mers, FAM-labeled
    - Locked Nucleic Acids (LNAs) incorporated



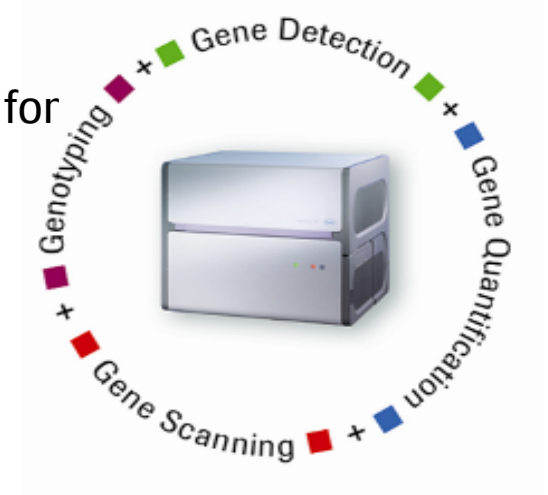
  - The UPL probe and the PCR primers together constitute the PCR assay for a given target gene.
- Design UPL assays using ProbeFinder Software available free at the web-based Assay Design Center at www.universalprobelibrary.com
- UPL assays are compatible with all real-time PCR instruments capable of detecting fluorescein, FITC, FAM and/or SYBR Green I. Use a standard real-time PCR protocol for hydrolysis probes.

LightCycler® 480 System
Application Overview

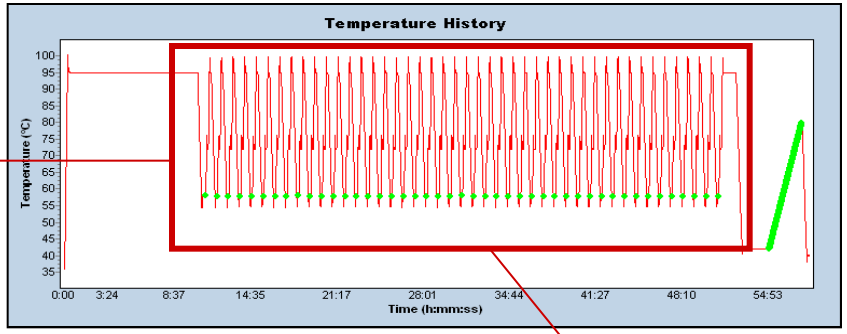
- It allows measurement during amplification for **quantitative** or **qualitative** results
  - **Qualitative detection** (Yes/No answer)
  - **Absolute Quantification** (result: e.g., copies/ml)
  - **Relative Quantification** (result: e.g., relative ratio)

- It allows measurements at the endpoint of PCR
  - **Endpoint Genotyping** (allelic discrimination)

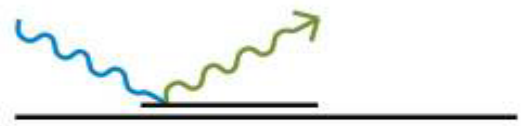
- It allows **melting curve analysis** subsequent to PCR amplification for
  - Product identification with SYBR Green I
  - Detection of known mutations using e.g., HybProbes
  - Detection of unknown mutations with Gene scanning



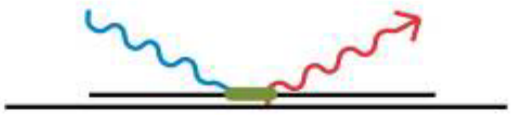
Amplification Analysis



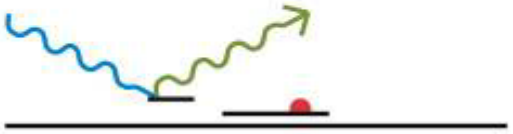
Any probe type



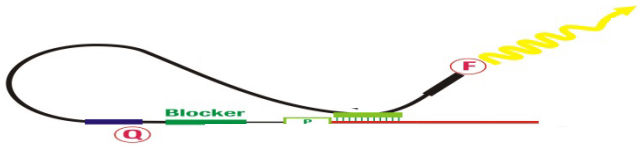
Simple Probes



Hybridization probes



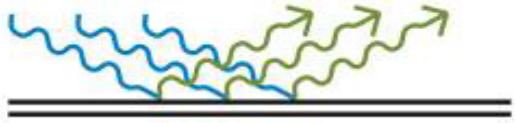
Hydrolysis probes



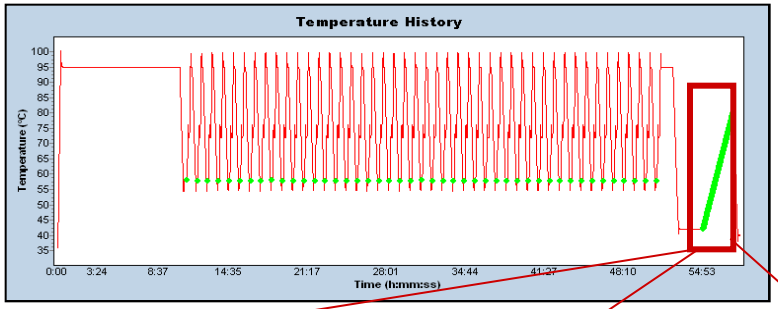
Scorpion Probes

dsDNA binding dye

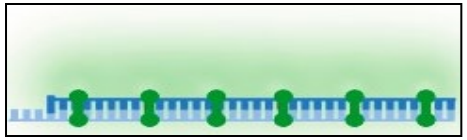
SYBR Green

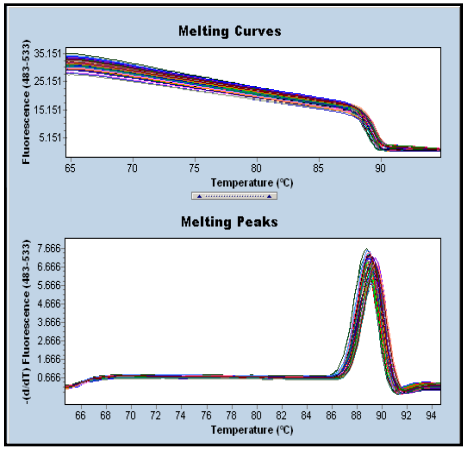


Melting Curve Analysis

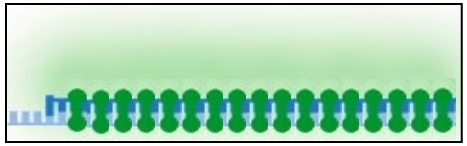


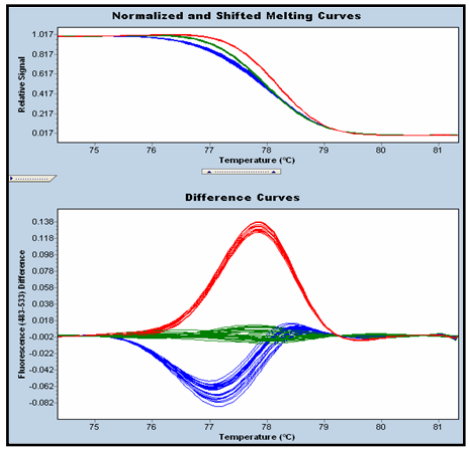
SYBR Green I



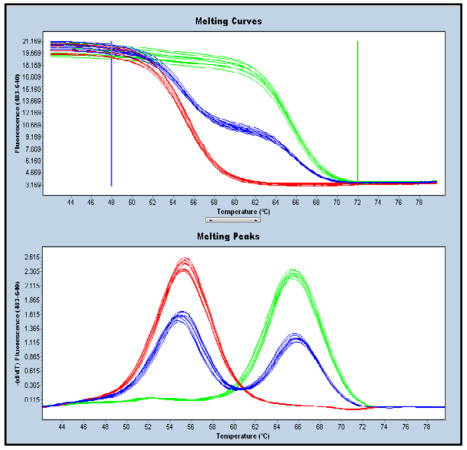


High Resolution Melting Dye



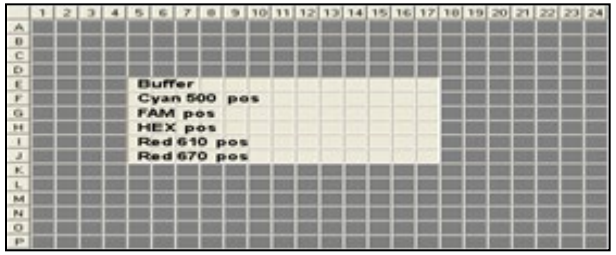


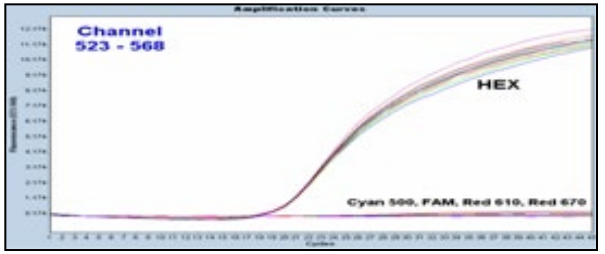
Hybridization Probes

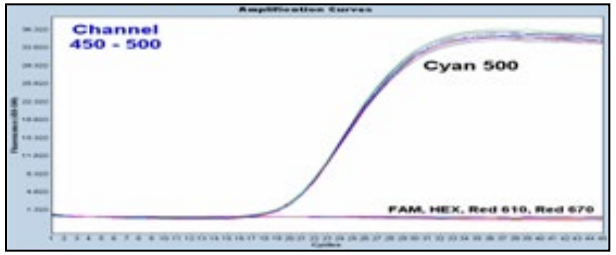


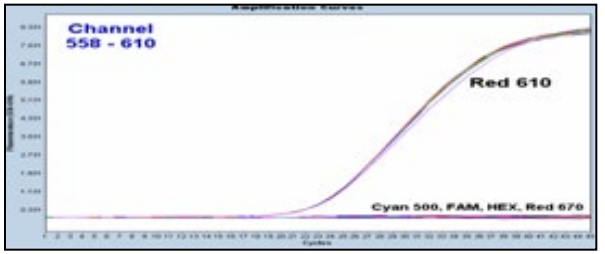
Multi Color Assay, Hydrolysis Probes

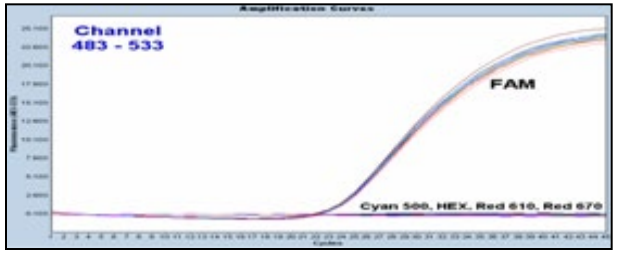
Color Compensation

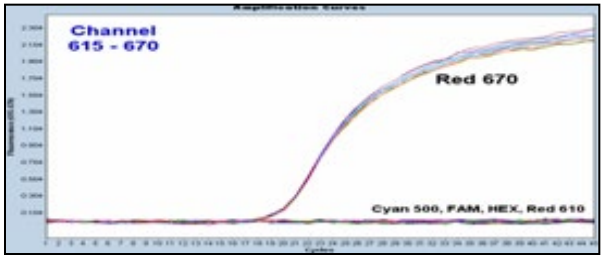












- **LightCycler® CYAN 500**
- **FAM**
- **HEX**
- **LightCycler® RED 610**
- **LightCycler® RED 670**

- **One target, dye per well**
- **All wells analyzed in all channels**

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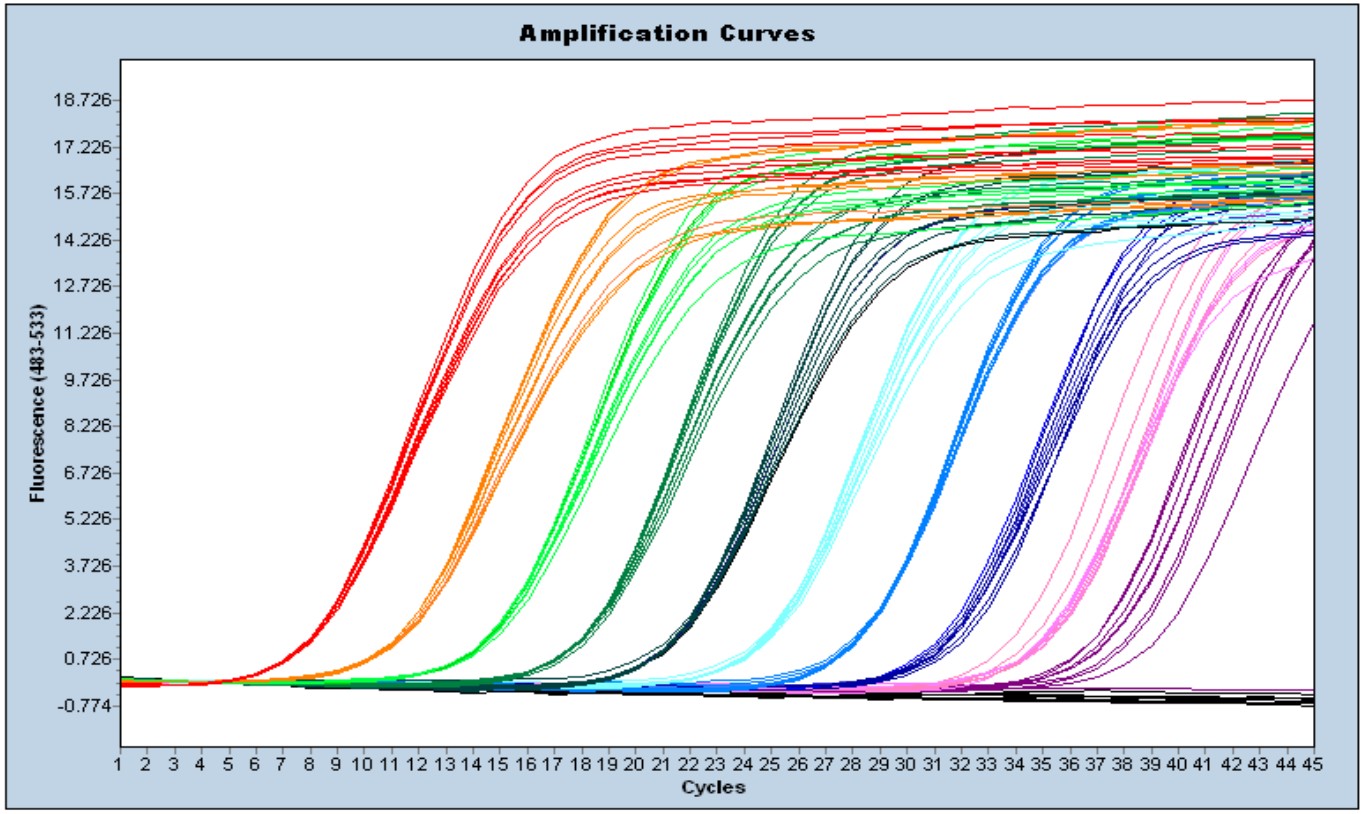
LightCycler® 480 System

Dynamic Range



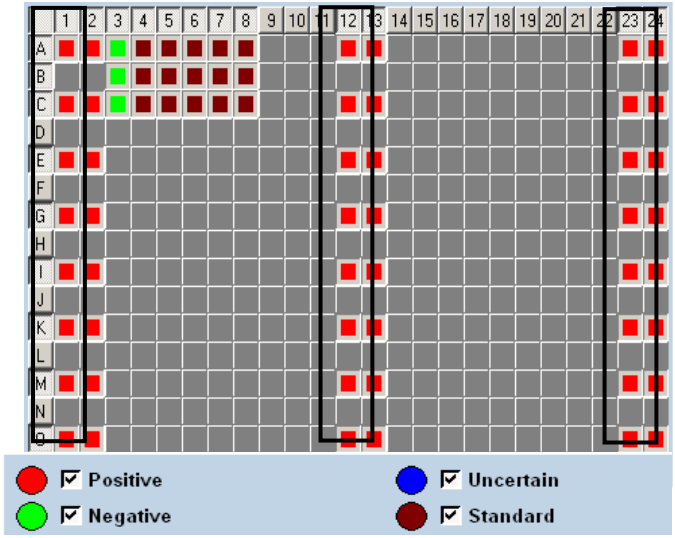
Samples	Statistics	
	Mean Cp	STD Cp
NTC		
1E0	37.69	1.08
1E1	34.94	0.49
1E2	31.65	0.27
1E3	28.21	0.05
1E4	24.85	0.01
1E5	21.56	0.02
1E6	18.17	0.01
1E7	14.81	0.01
1E8	11.46	0.02
1E9	8.18	0.02

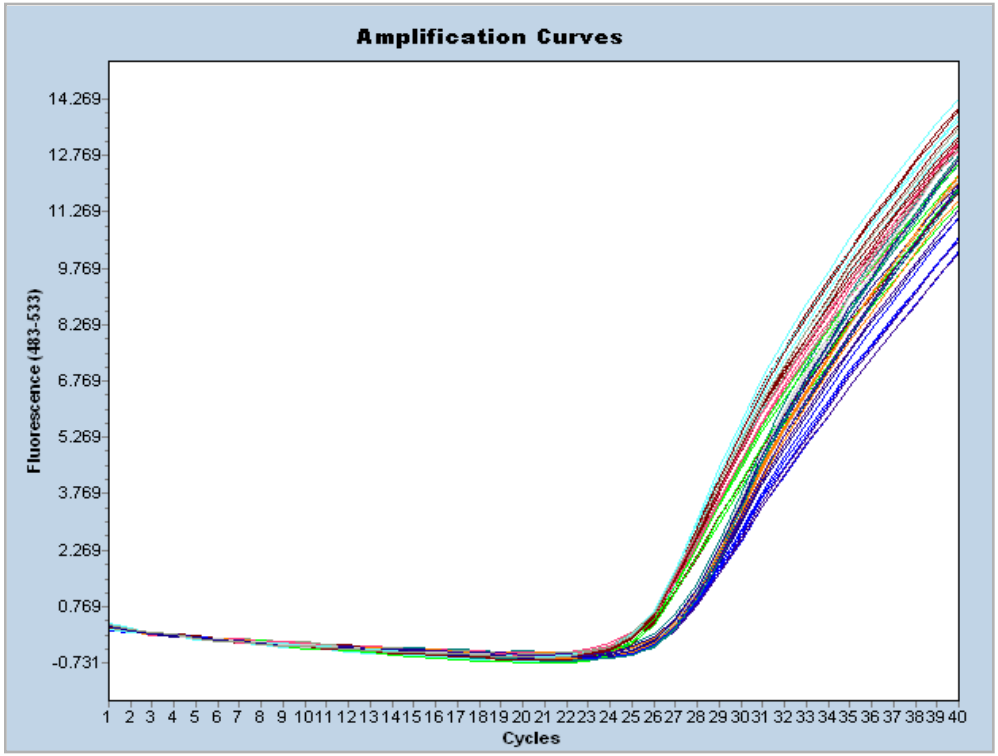
- Serial dilution of plasmid DNA (1 copy - 10^9 copies)
- 9 replicates each
- Detection format: UPL (FAM)
- Target: GAPDH

LightCycler® 480 Control Kit

Detection of a two-fold Difference

- 1000 vs. 2000 copies
- 24 replicates each
- detection format: UPL (FAM)



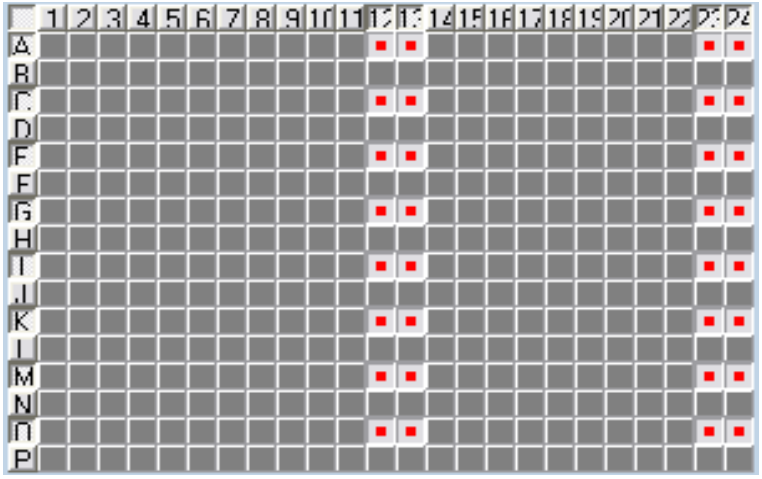


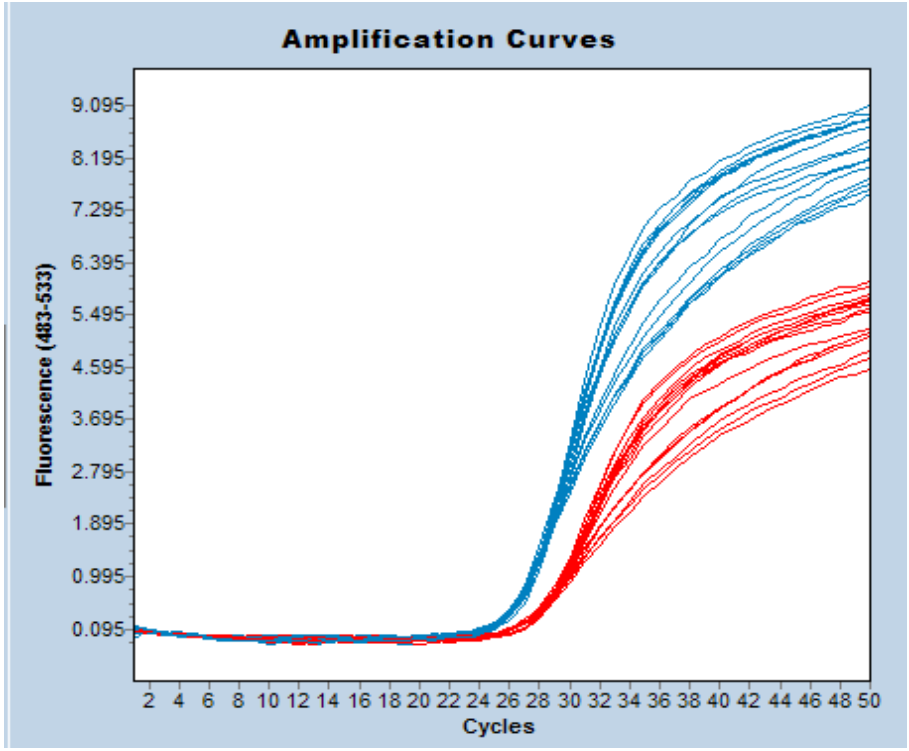
Samples	Mean Cp	STD Cp	Mean conc	STD conc
Unknown 1	26.69	0.08	9.70E2	4.93E1
Unknown 2	25.65	0.04	1.98E3	5.85E1

LightCycler® 480

Detection of a two-fold Differencec

- **1000 vs. 2000 copies**
- **16 replicates each**
- **detection format: one step, Hydrolysis Probes**



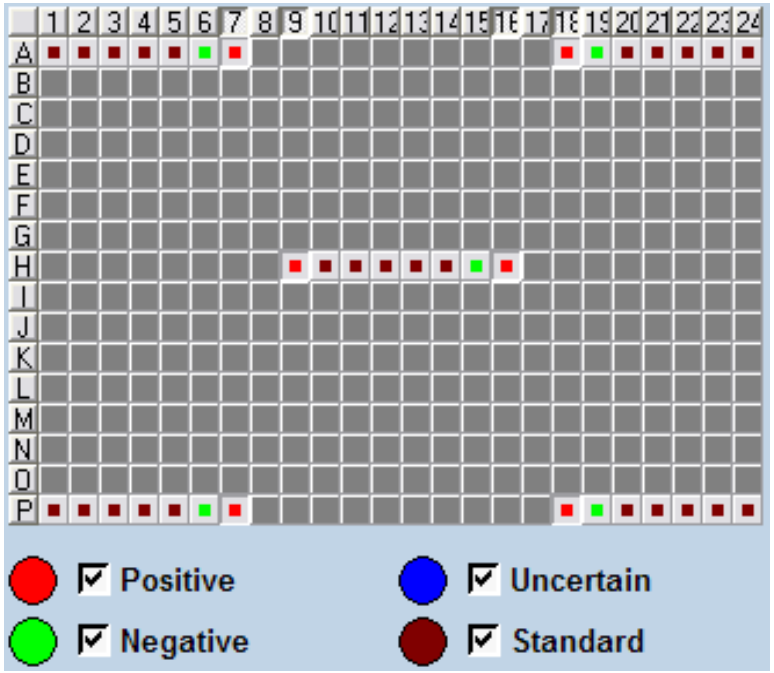


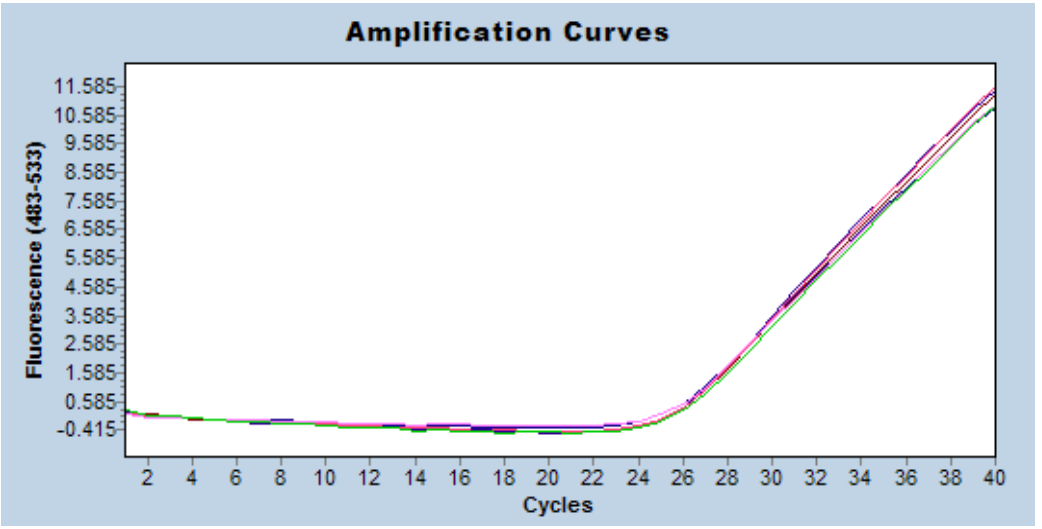
Samples	Mean Cp	STD Cp
A13, A24, C13, C24, E13, E24, G1	26.36	0.38
A23, C12, C23, E12, E23, G12, G2	27.52	0.40

LightCycler® 480

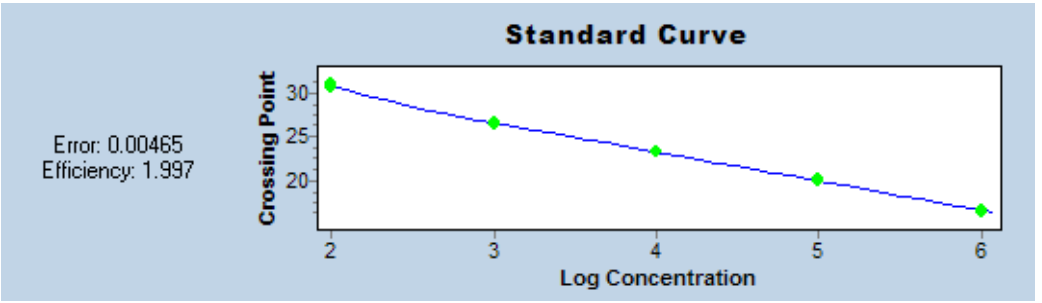
Walk Around the Block

- detection format: Hydrolysis Probes



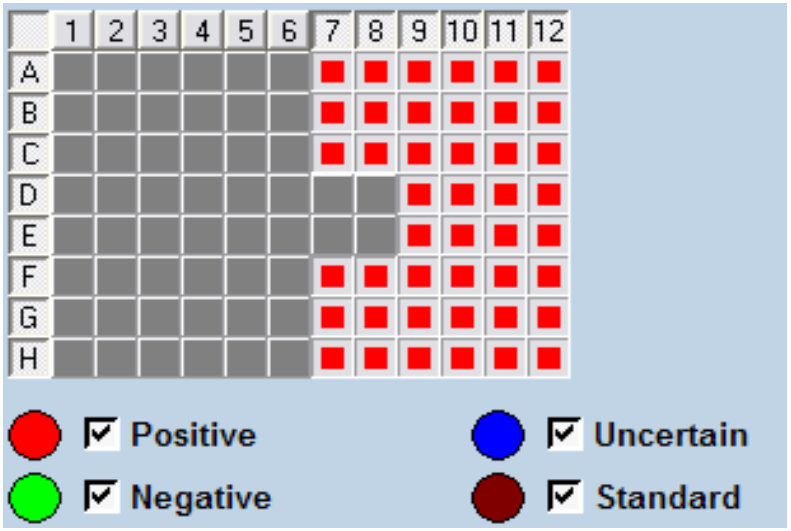


Statistics		
Samples	Mean Cp	STD Cp
A7, A18, H9, H16, P7, P18	25.48	0.09



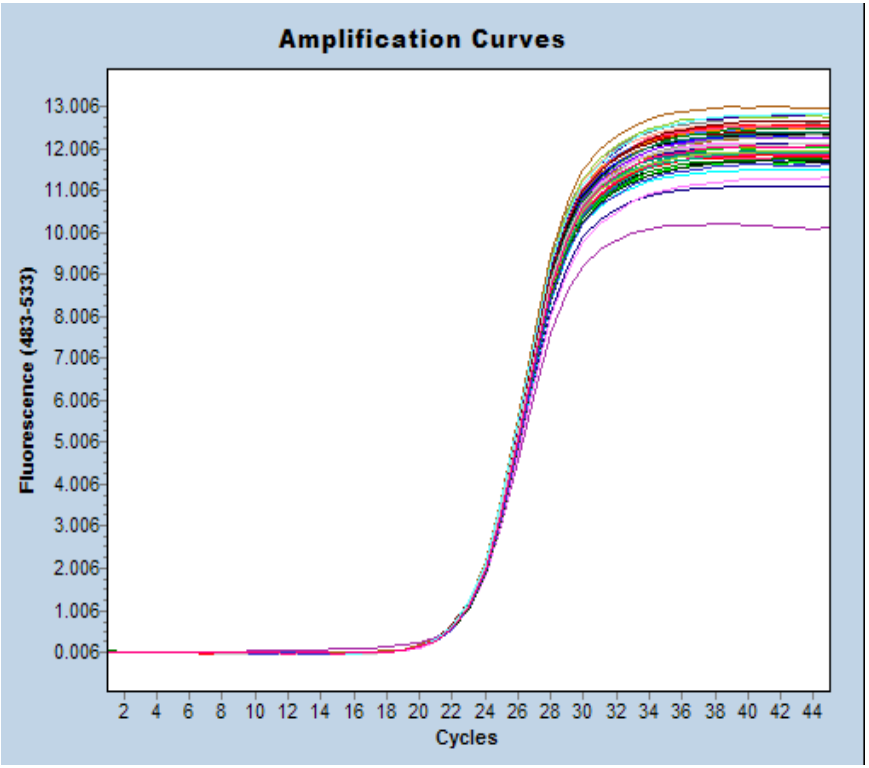
LightCycler® 480
Reproducibility

- **detection format: SYBR Green I**
- **44 replicates of sample**



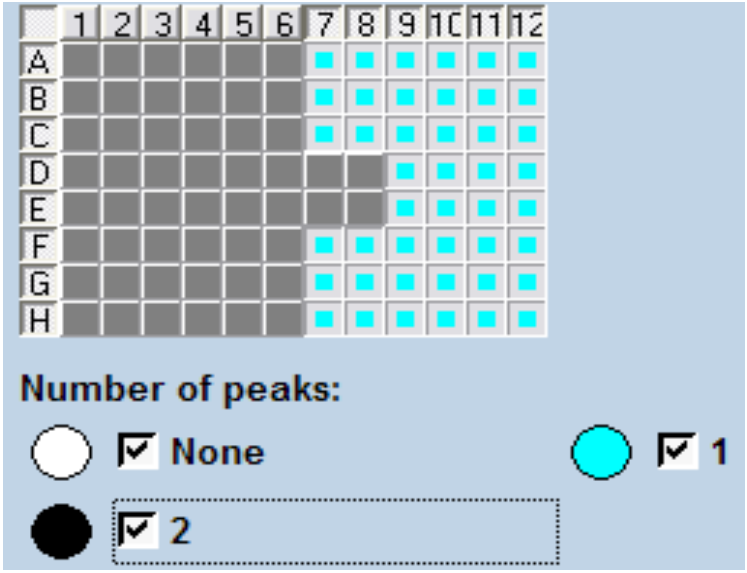
Statistics

Samples	Mean Cp	STD Cp	Mean co...	STD conc
A7, A8, A	22.97	0.04		

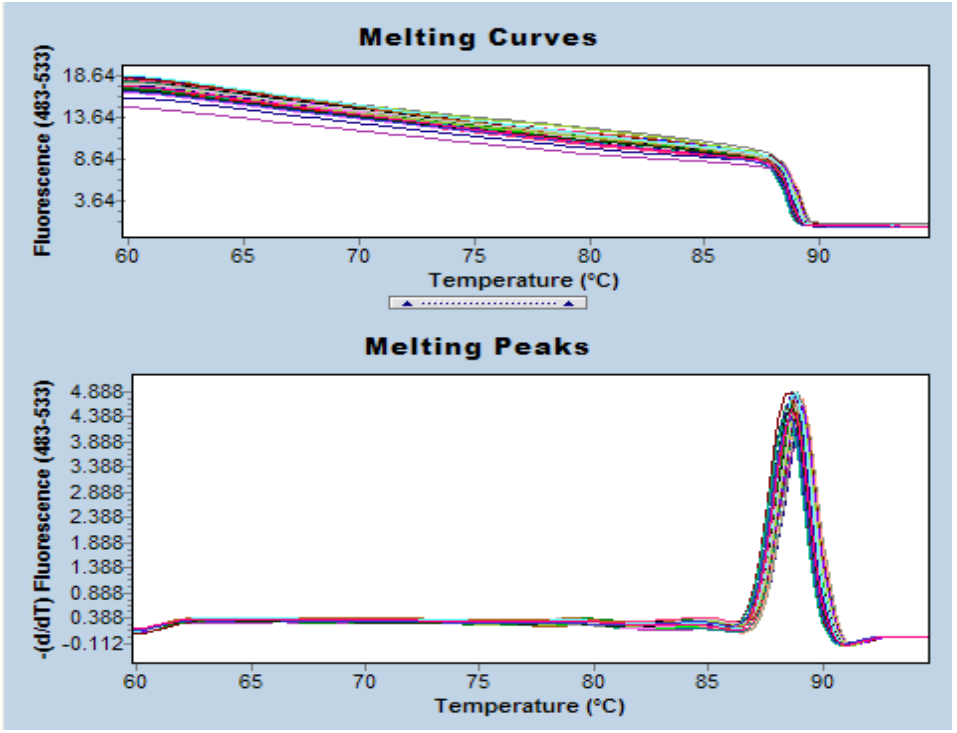


LightCycler® 480
Reproducibility

- **detection format: SYBR Green I**
- **44 replicates of sample**



Samples				Meltin...
Include	Color	Pos	Name	Tm1
☑		A7	Gene x	89.14
☑		A8	Repl. of Gene x	89.11
☑		A9	Repl. of Gene x	89.06
☑		A10	Repl. of Gene x	88.86
☑		A11	Repl. of Gene x	88.94



Average Tm = 88.798 STD=0.16

LightCycler® 480 System
Overview

1. General Description and Specifications

2. LightCycler® 480 Instrument

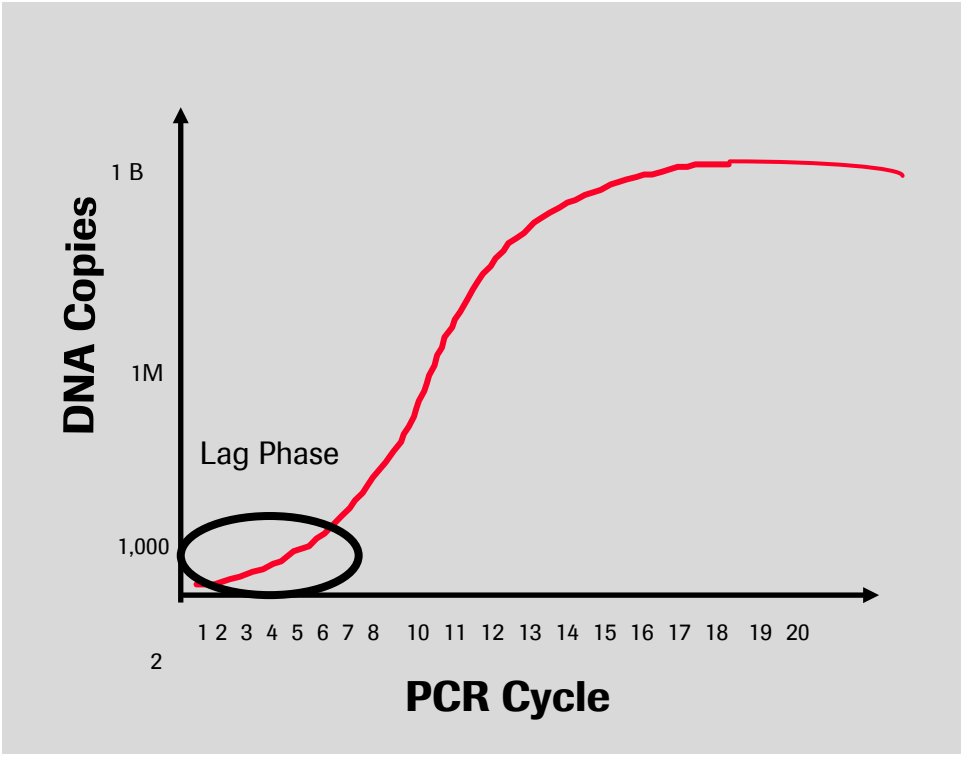
3. Assay Formats, Dyes, and Applications

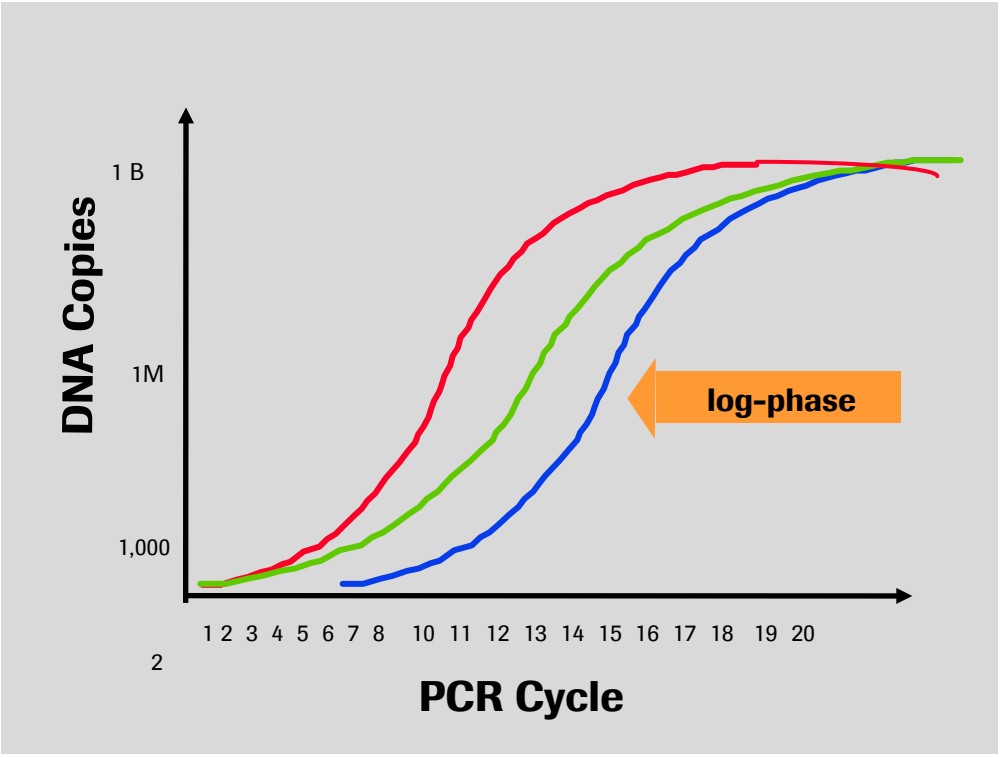
4. LightCycler® 480 Performance

5. LightCycler® 480 Software

Crossing Point Determination

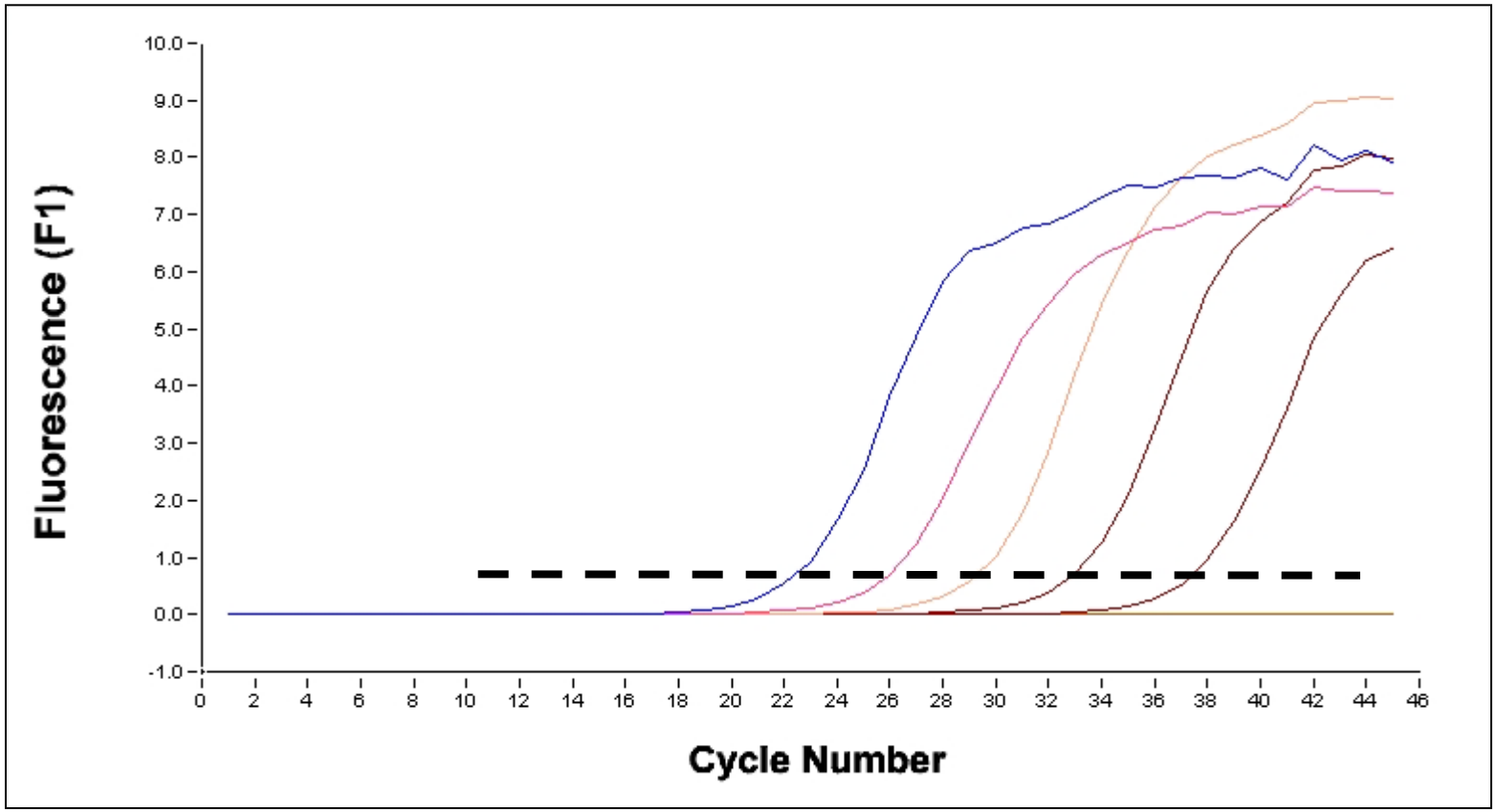
At what point does the amplification reaction transition from lag phase to log-linear phase?





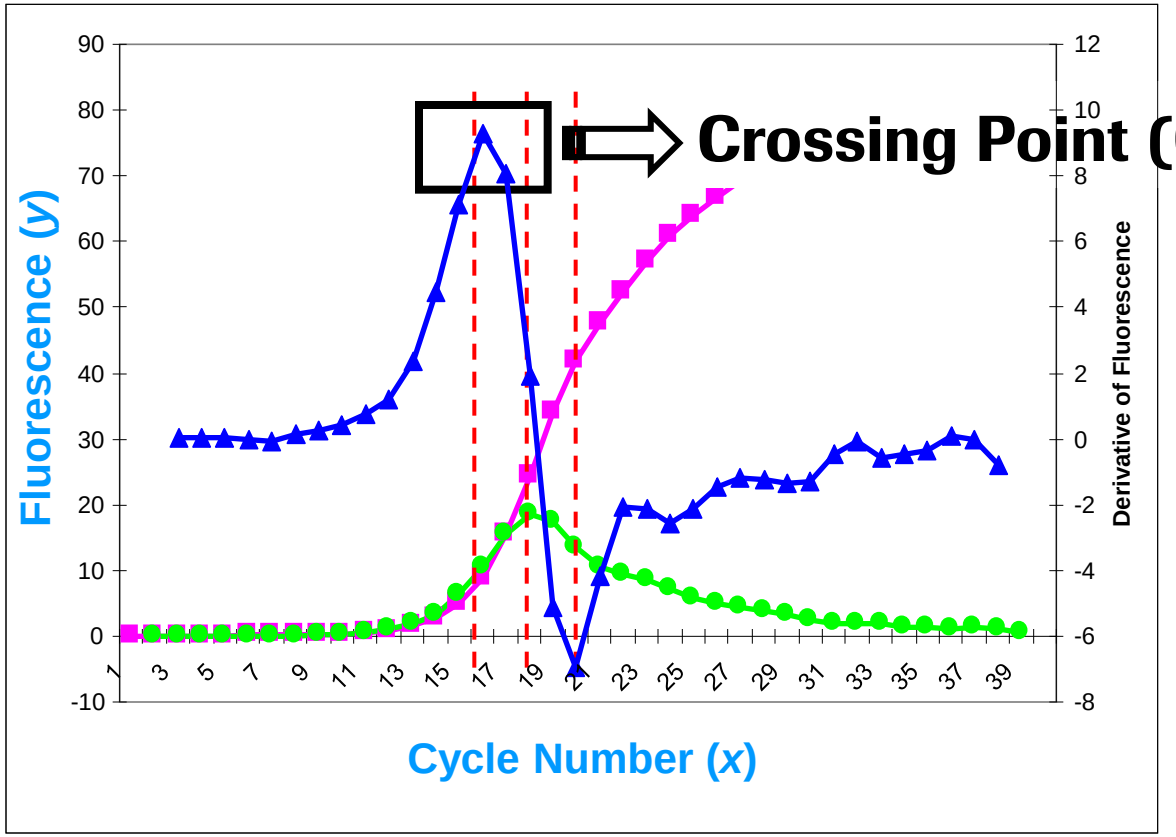
Crossing Point Determination

The Fit Points Method



Crossing Point Determination

The Second Derivative Maximum



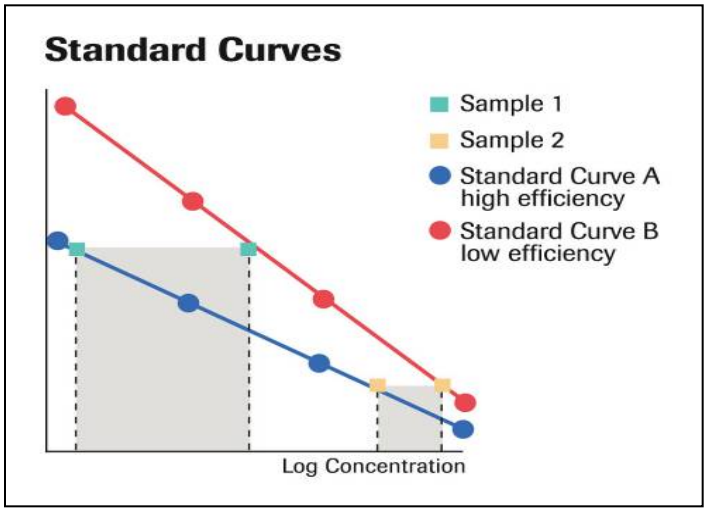
Amplification Data (*x*, *y*)

1st Derivative d*y*/d*x*

2nd Derivative d²*y*/d*x*²

LightCycler® 480 System
Software Innovations(2)

- **PCR efficiency consideration**

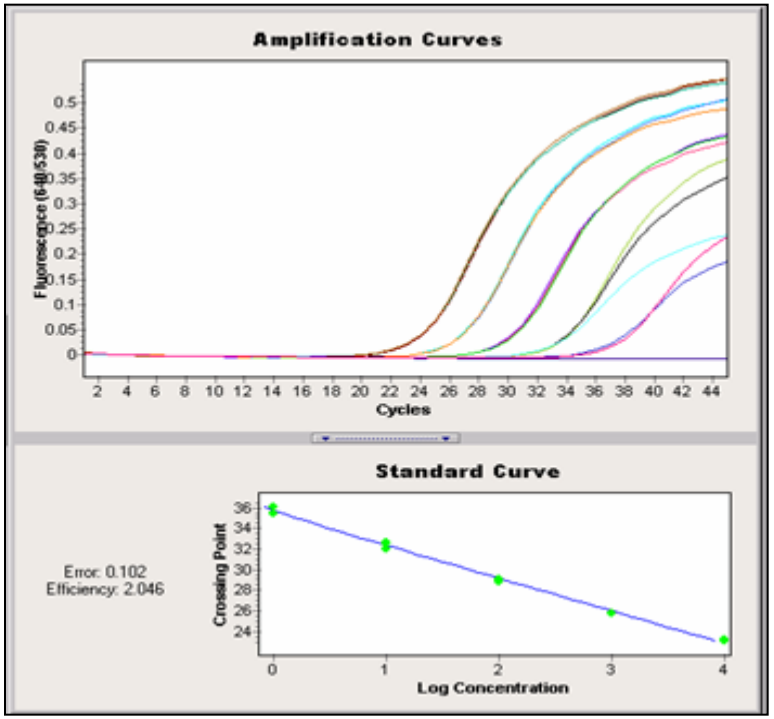


- **Calibrator-normalized PCR**

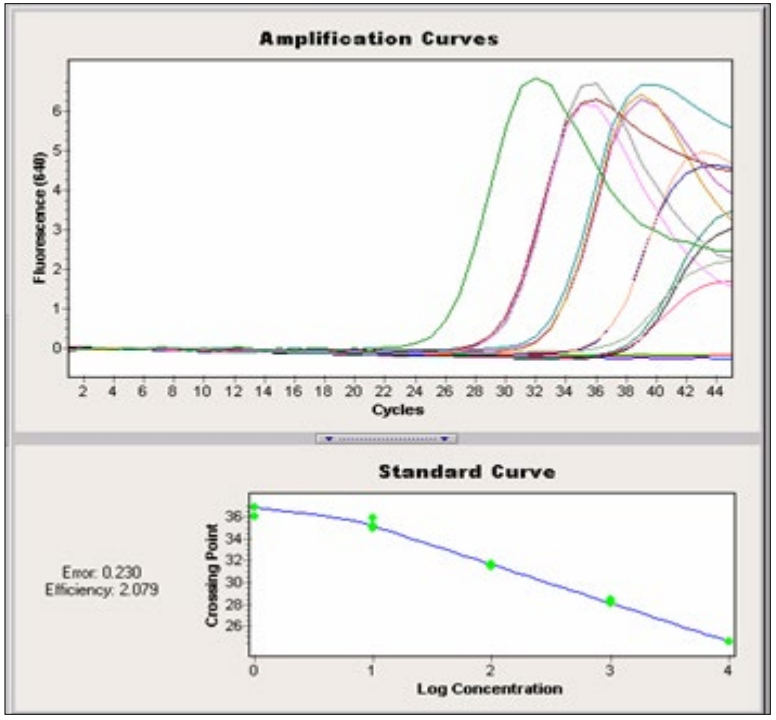
$$\text{normalized ratio} = \frac{\text{target/reference}_{\text{(unknown sample)}}}{\text{target/reference}_{\text{(run calibrator)}}}$$

LightCycler® 480 System
Software Innovations(3)

- **Standard curve calculation truly based on data**

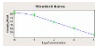


linear fit



non-linear fit

LightCycler® 480 System
Software Innovations

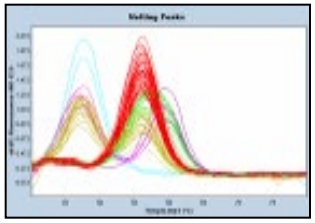
	Without efficiency correction	Efficiency correction with linear fit	Efficiency correction with non-linear fit 
Adrenal Gland RNA			
40 ng	1.03	1.18	**1.41**
8 ng	2.21	1.79	**1.01**
1.6 ng	6.00	4.17	**1.17**
Mean value	3.08	2.38	**1.21**
Standard deviation	2.5967	1.5799	**0.2173**
Coefficient of variation	84.3%	66.4%	**18.0%**

Test System: **Total RNA from - human adrenal gland tissue (sample)**
- HeLa cell line (calibrator)

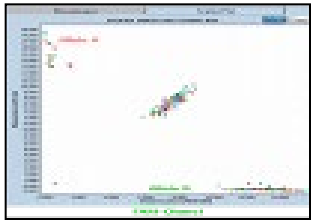
Duplicate measurements for each data point

Data Analysis: **Calibrator-normalized target/housekeeping ratios**

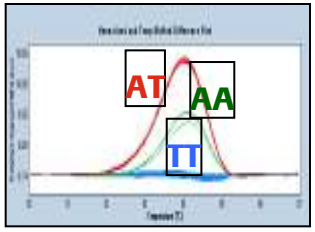
LightCycler® 480 Software
Flexible Analysis Options



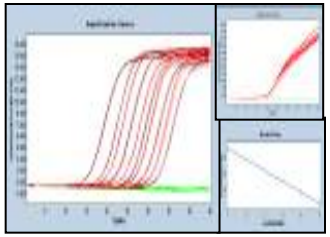
Melting Curve Genotyping :SNP analysis with HybProbe probes
- Robust melting curve analysis
- Automated annotation
- Ideal for multiplex assays and haplotyping



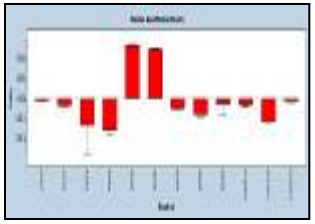
Endpoint Genotyping
- Using hydrolysis probes
- For known SNPs in known regions



Gene Scanning-HRM
- Discovery of unknown genetic and epigenetic changes
- Homo-/heterozygote differentiation

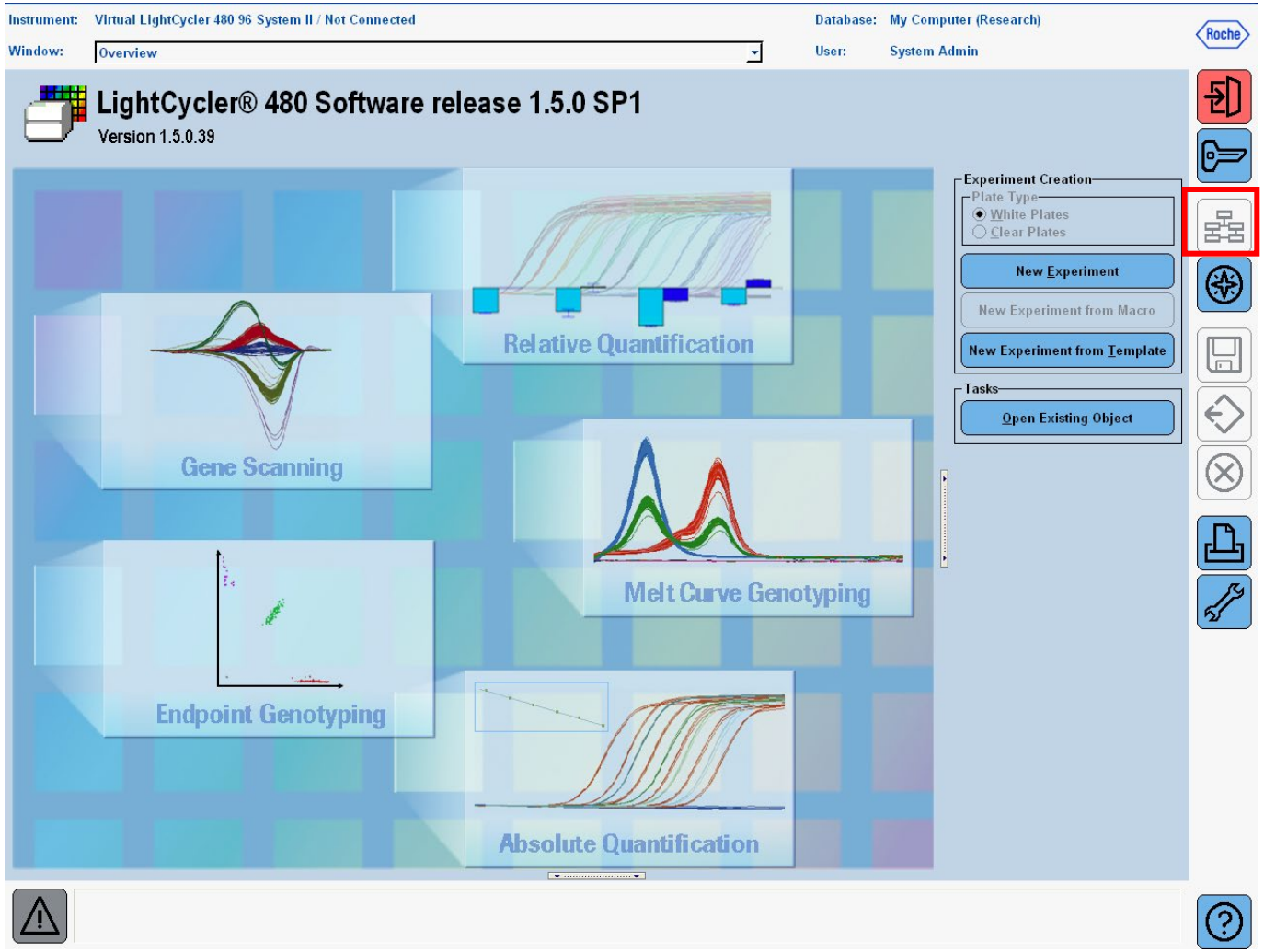


Absolute Quantification
- Dynamic range: 1–10^{10} copies
- External standard curves



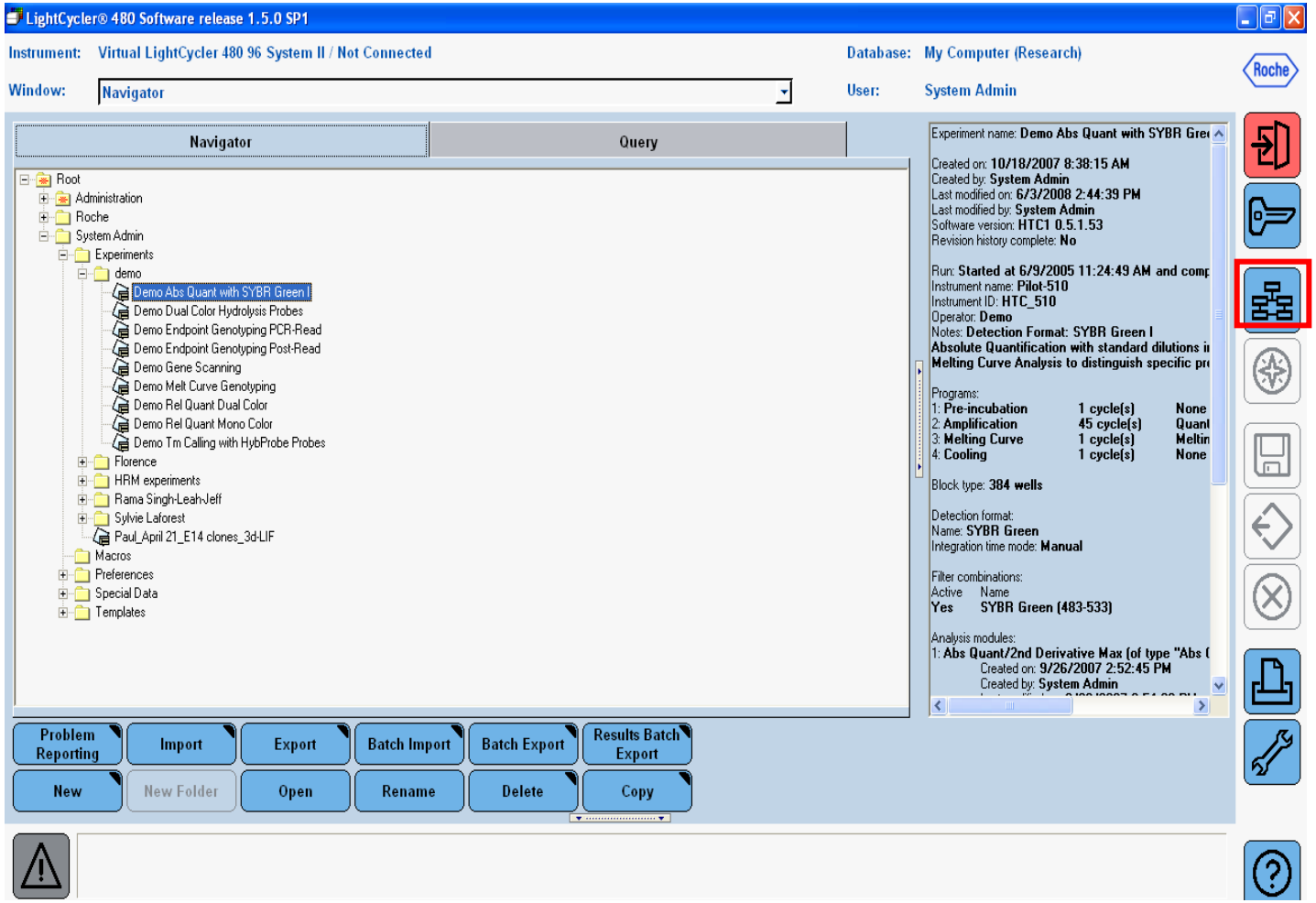
Relative Quantification
- Basic analysis: one click to result
- Advanced method for utmost accuracy
- Accurate results using efficiency correction
- Use of reference genes

LightCycler® 480 Software
Overview



- **New Experiment**
- **Macros are used to run an experiment automatically**
- **Templates**

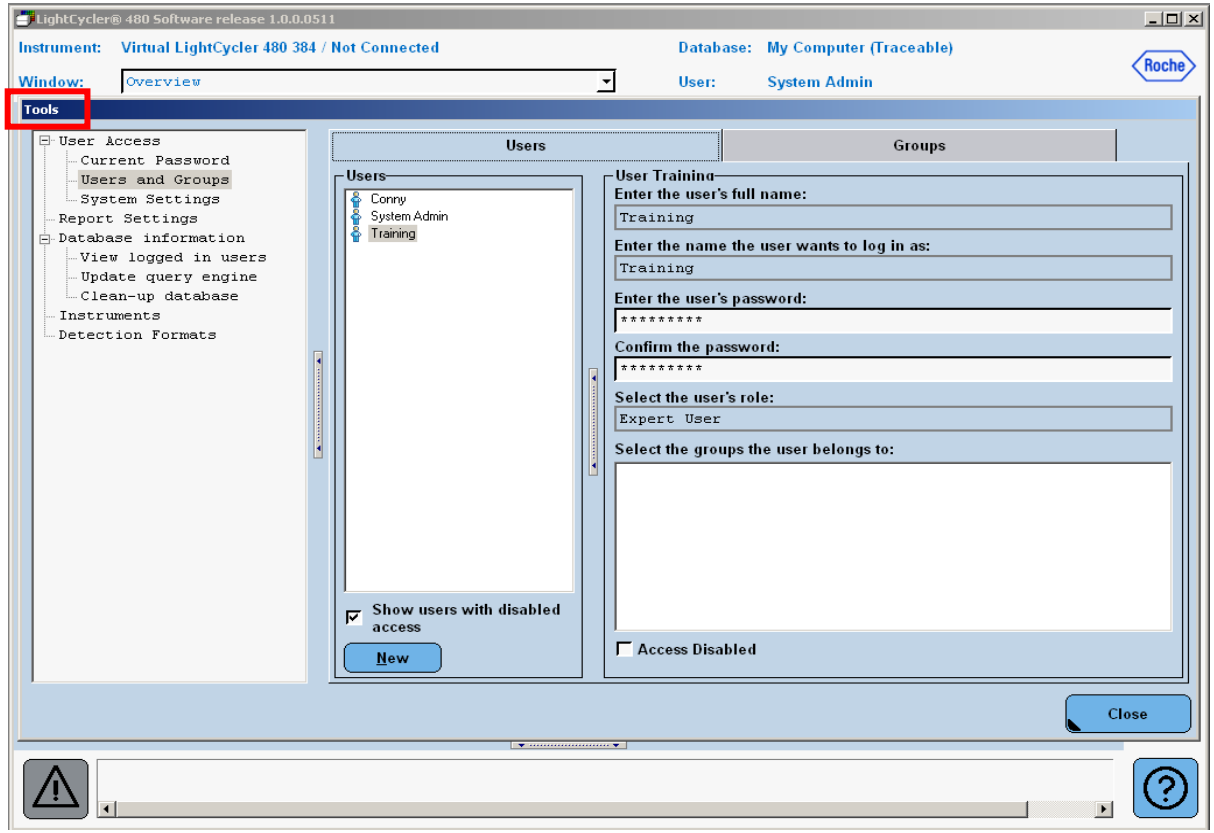
LightCycler® 480 Software
Data Management



- Data stored in a traceable or a research database
- Navigator for data base entries
- Query possibility to find information
- Export and Import functions

LightCycler® 480 Software

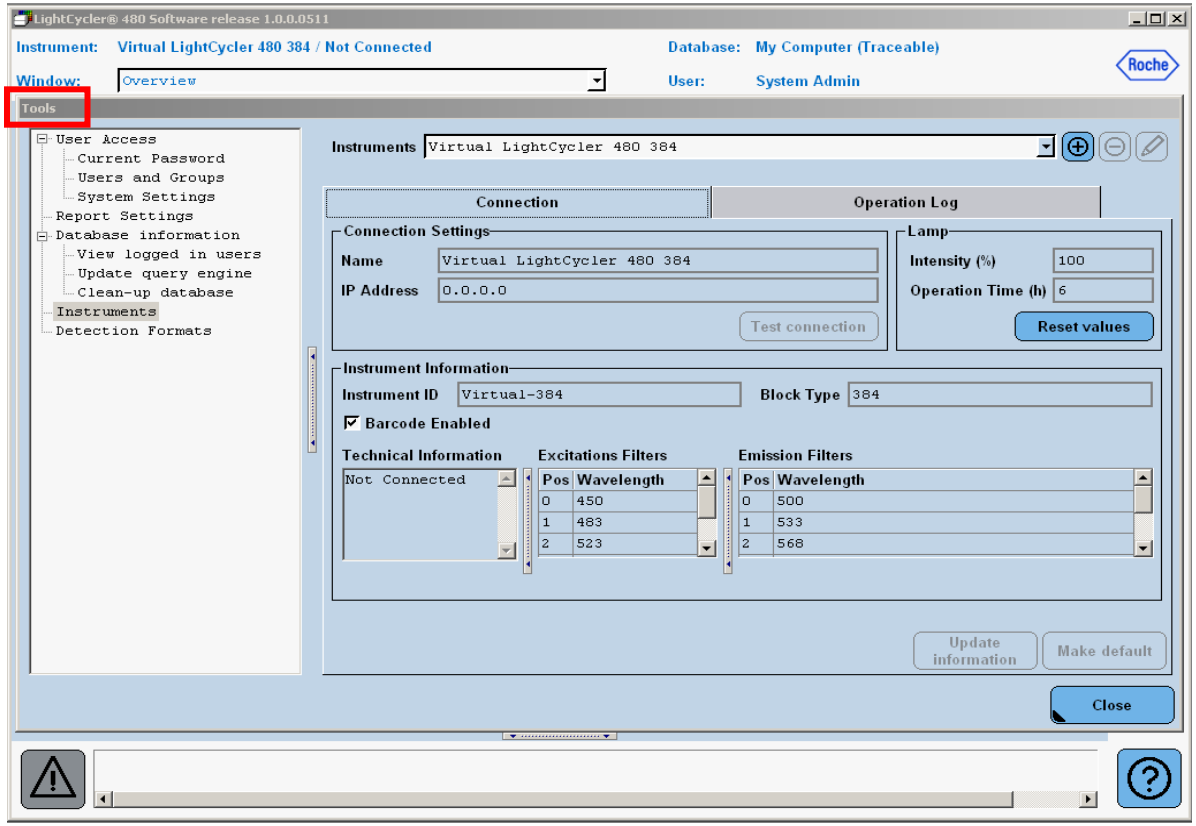
User Management



- **Different user levels (admin and expert user)**
- **Users may be organized in groups**

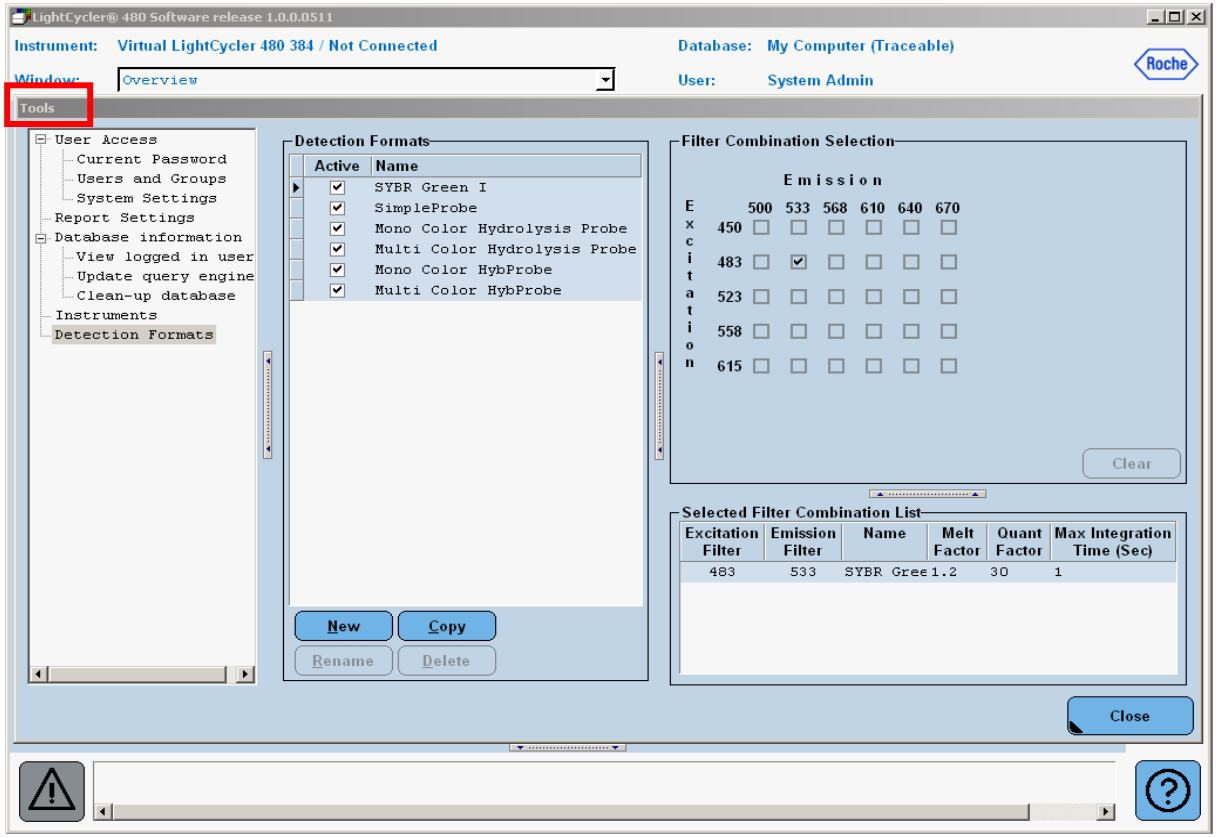
LightCycler® 480 Software

Instrument



- **Connected instrument**
- **Block type,**
- **Lamp status,**
- **Operation log**

LightCycler® 480 Software
Detection Format

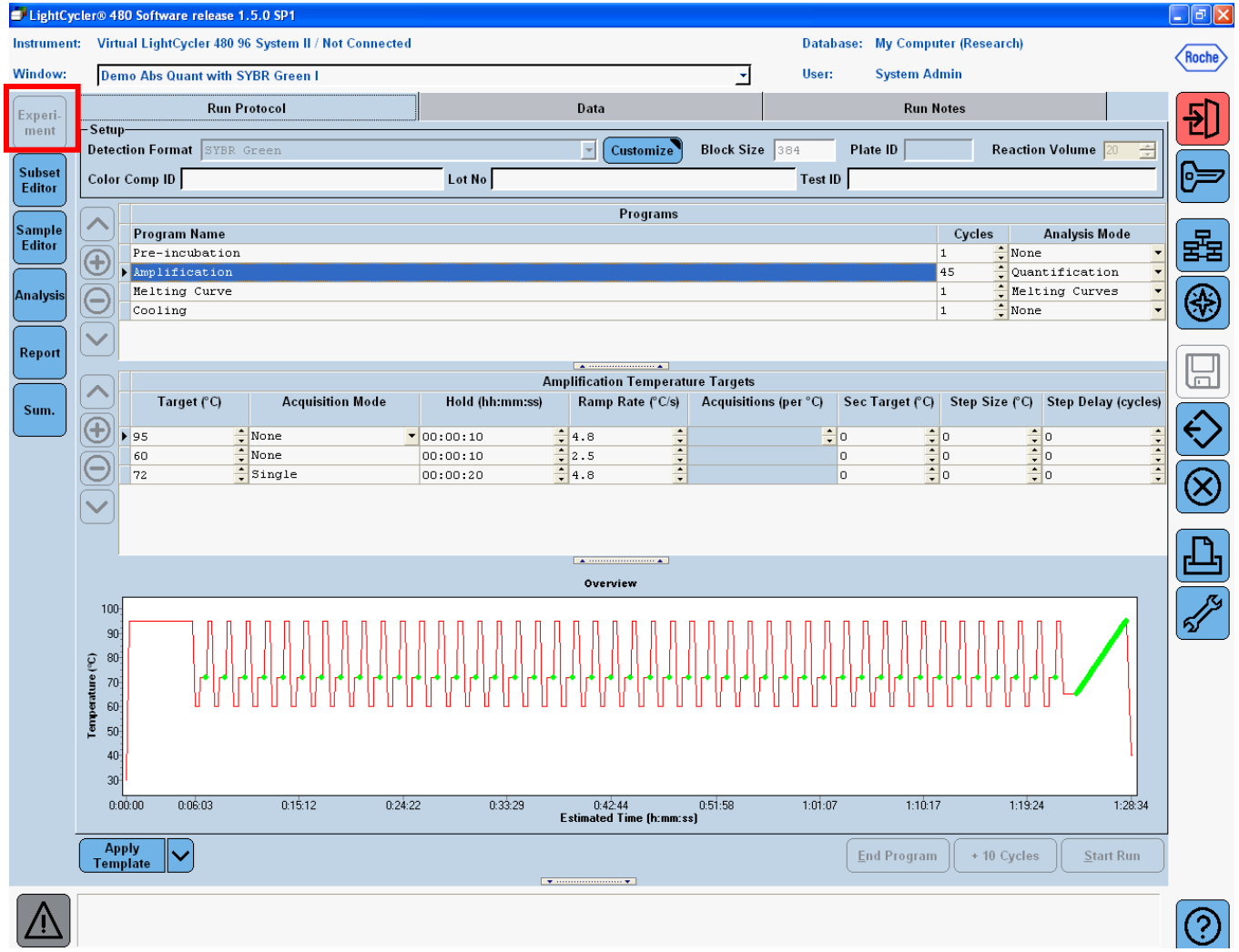


- **5 Excitation filters**
- **6 Detection filters**

- **Roche-defined detection formats**
- **User-defined detection formats possible**

LightCycler® 480 Software

Experiments

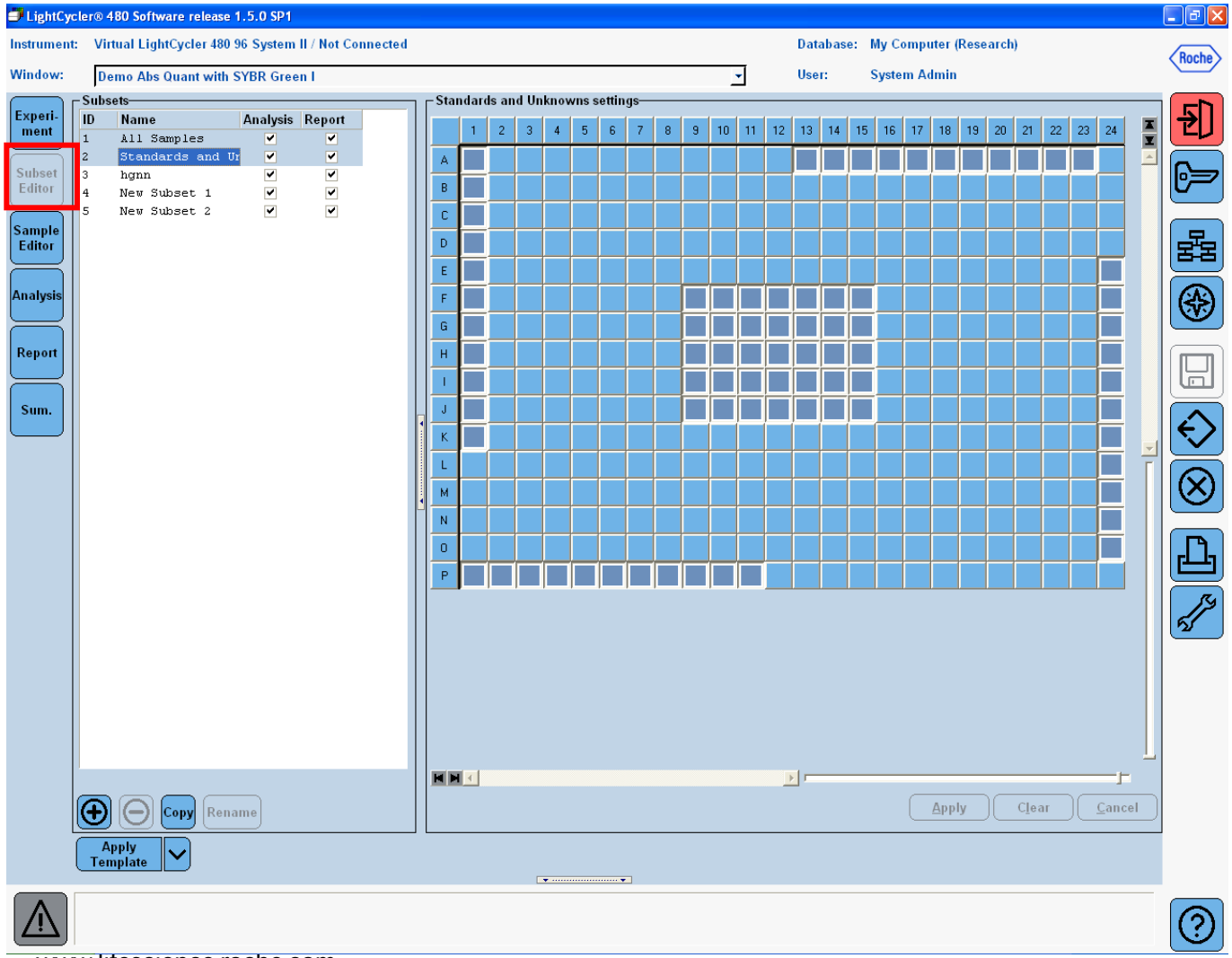


- **Flexible run programming**
- **Online data display of fluorescence and temperature**
- **Create or apply Templates**

  All parts and settings of an experiment (run, sample management, analysis and report) can be stored in templates and easily applied to experiments

LightCycler® 480 Software

Sample Management (1): Subset Editor

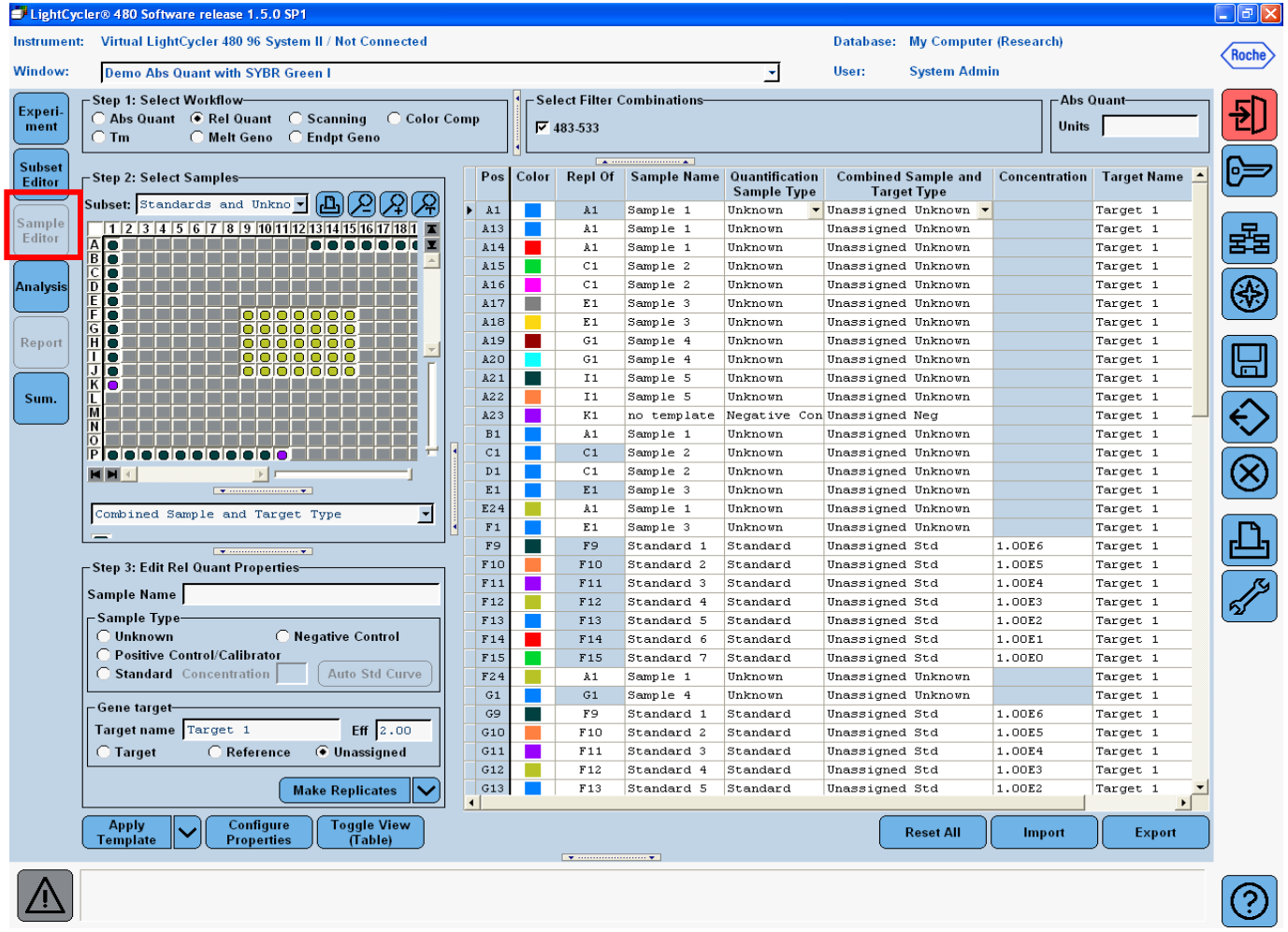


- **Subset Editor**
- **Sample Editor**
- **Plate view**

- **Create or apply Template**

LightCycler® 480 Software

Sample Management (2): Sample Editor

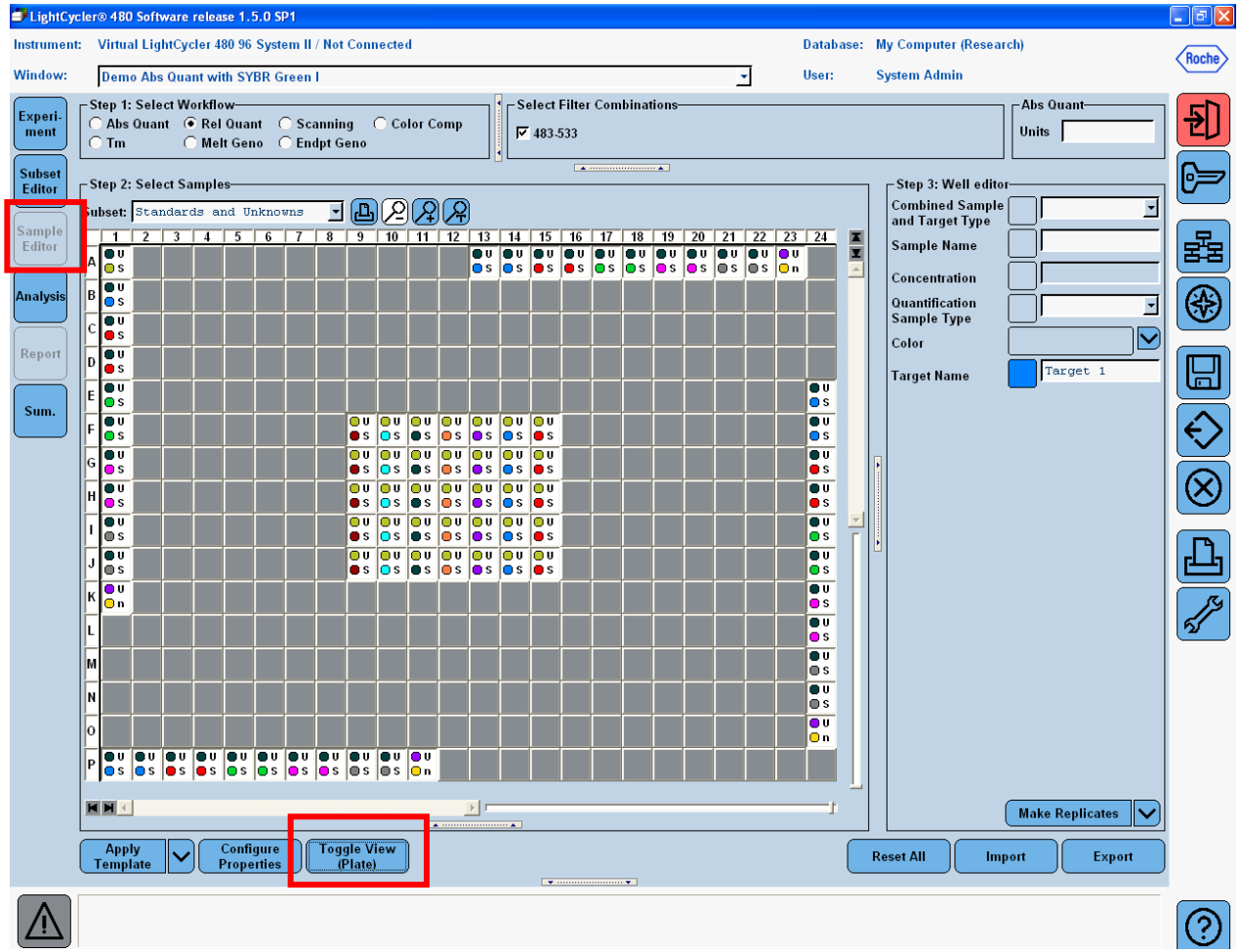


- **Subset Editor**
- **Sample Editor**
- **Plate View**

- **Create or apply Template**

LightCycler® 480 Software

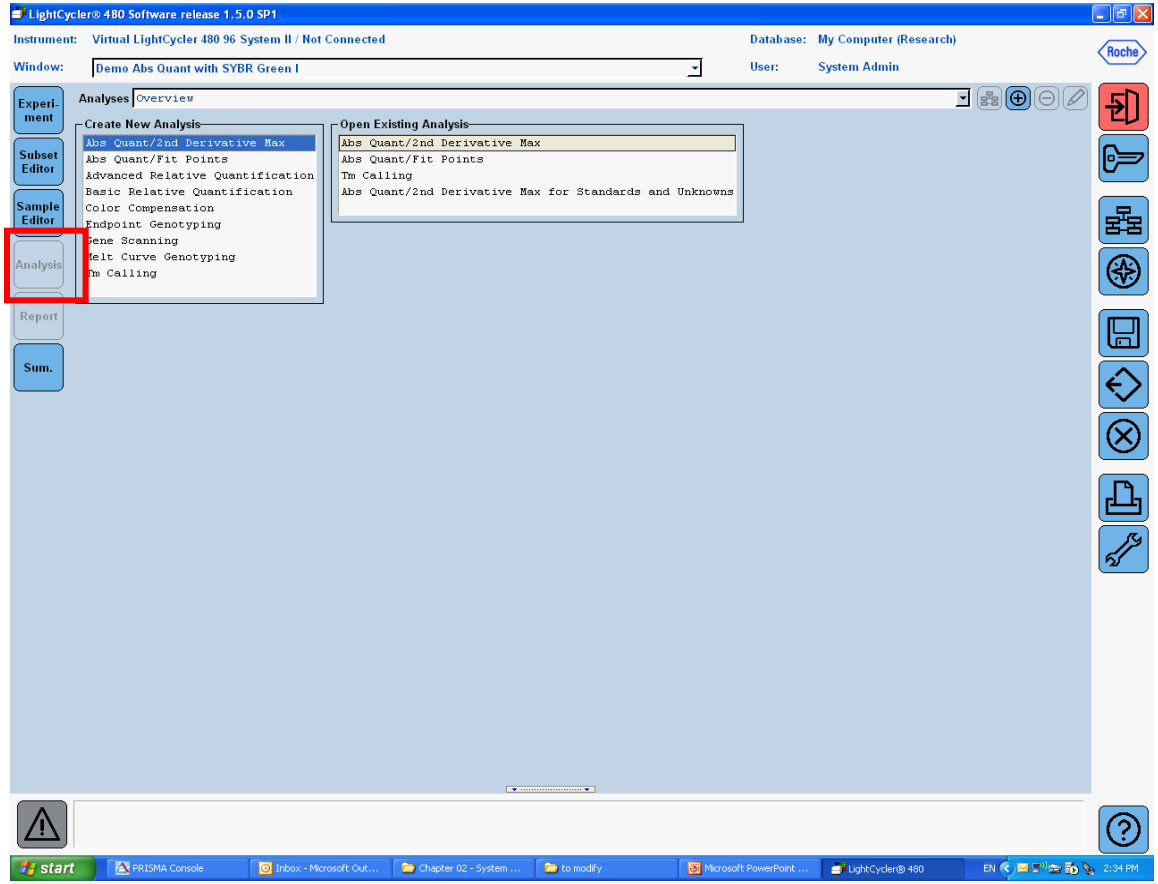
Sample Management (3): Plate View



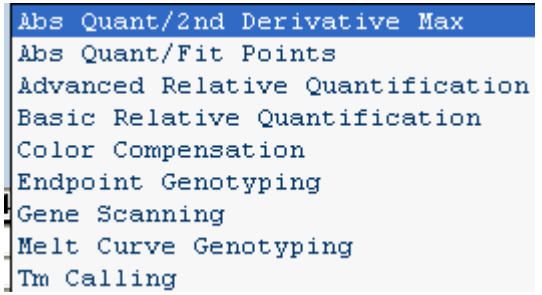
- **Subset Editor**
- **Sample Editor**
- **Plate view**

- **Create or apply Template**

LightCycler® 480 Software
Analysis Modules



Analysis Modules

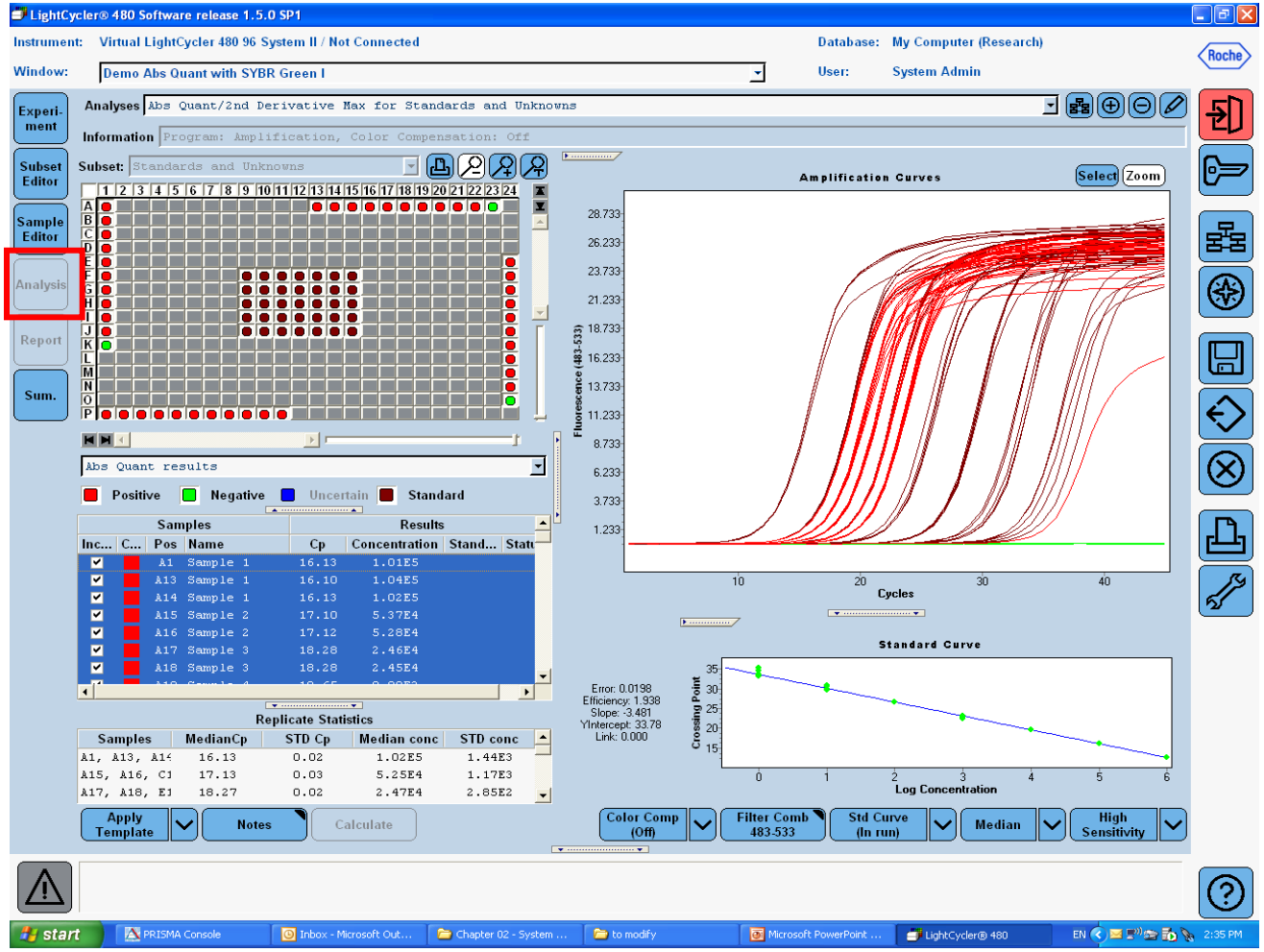


Optional Modules

- **LIMS/Barcode module**
- **Gene Scanning**
- **Multiple Plate Analysis Software**

LightCycler® 480 Software

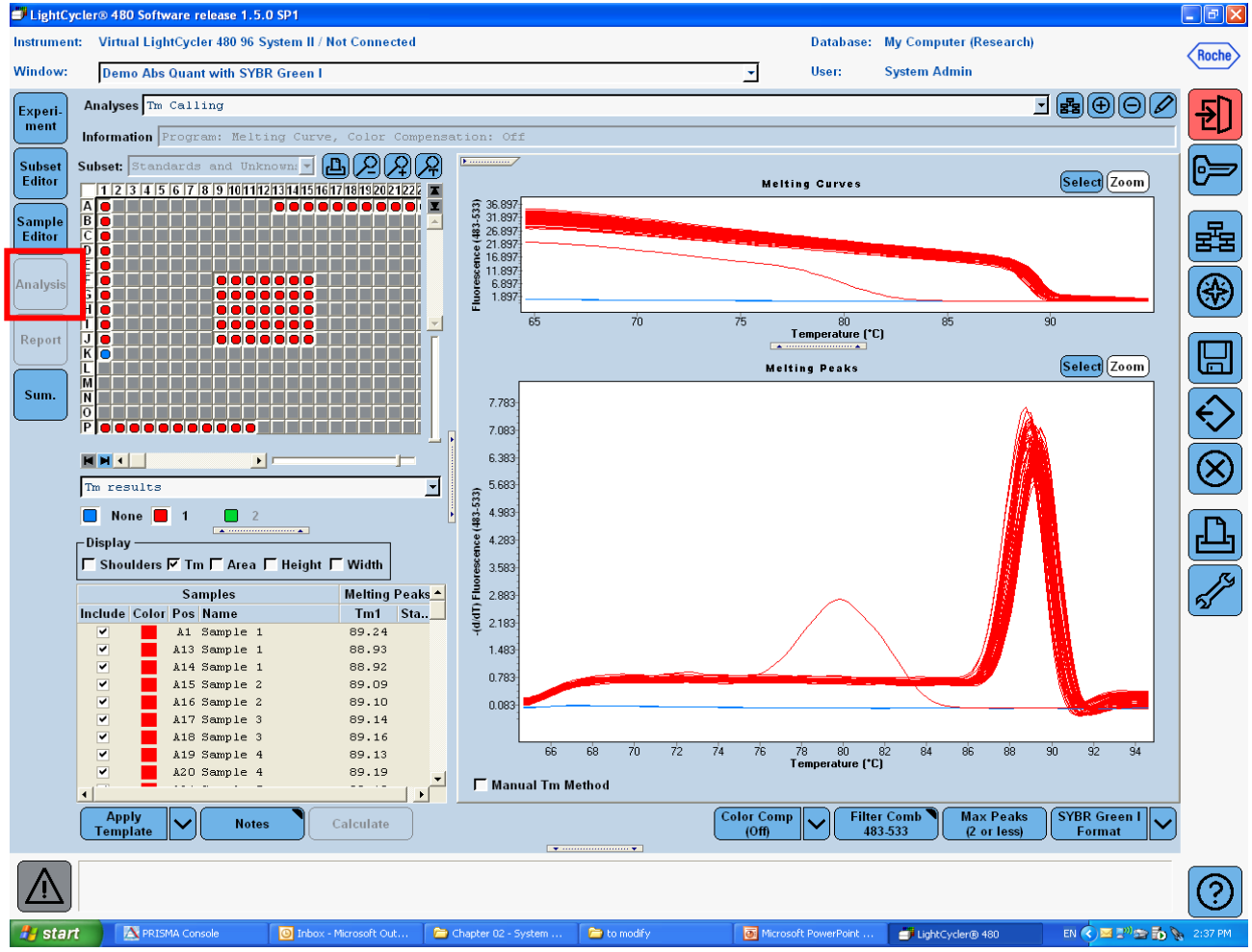
Analysis Module: Absolute Quantification



- Automated calculation method of Crossing Points (Cp)
- Standard curve:
  - Standards in the same experiment
  - import of a previous saved standard curve object
- Create or apply Template

LightCycler® 480 Software

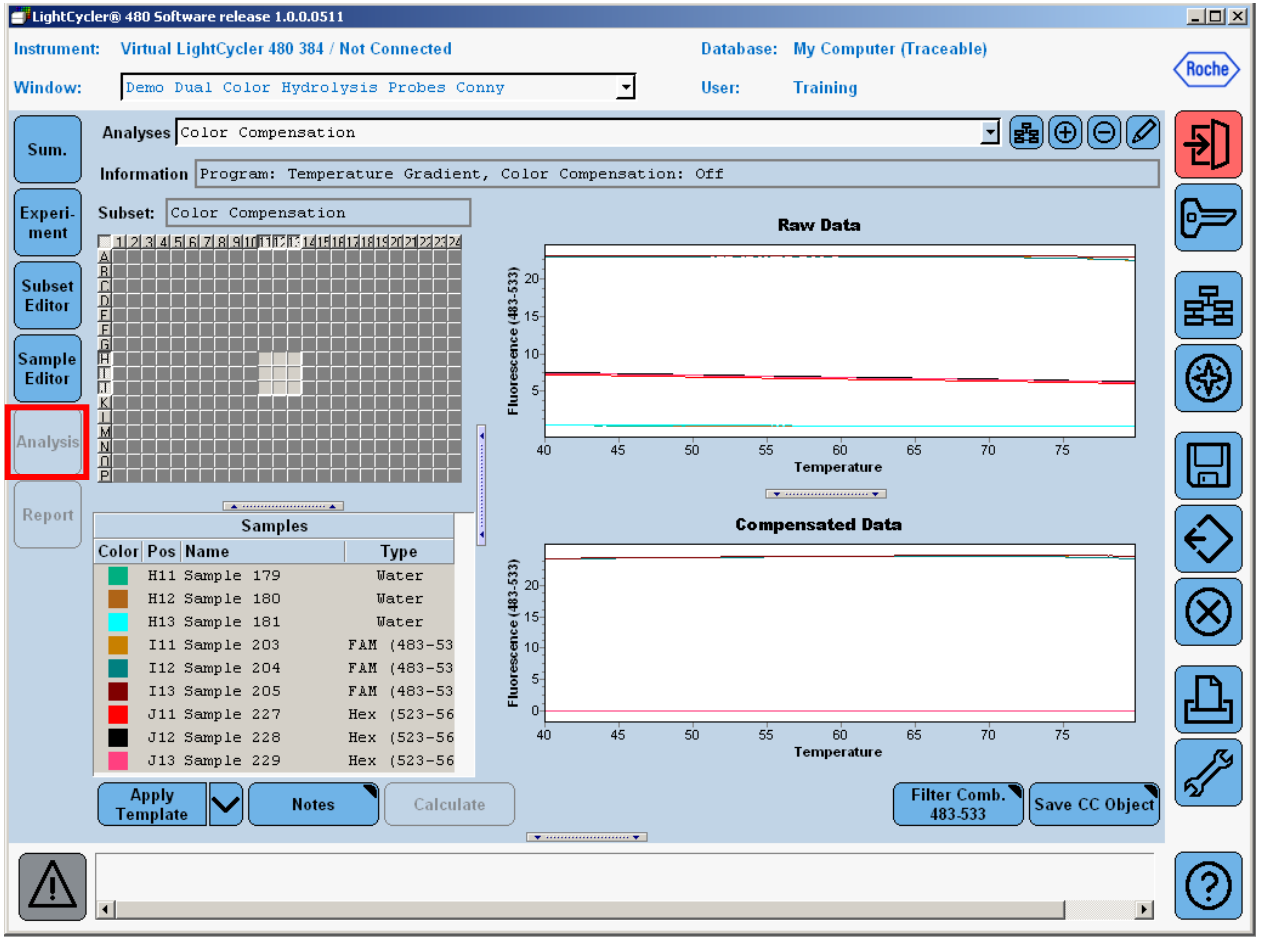
Analysis Module: Tm Calling



- Automated calling of melting temperatures (Tm)
- Improved algorithms for
  - HybProbe Format
  - SYBR Green I Format
  - SimpleProbe Format
- Optional
  - Manual Tm Editing
  - Display settings
- Create or apply Template

LightCycler® 480 Software

Analysis Module: Color Compensation



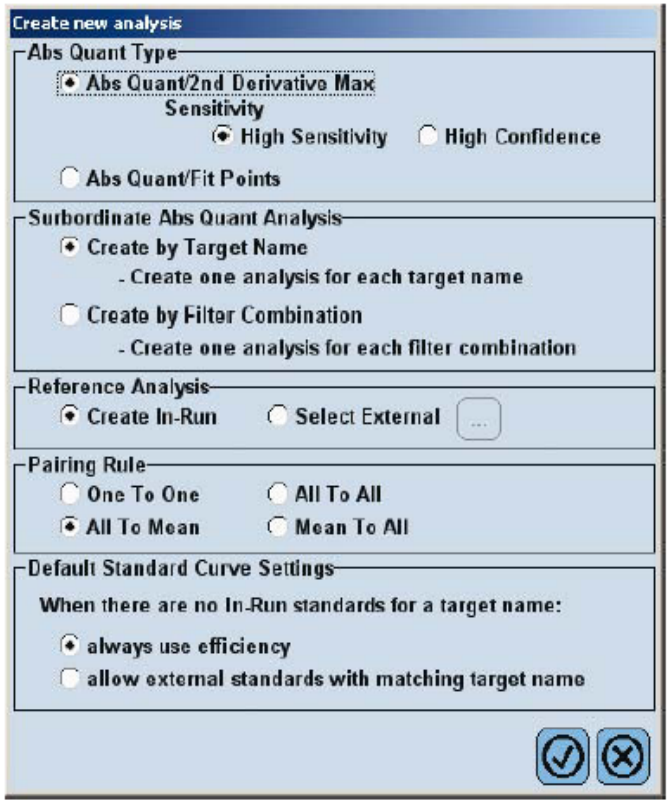
- Raw Data are generated with a Color Compensation run
- Analyzed data are saved as CC Object
- Color Compensation objects are applied to dual or multi color experiments
- Create or apply Template

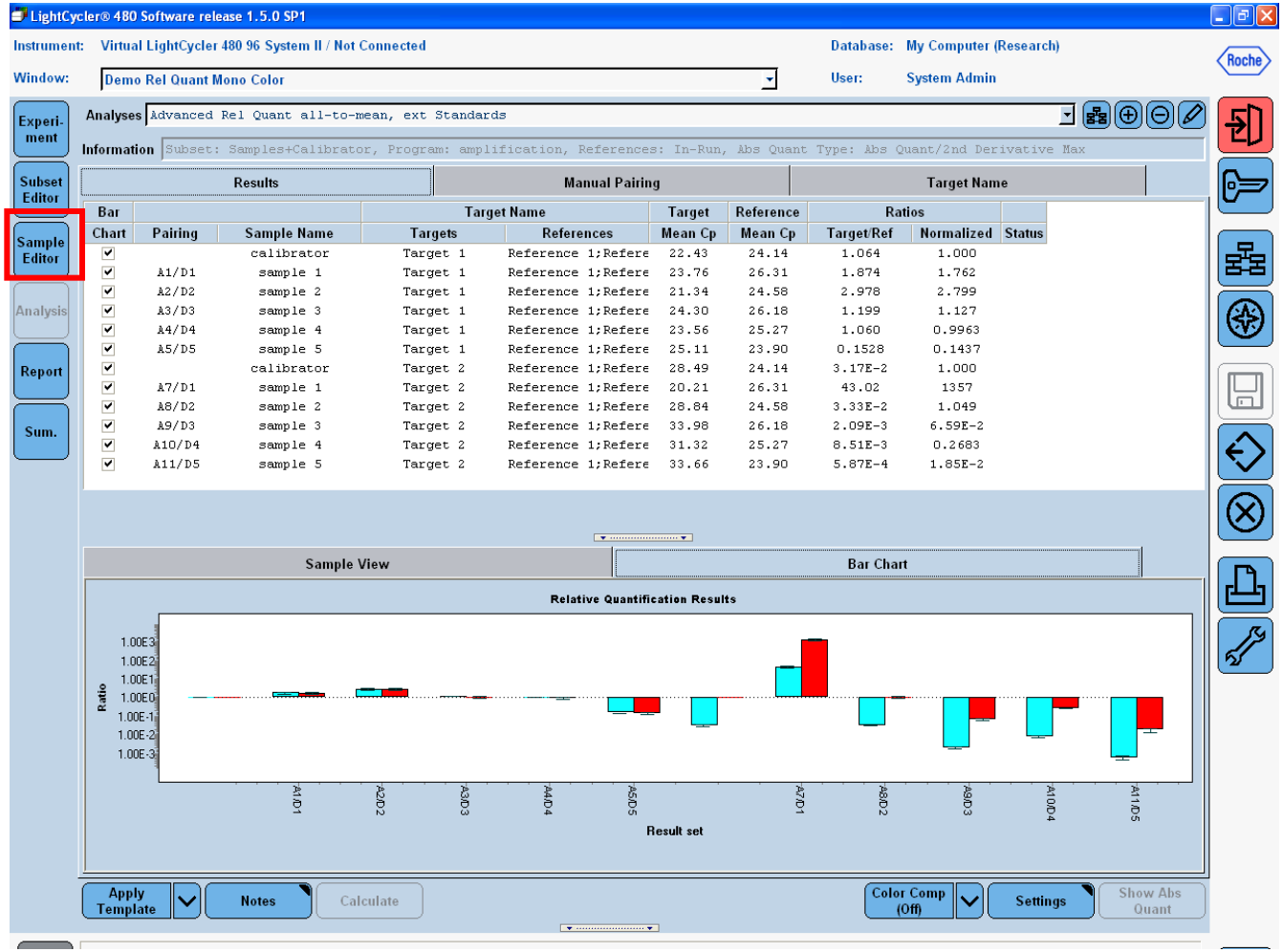
LightCycler® 480 Software

Analysis Module: Relative Quantification

-Basic Relative Quantification Analysis: automated, results with one click

-Advanced Relative Quantification Analysis: according to your ***needs***



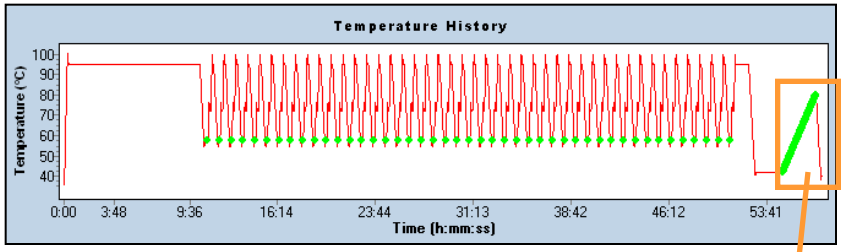


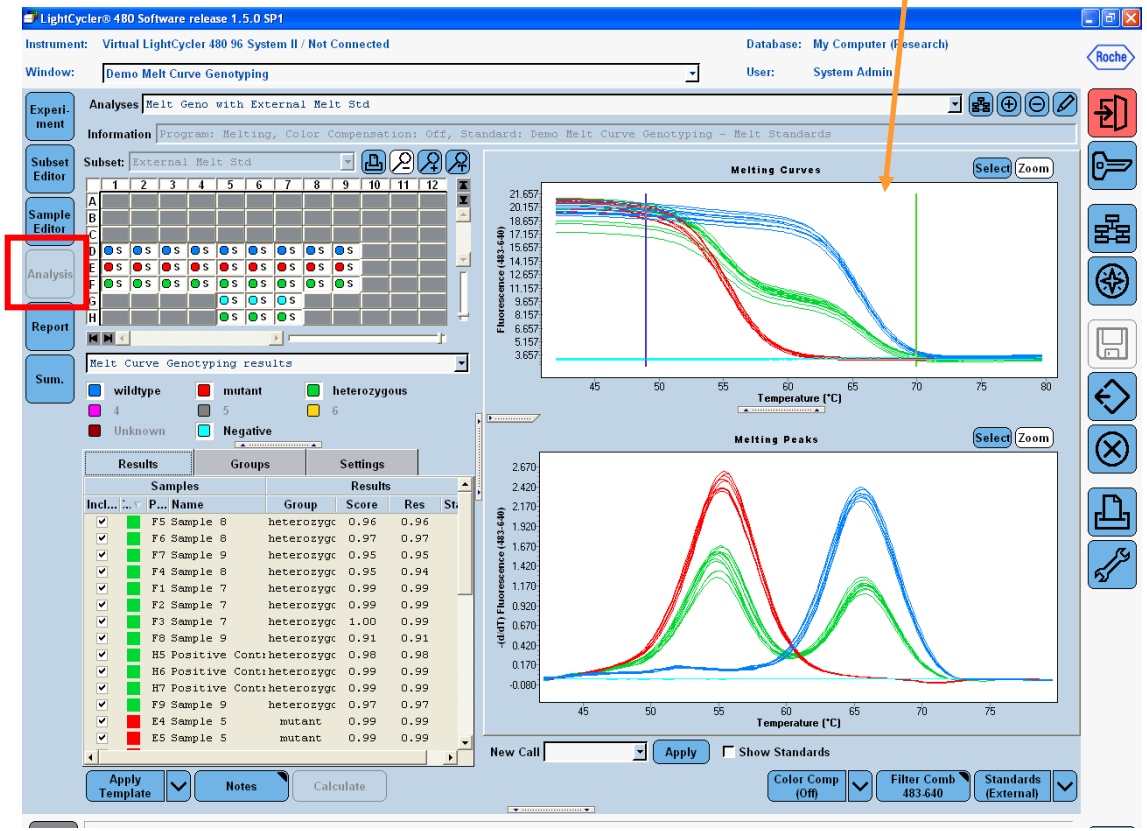
LightCycler® 480 Software

Analysis Module: Melting Curve Genotyping

Detection of known variants

Genotyping with HybProbe-/Simple Probe probes



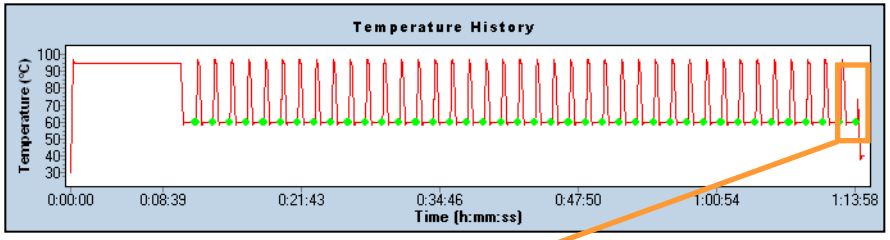


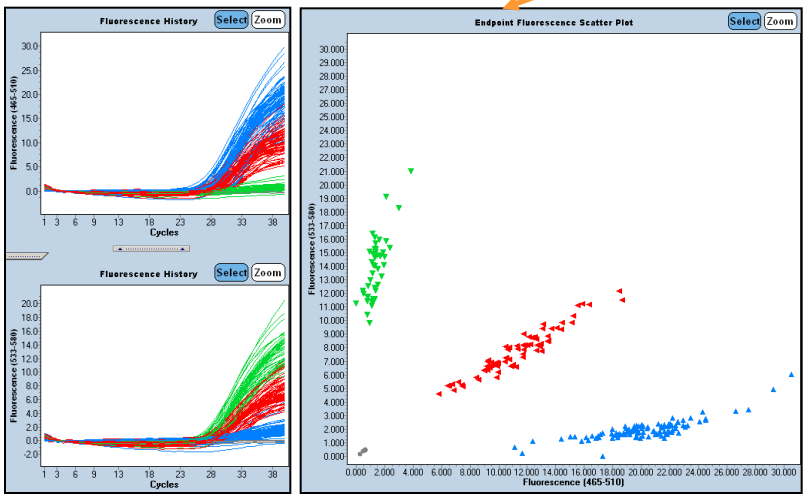
LightCycler® 480 Software

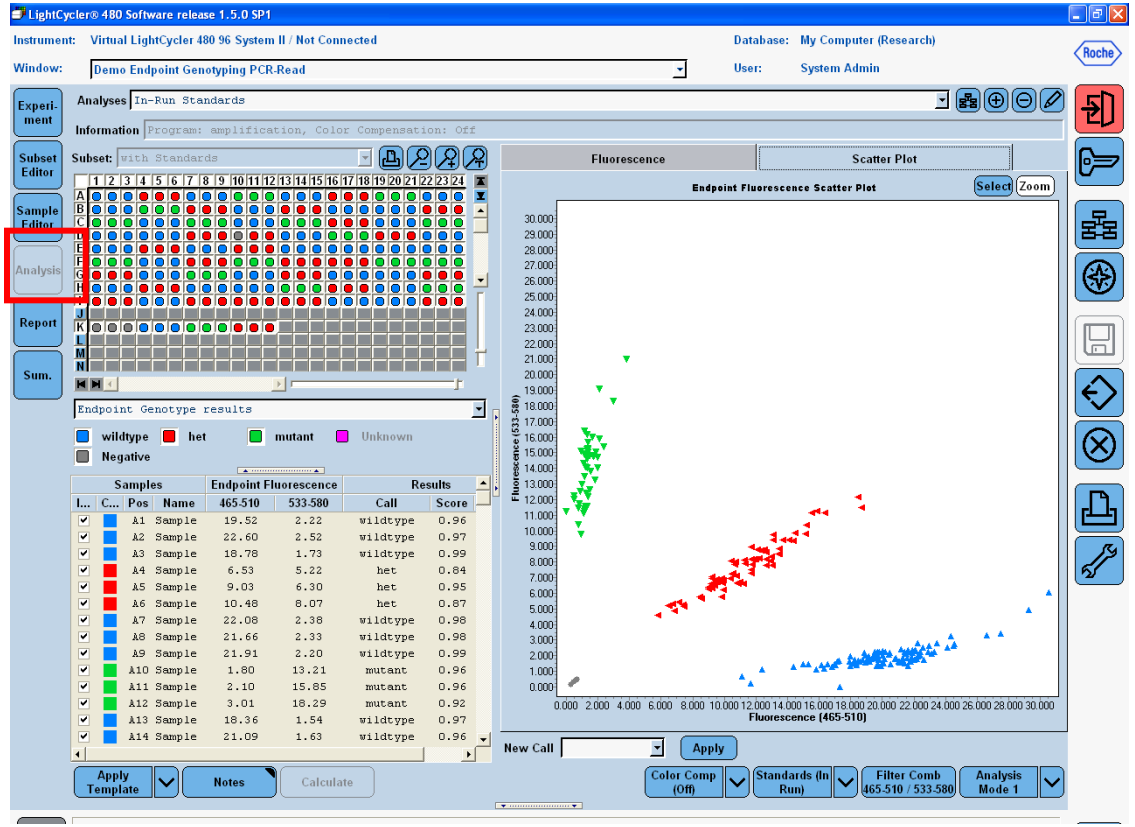
Analysis Module: Endpoint Genotyping

Detection of known variants

Genotyping with Hydrolysis probes



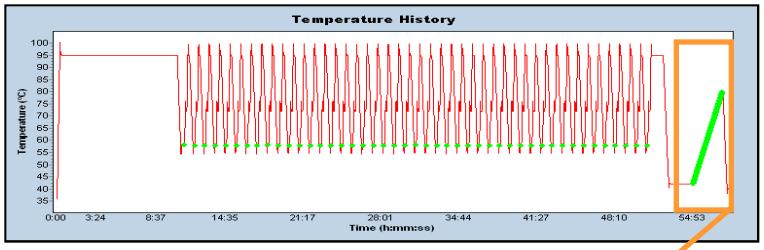


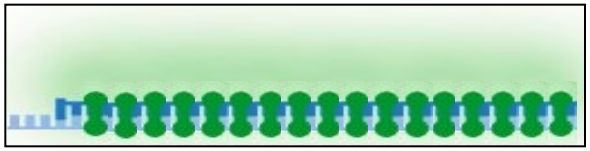


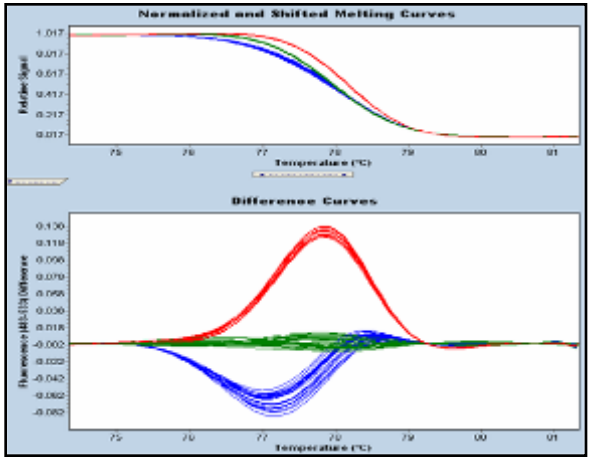
LightCycler® 480 Software

Analysis Module: Gene Scanning

Detection of any variants

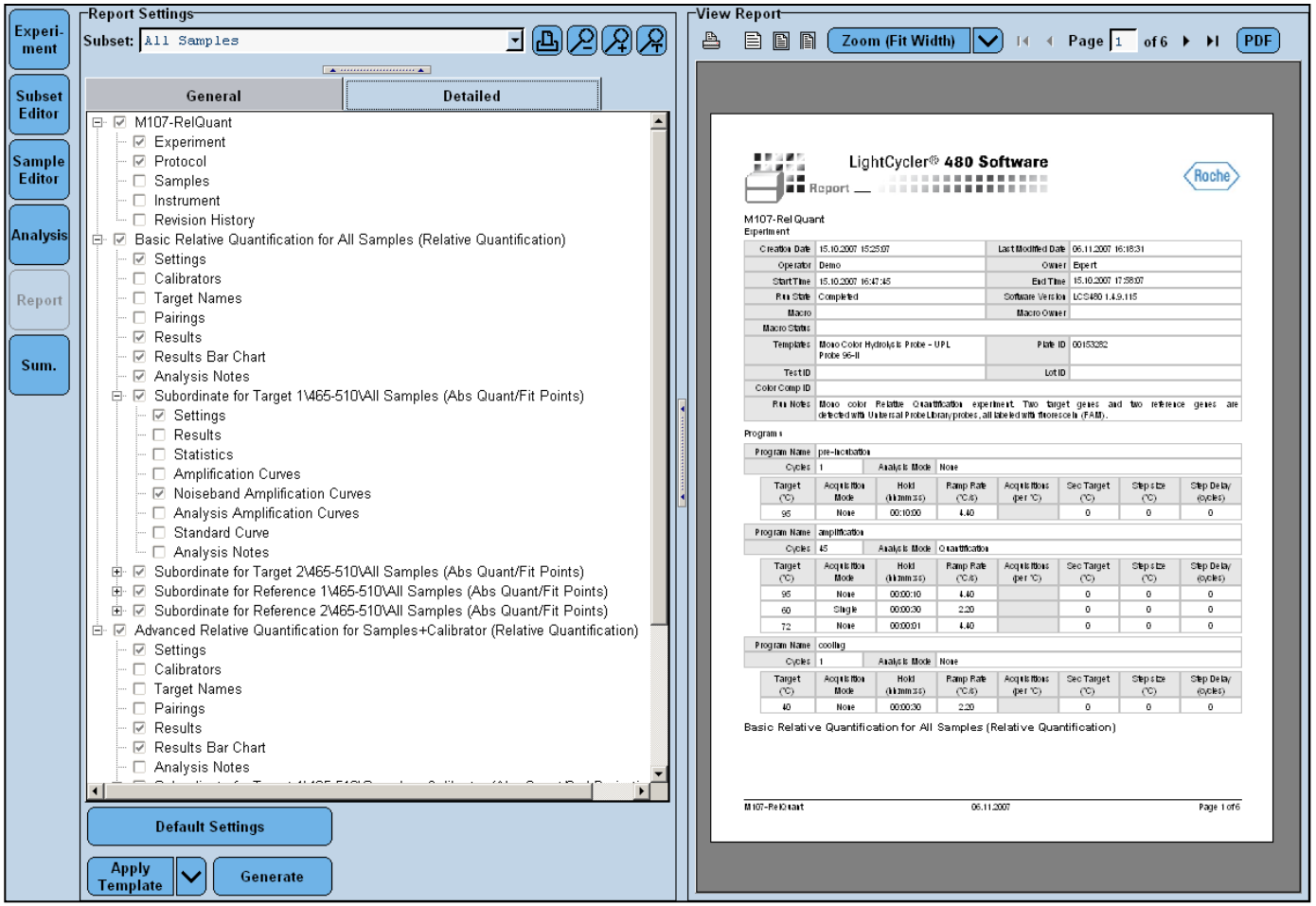






LightCycler® 480 Software

Report



- Flexible selection of report elements
- Different report settings possible
- Create or apply template

LightCycler® 480 Real-Time PCR System

Summary (1)

- Unique thermal block cycler technology for exceptional well-to-well data homogeneity.
- Easily interchangeable 96- and 384-well thermal block cycler units.
- Optional clear or white plates, depending on your workflow and sensitivity needs.
- Advanced high-performance optical system for accurate data capturing.
- Highest flexibility with fluorescence dyes and detection formats.
- Intuitive, user-friendly LightCycler® 480 software interface.
- Versatile genotyping based on endpoint analysis or melting curves.
- Multi-function database with research and traceable modes.

LightCycler® 480 Real-Time PCR System

Summary (2)

- Step-saving short cuts for high-throughput sample and data handling.
- Fast and easy assay setup with the new sample editor.
- Options for basic and advanced gene expression and genotyping methods.
- One-click experiment setup with options to refine results later.
- Excellent PCR sensitivity with high-value LightCycler® 480 reagents and disposables.
- State-of-the-art LIMS connectibility.
- 21 CFR part 11 compliance data protection.
- Premium **customer support and instrument service**

LightCycler® 480 System Support and Service

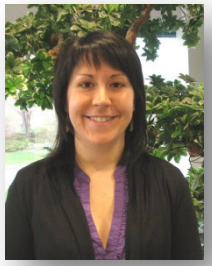
- Roche Diagnostics head office based in Laval, Quebec.
- Ontario Service Team (Instruments and Applications) for Life Sciences Research and Molecular Diagnostics:

Roche Care Centre

Technical Support Specialists

biochem.ca@roche.com
1-800-870-7050





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