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The LightCycler® 480 Real-Time PCR System

Unleash the Power of Real-Time PCR

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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



LightCycler® Systems

Technological (R)Evolution

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



**Carousel-Based
LightCycler® System**



LightCycler® 480 System

LightCycler® 480 System *Overview*



1. General Description

2. LightCycler® 480 Instrument and Specifications

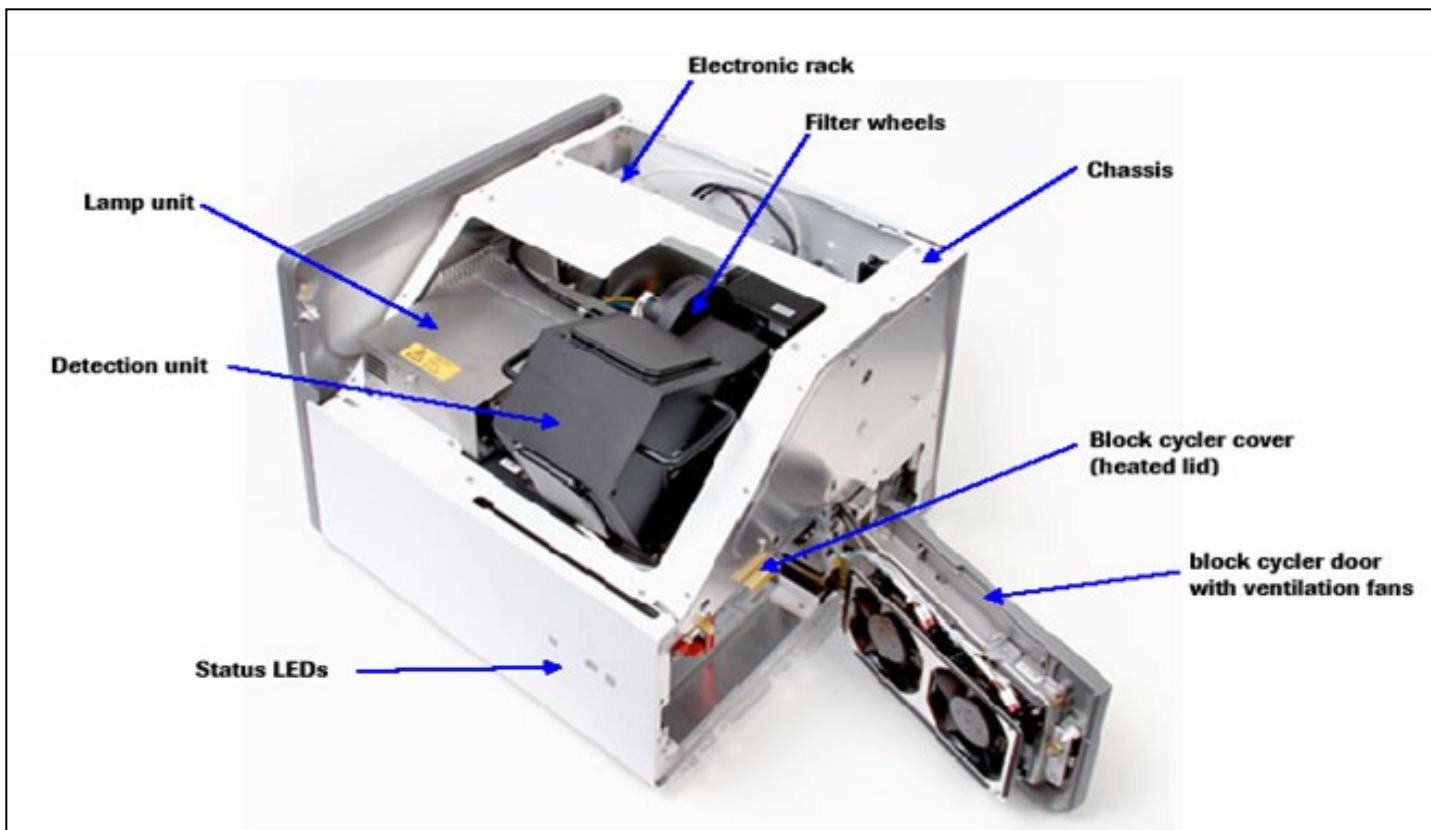
3. Assay Formats, Dyes, and Applications

4. LightCycler® 480 Performance

5. LightCycler® 480 Software

LightCycler[®] 480 Instrument

General Architecture



LightCycler[®] 480 System

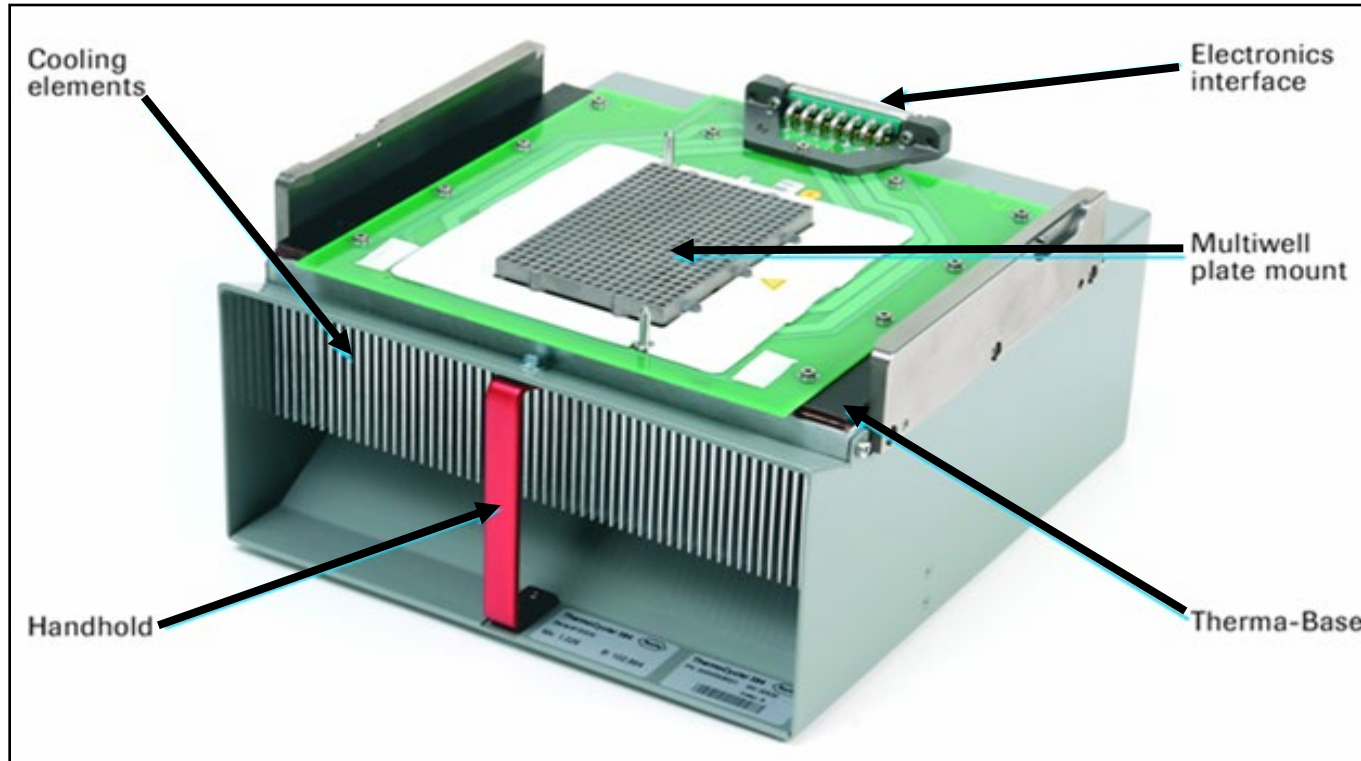
96 ↔ 384 Switch



- 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

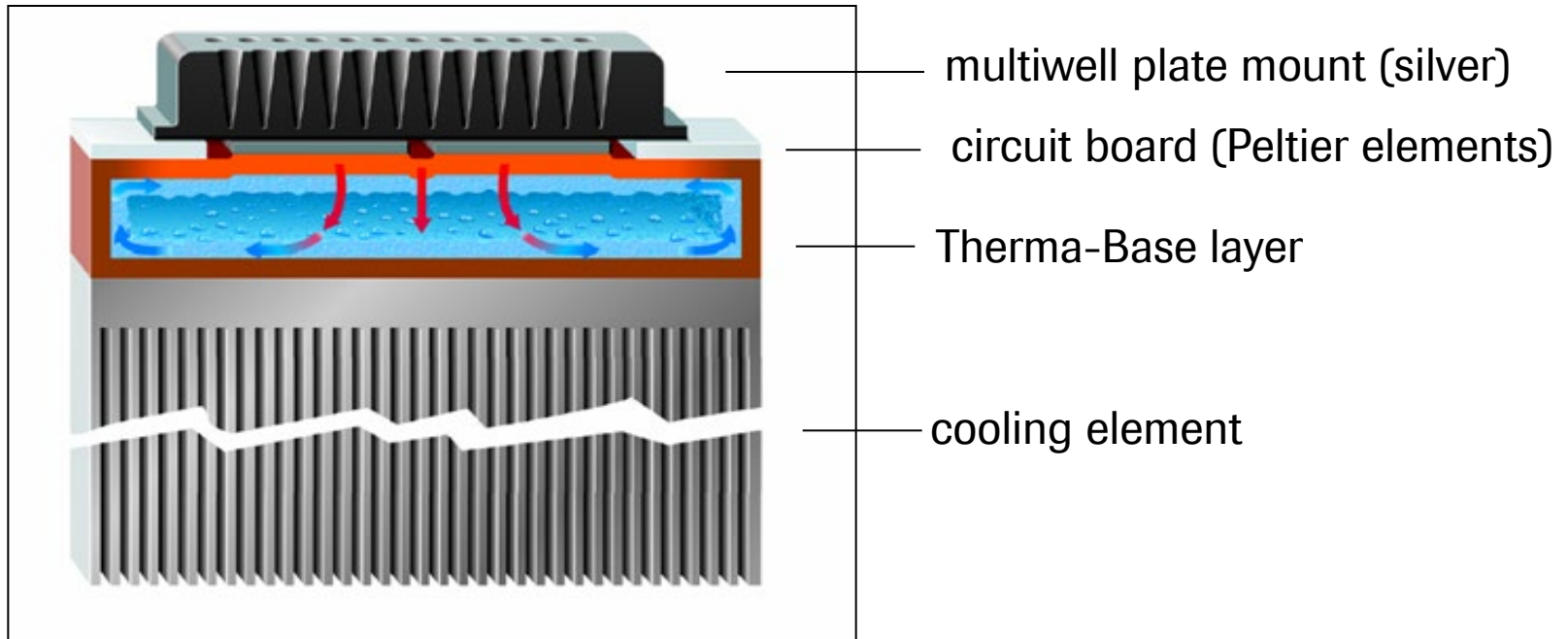


Therma-Base for optimized heat equalization

- 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

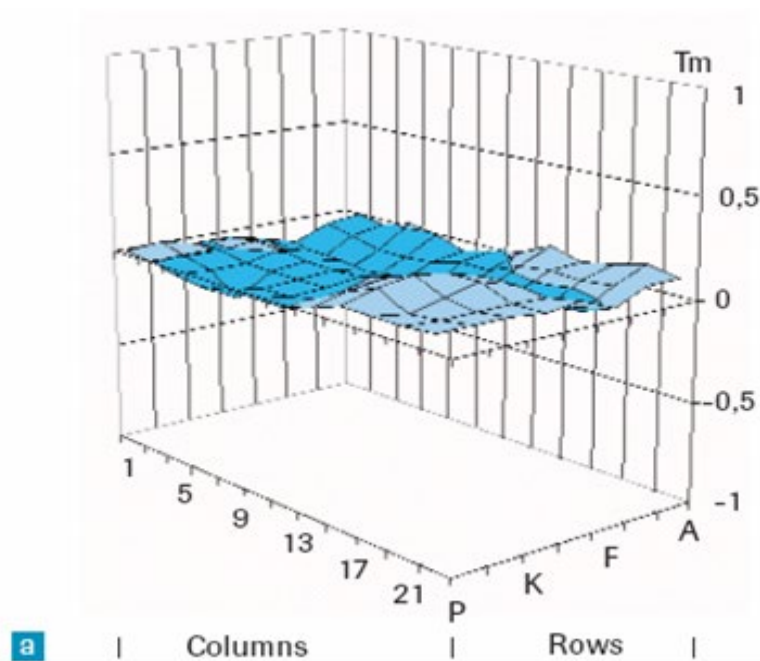


- Thin sealed vacuum vessel with working fluid in a wick structure
- Rapidly transfers heat by evaporation and condensation

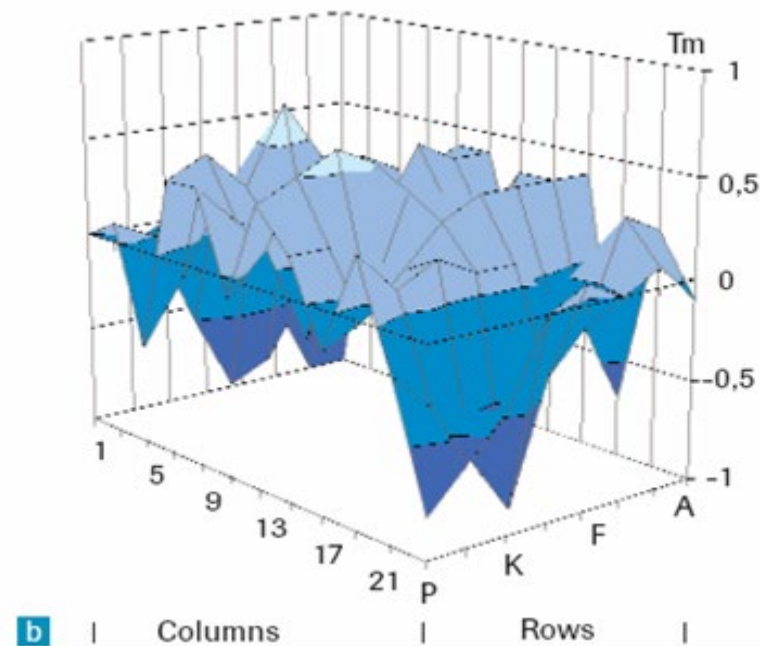
➔ **Enables both rapid and accurate cycling!**

Thermal Uniformity *Instrument Comparison*

LightCycler® 480 Instrument

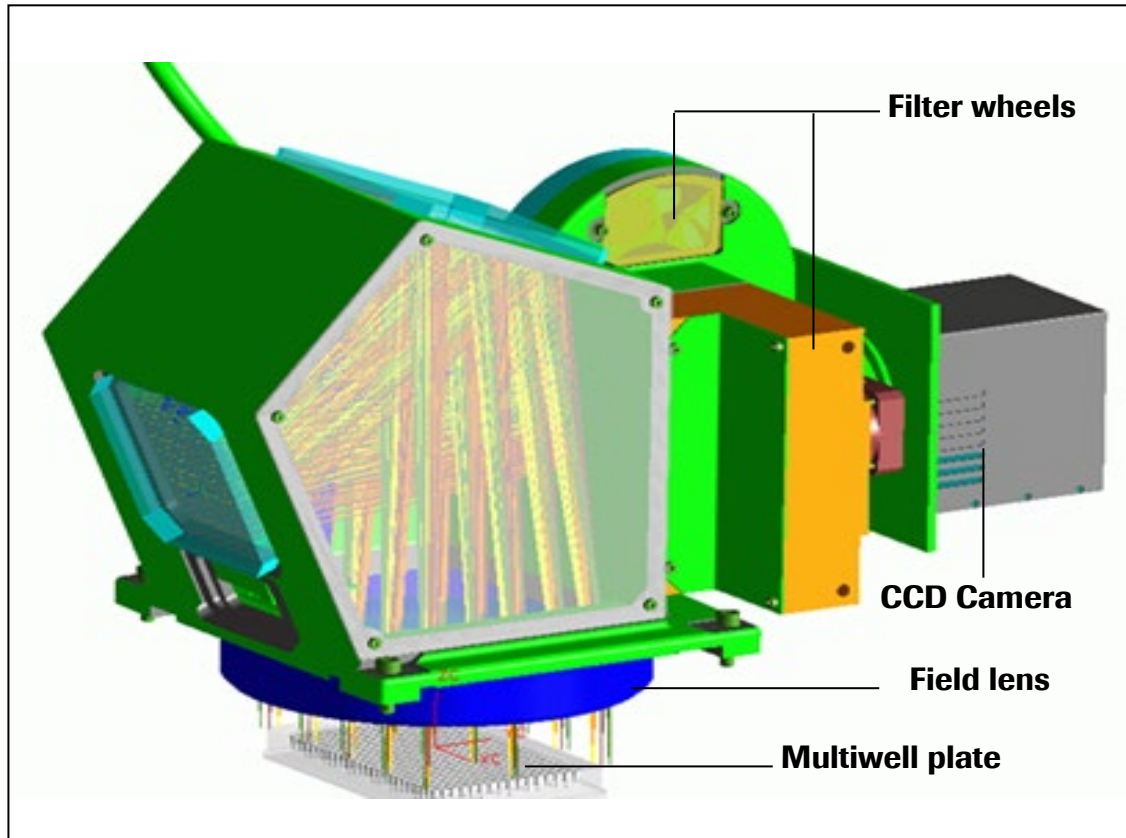


Standard Instrument



LightCycler® 480 Optical System

Sensitivity and Homogeneity

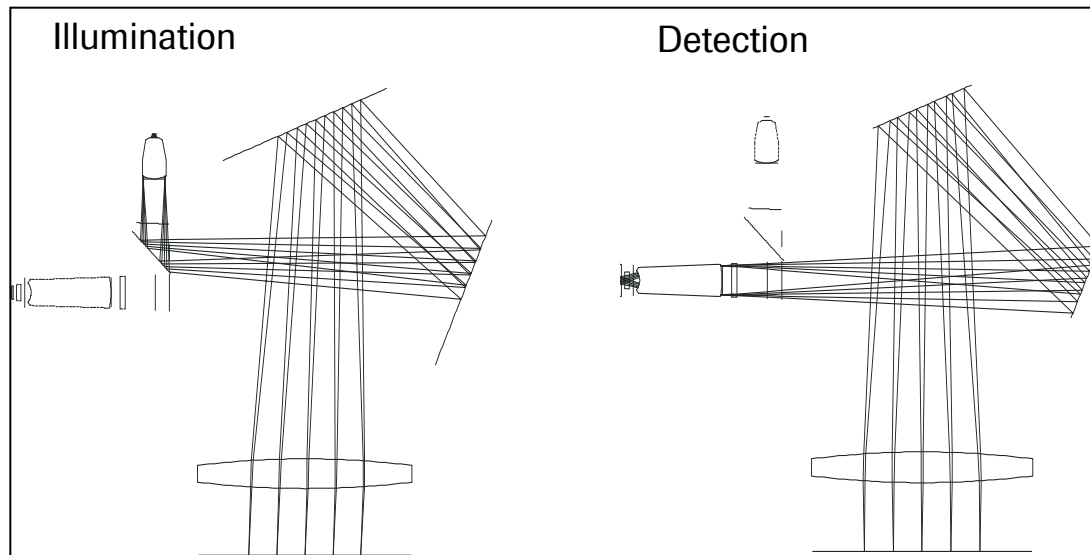


- LED
 - high intensity
 - broad dynamic range
 - lifetime: 10 000 hrs
- CCD camera
- Five excitation filters
- Six detection filters
- Optimized arrangement of optical components
- Homogeneous excitation and fluorescence detection

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 lens 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

LightCycler[®] 480 System

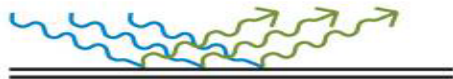
Overview



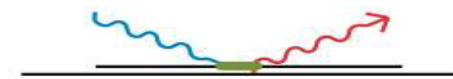
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LightCycler® Assay Formats

... *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	•		•	•		•

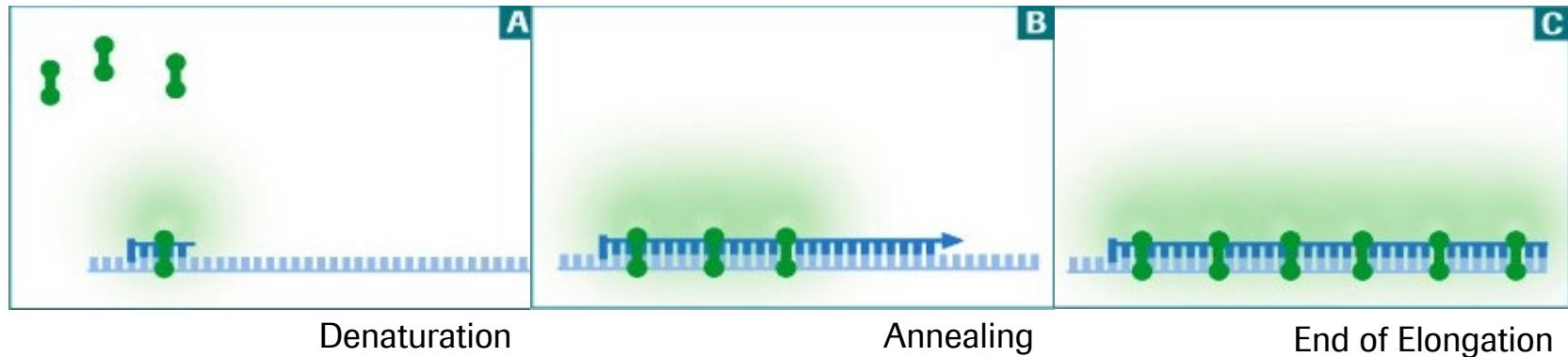
LightCycler® 480 System

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

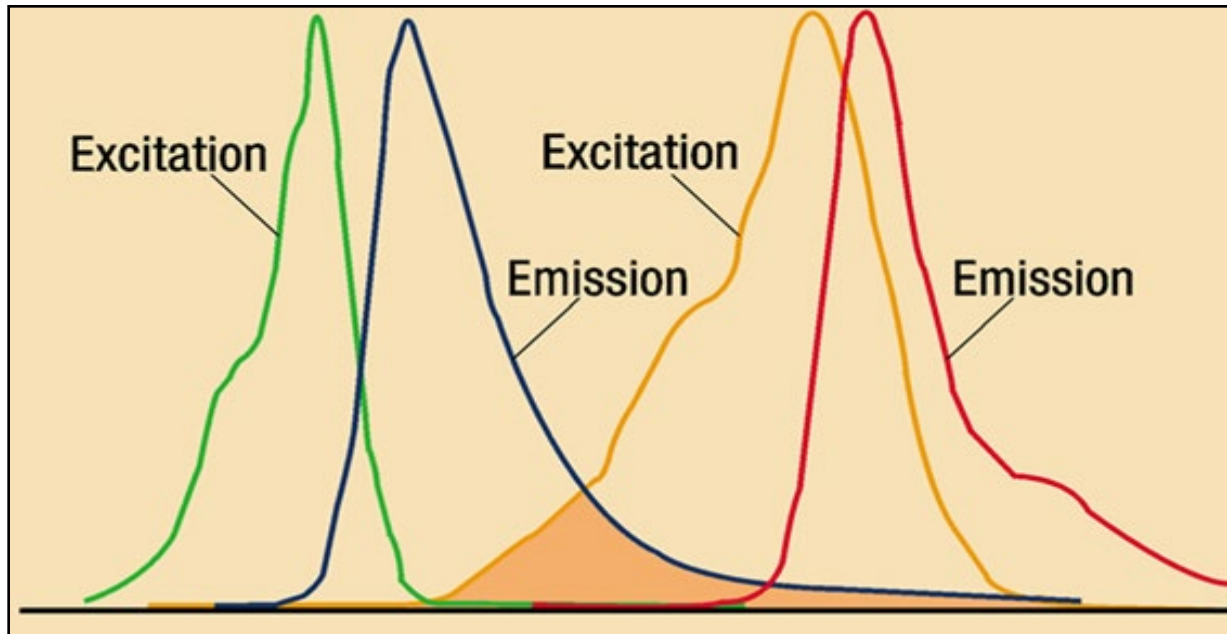
SYBR Green I



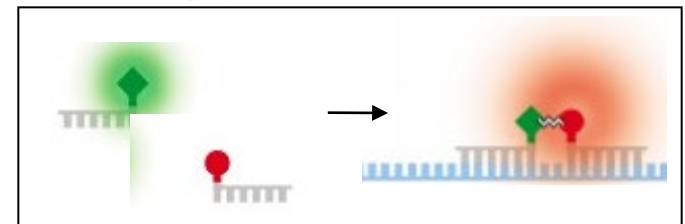
- When SYBR Green I dye **intercalates** into **dsDNA**, its fluorescence greatly increases
- All SYBR Green I assays have to be verified by a **Melting Curve Analysis** for side products other than the specific amplicon
- Dyes like the **Roche Dye ResoLight** completely **saturate** the double stranded 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)

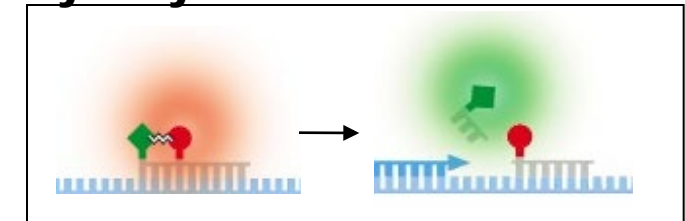


HybProbe Probe



FRET reporting

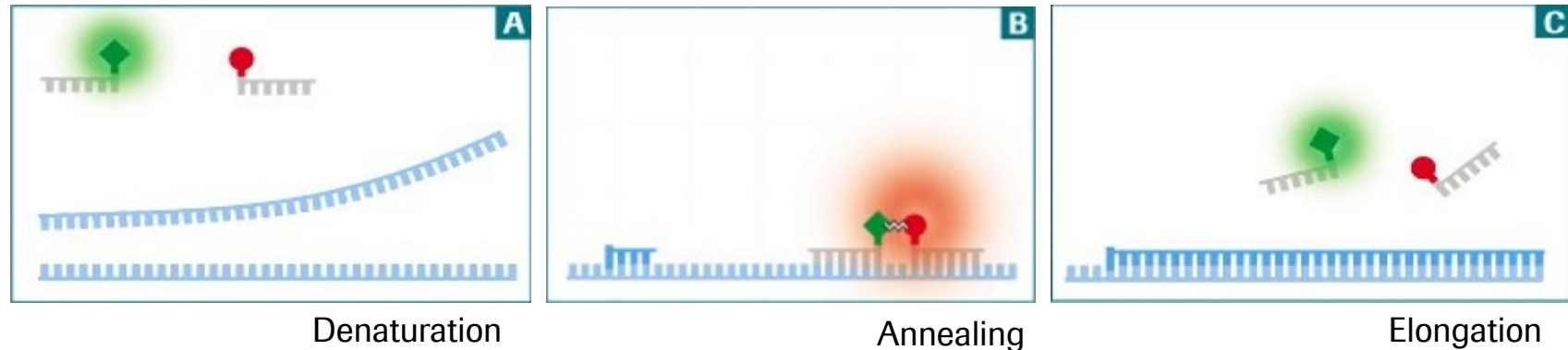
Hydrolysis Probes



FRET quenching

Probe Based Assay Format

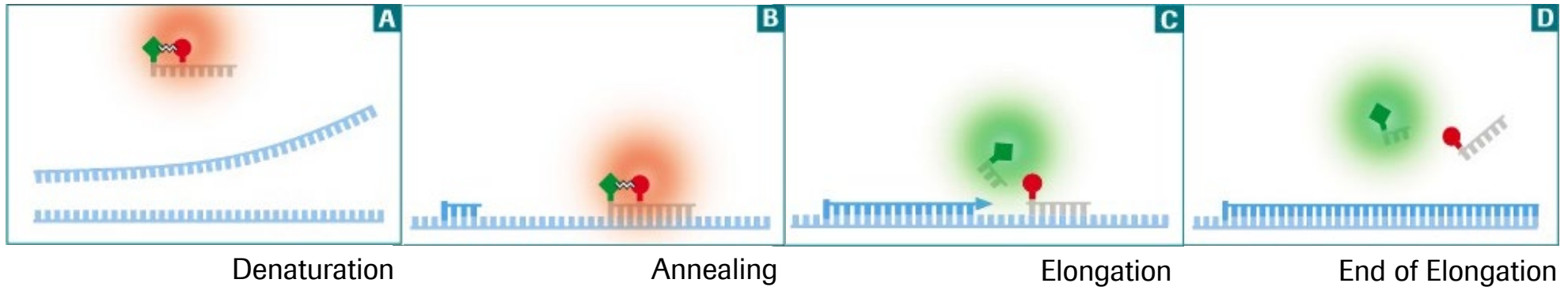
HybProbe Format



- 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)

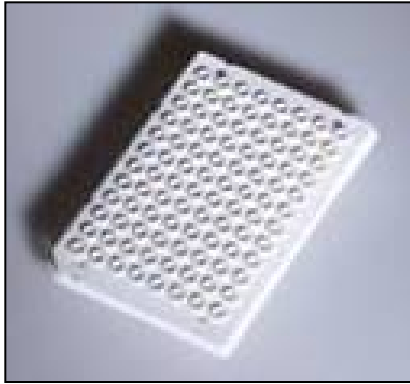
Hydrolysis Probe Format



- **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

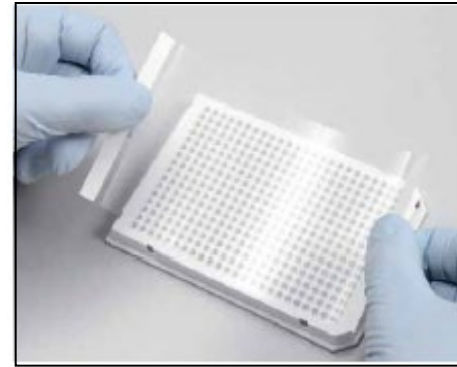
Disposables



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

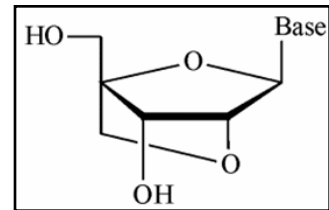
- LightCycler[®] 480 SYBR Green I Master
- LightCycler[®] 480 Probes Master
- LightCycler[®] 480 Genotyping Master
- LightCycler[®] 480 High Resolution Melting Master
- LightCycler[®] 480 ResoLight Dye
- LightCycler[®] 480 RNA Master Hydrolysis Probes
- RealTime ready RNA Virus Master
- LightCycler[®] 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.



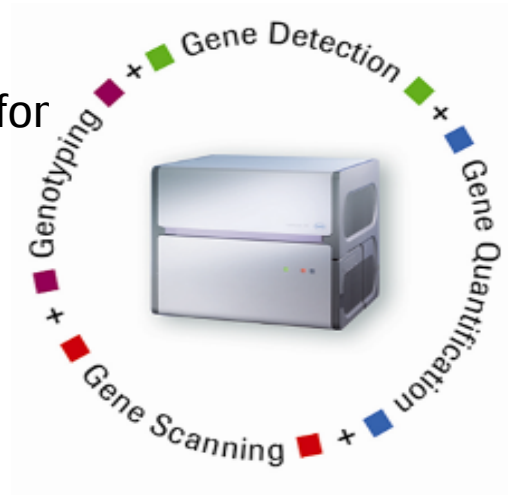
LightCycler® 480 System

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)

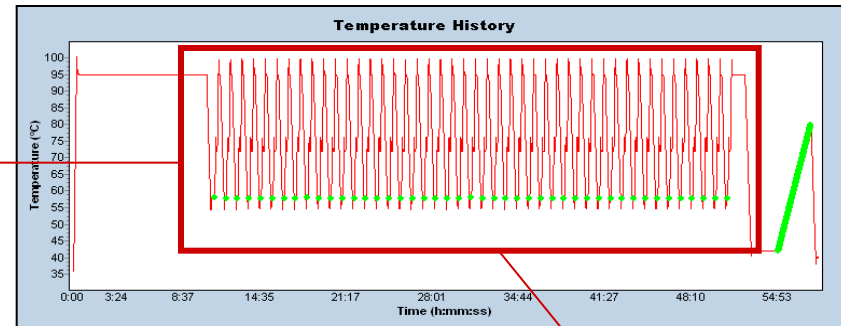
- It allows measurements at the endpoint of PCR
 - **Endpoint Genotyping** (allelic discrimination)

- 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



dsDNA binding dye

SYBR Green

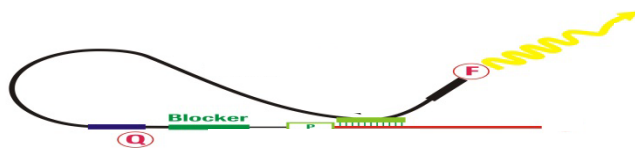
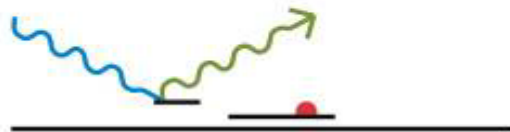
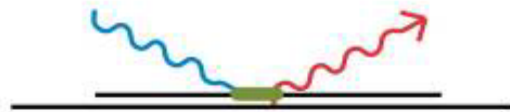


Simple Probes

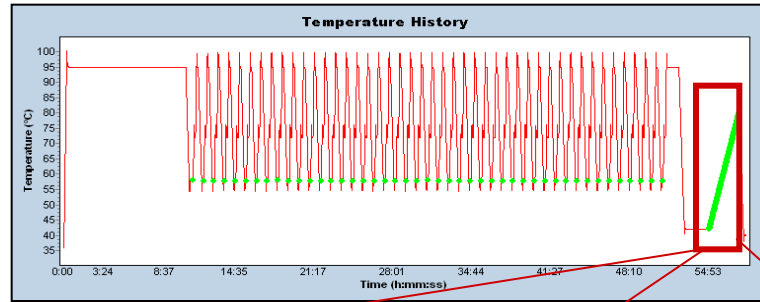
Hybridization probes

Hydrolysis probes

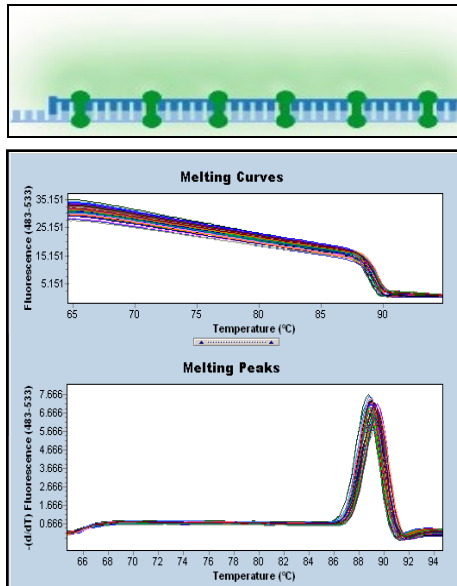
Scorpion Probes



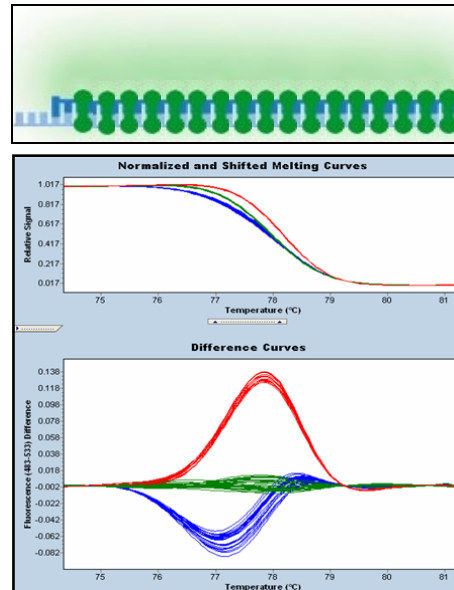
Melting Curve Analysis



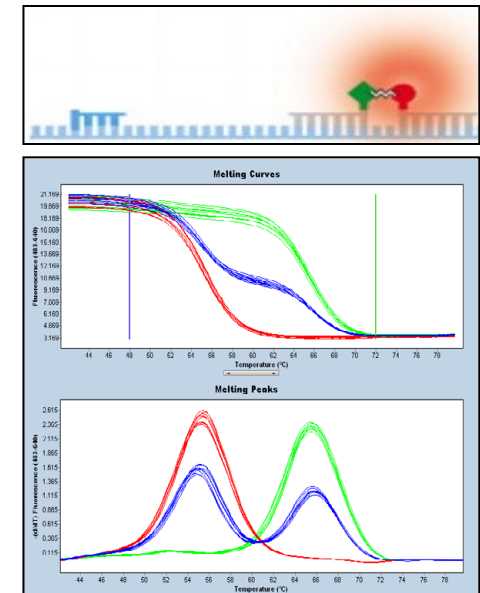
SYBR Green I



High Resolution Melting Dye

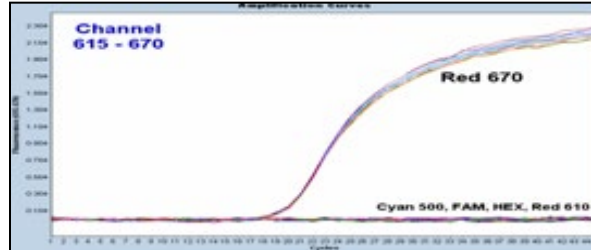
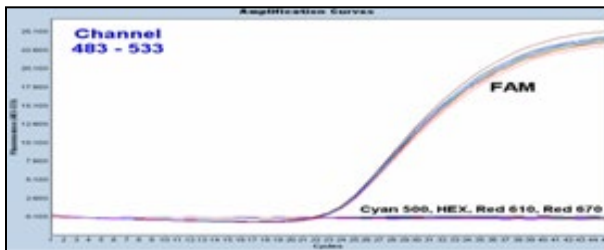
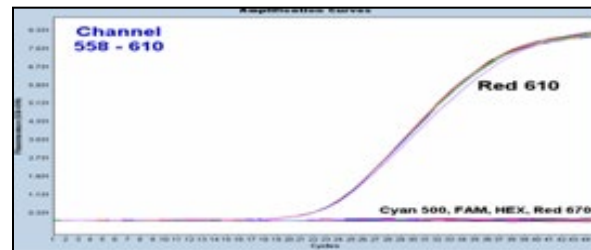
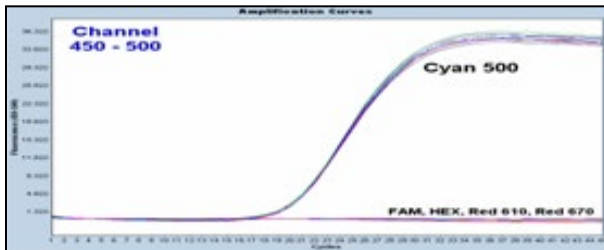
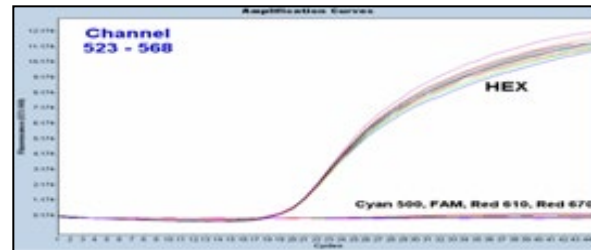
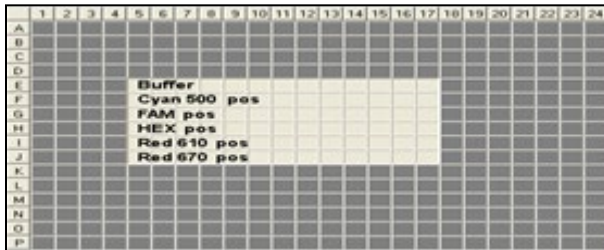


Hybridization Probes



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

LightCycler[®] 480 System

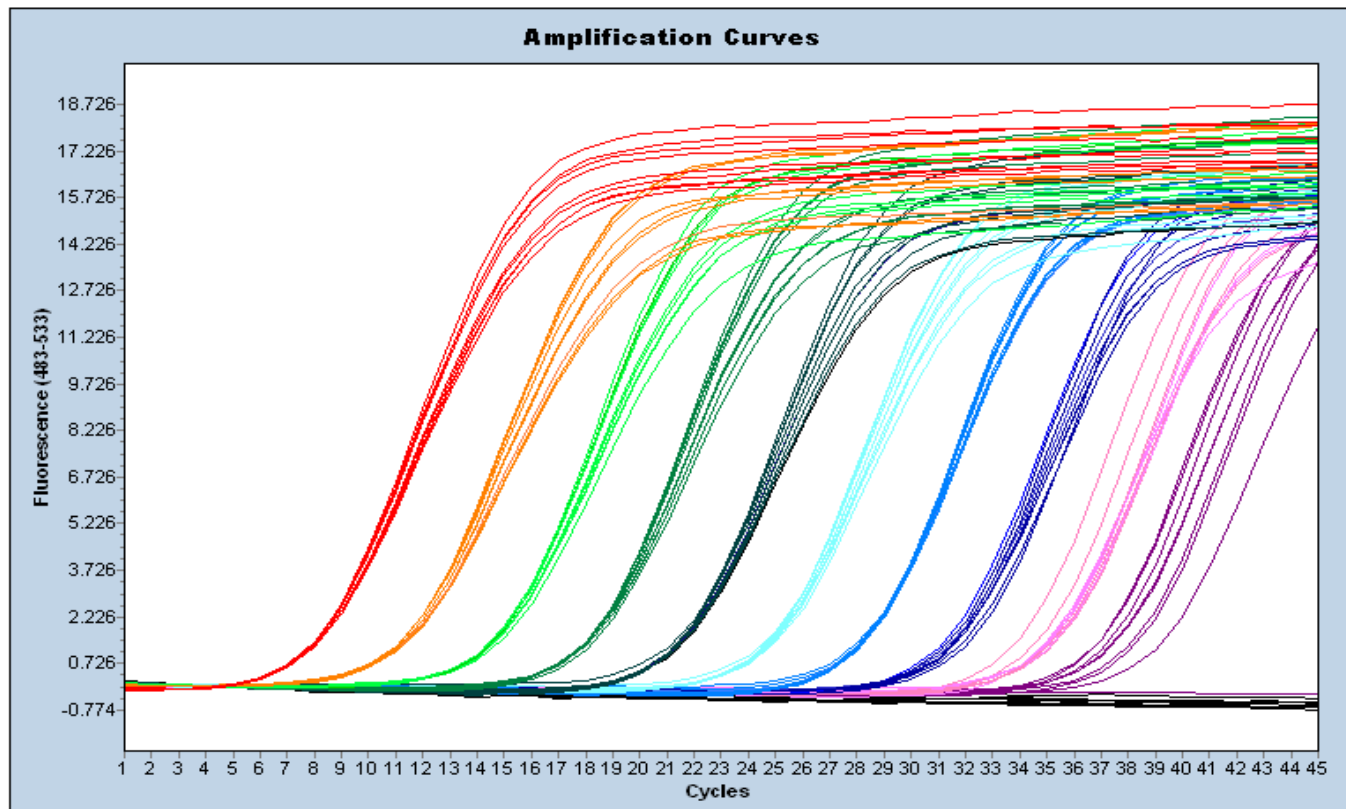
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LightCycler® 480 System

Dynamic Range



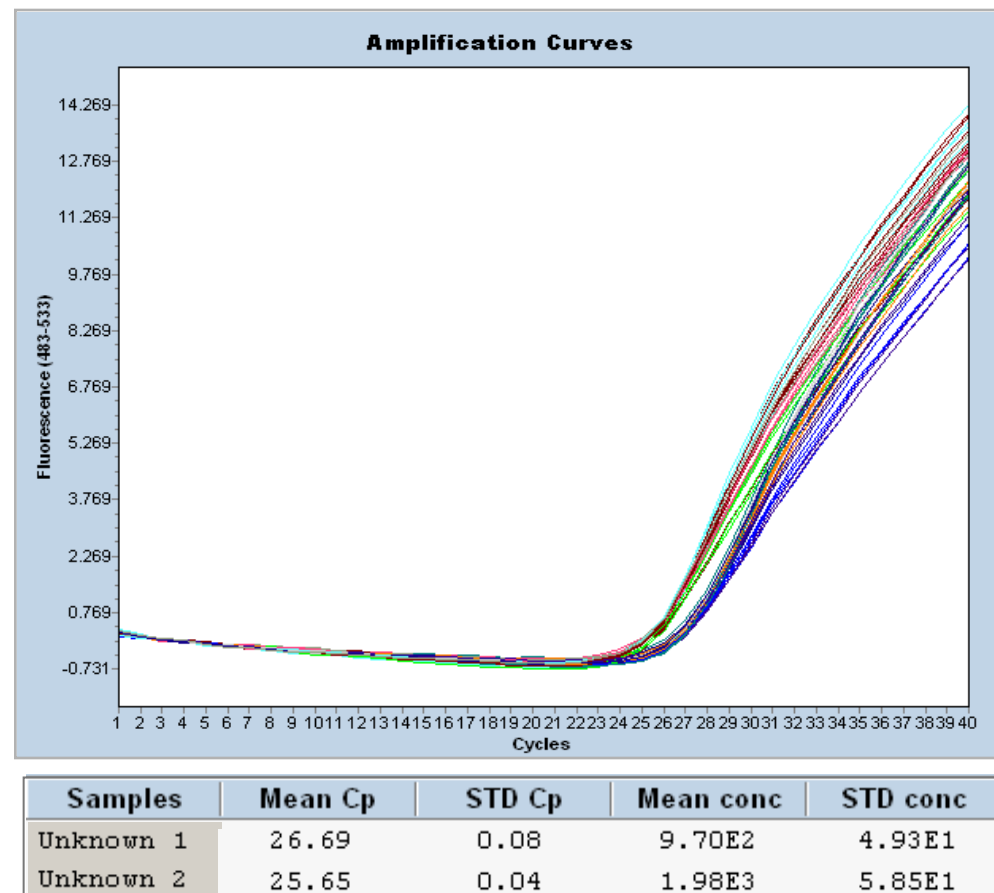
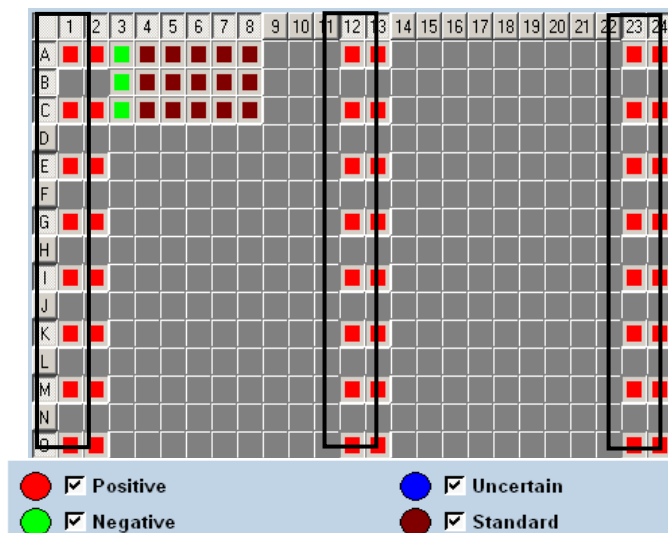
Samples	Statistics	
	Mean Cp	STD Cp
NTC		
1E0	37.69	1.08
1E1	34.94	0.49
1E2	31.65	0.27
1E3	28.21	0.05
1E4	24.85	0.01
1E5	21.56	0.02
1E6	18.17	0.01
1E7	14.81	0.01
1E8	11.46	0.02
1E9	8.18	0.02

- 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)

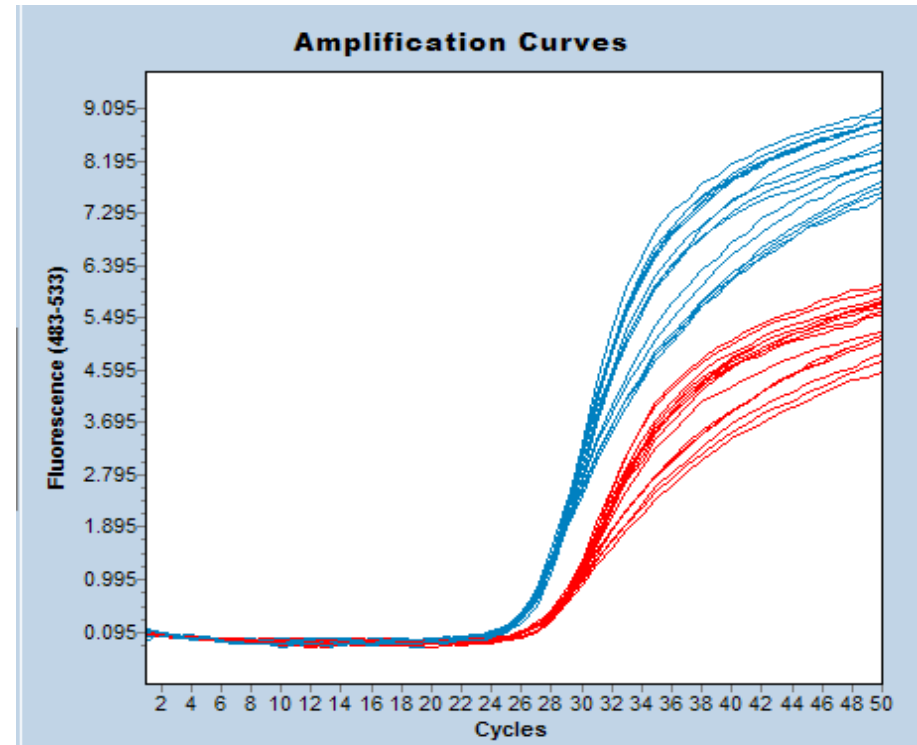
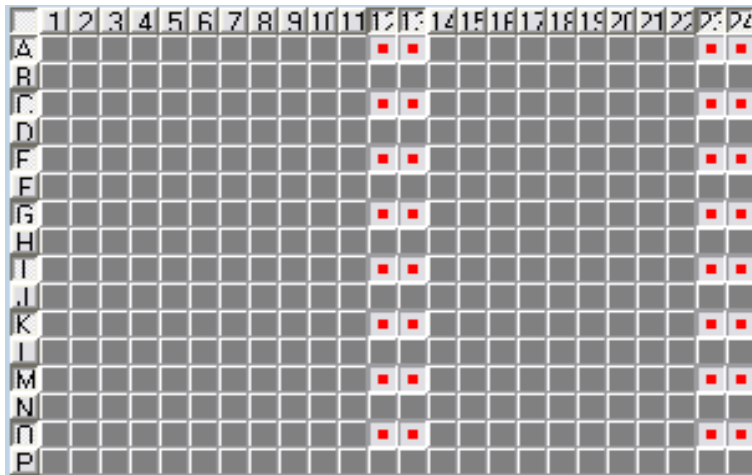


LightCycler® 480

Detection of a two-fold Difference



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



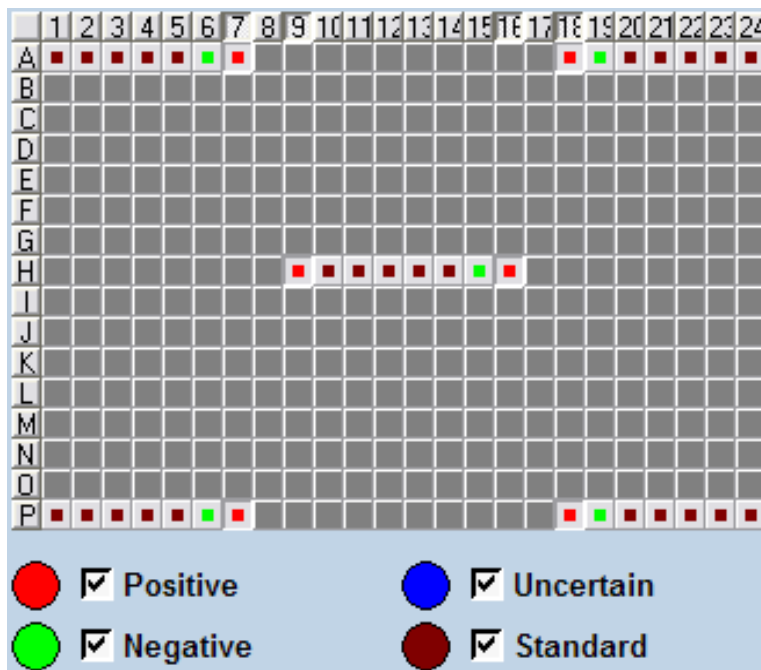
Samples	Mean Cp	STD Cp
A13, A24, C13, C24, E13, E24, G1	26.36	0.38
A23, C12, C23, E12, E23, G12, G2	27.52	0.40

LightCycler® 480

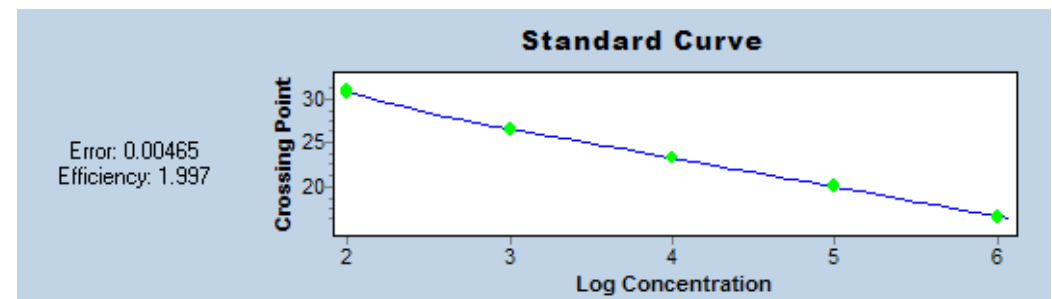
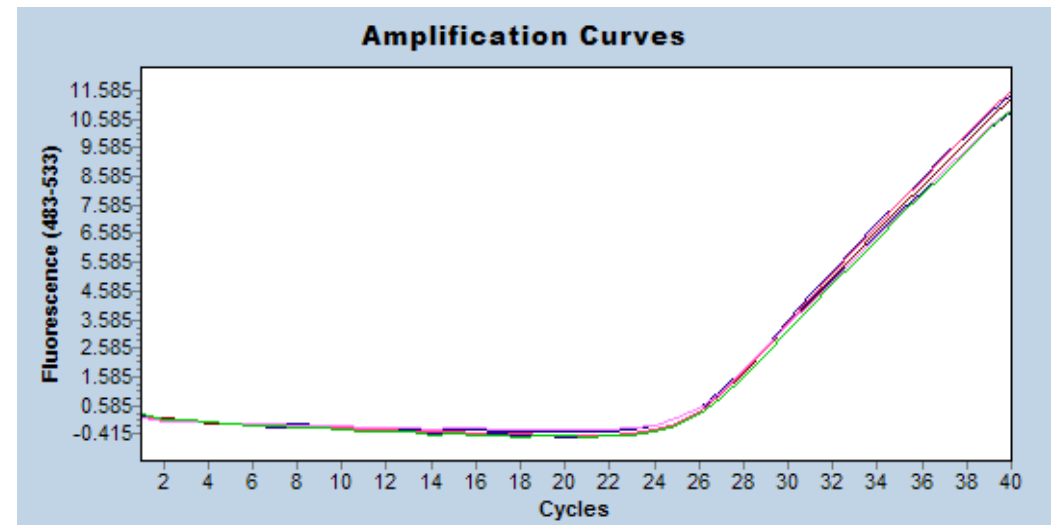
Walk Around the Block



•detection format: Hydrolysis Probes



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

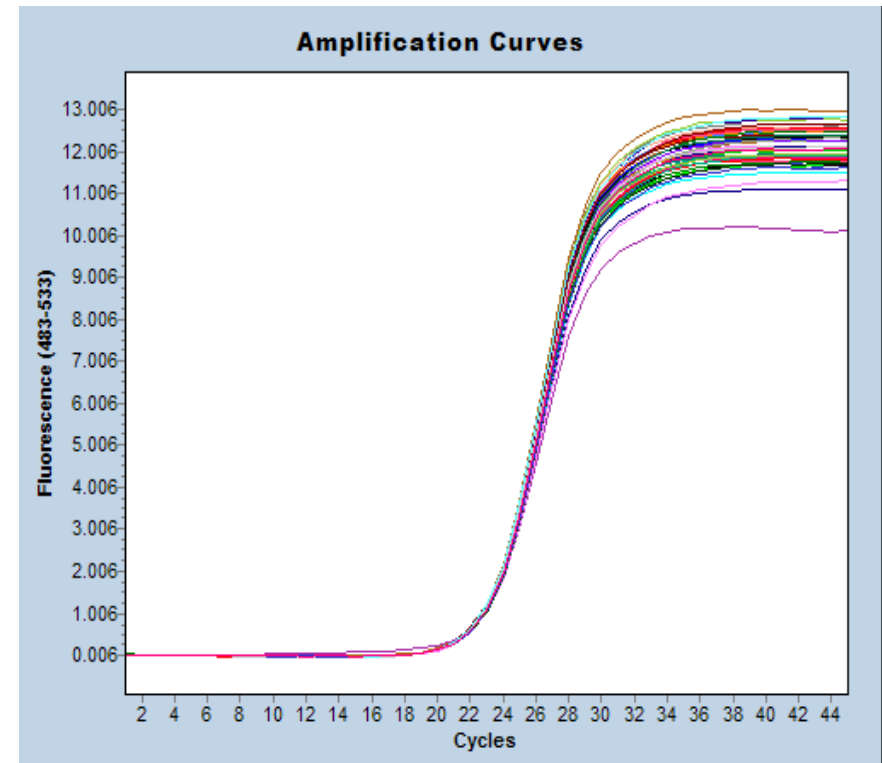
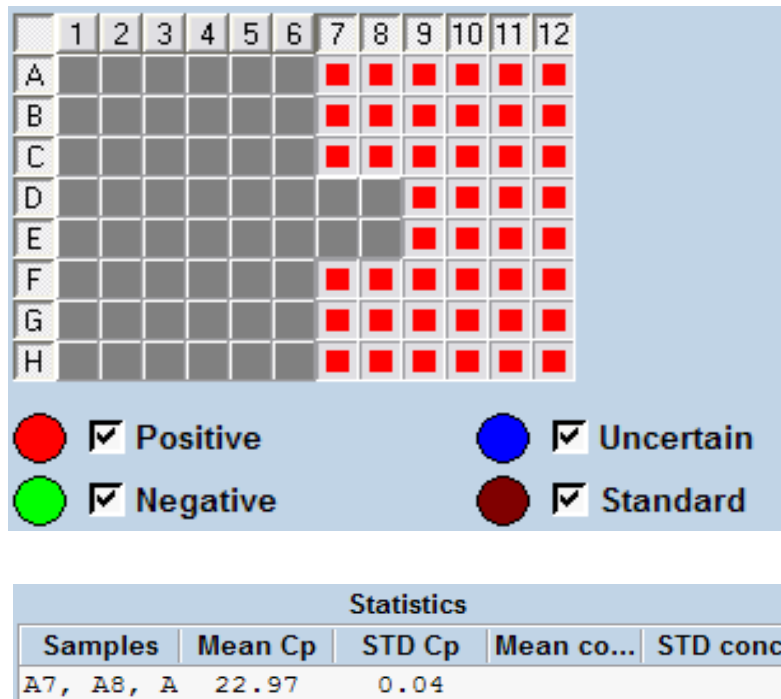


LightCycler® 480

Reproducibility



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

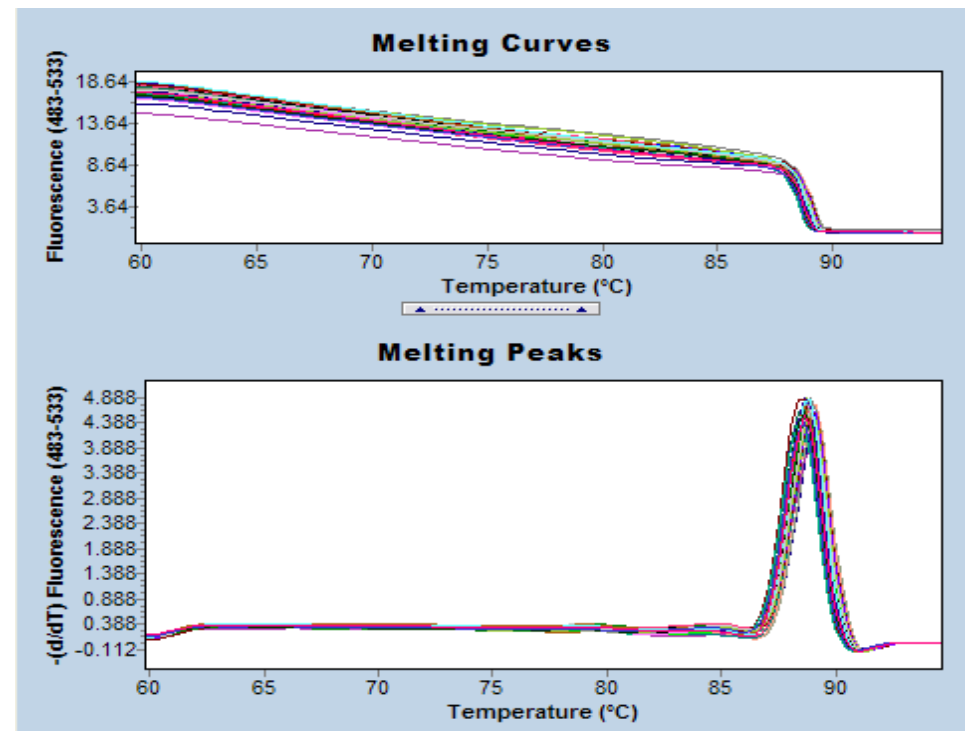
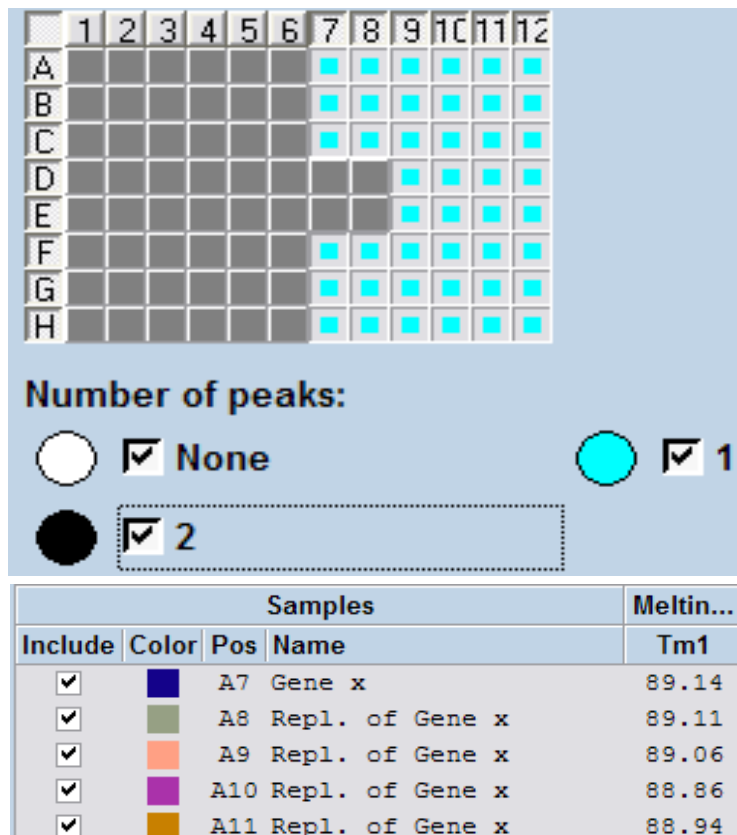


LightCycler® 480

Reproducibility



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



Average Tm = 88.798 STD=0.16

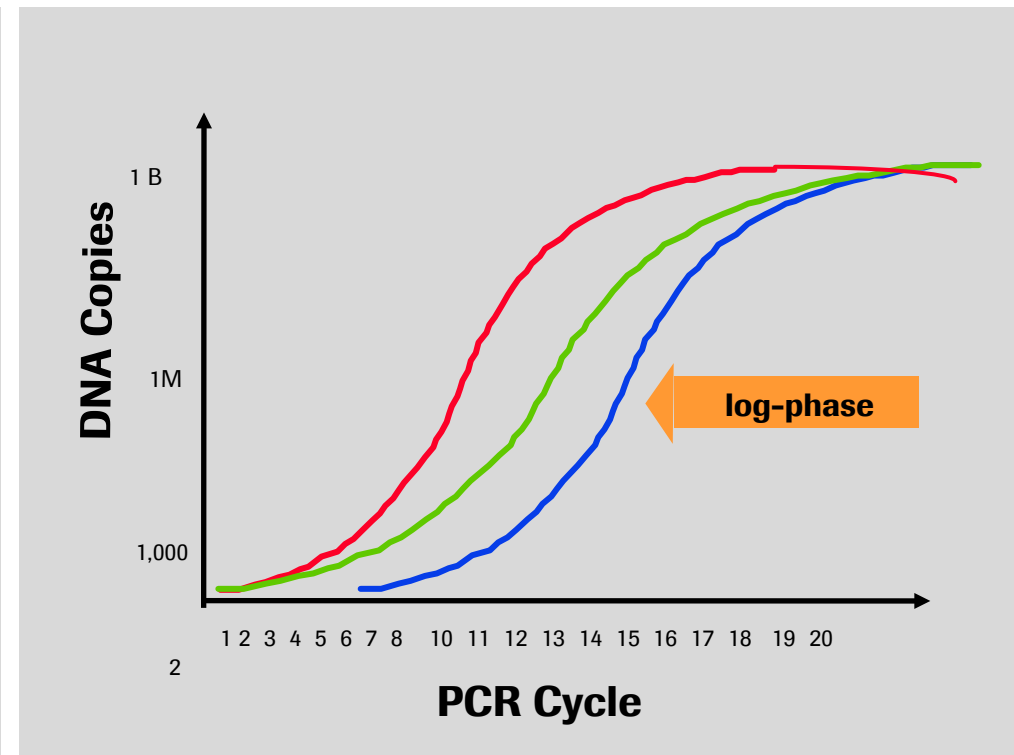
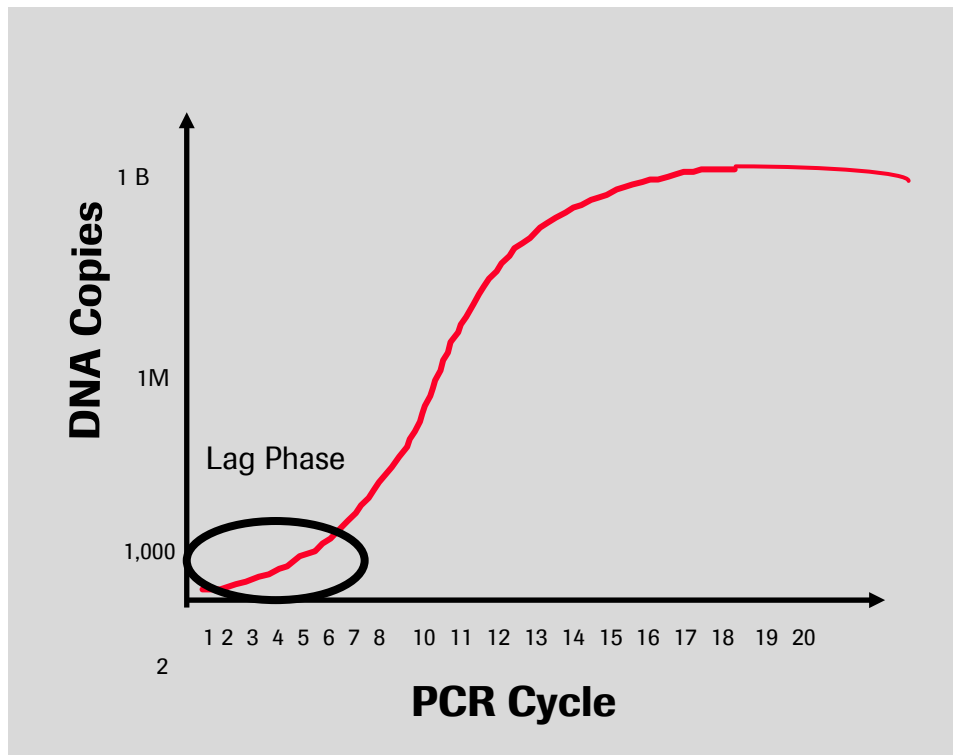
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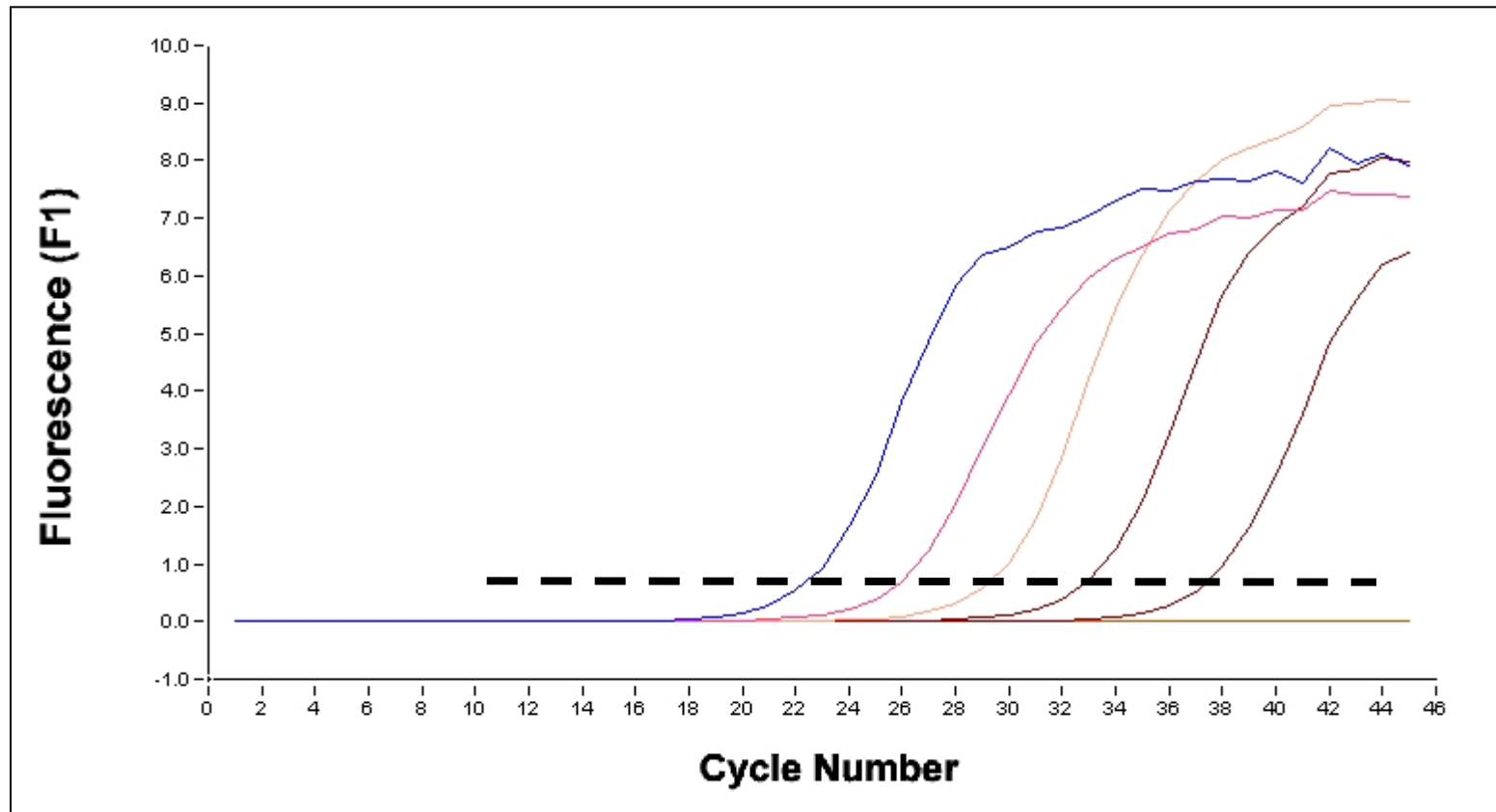
Crossing Point Determination

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



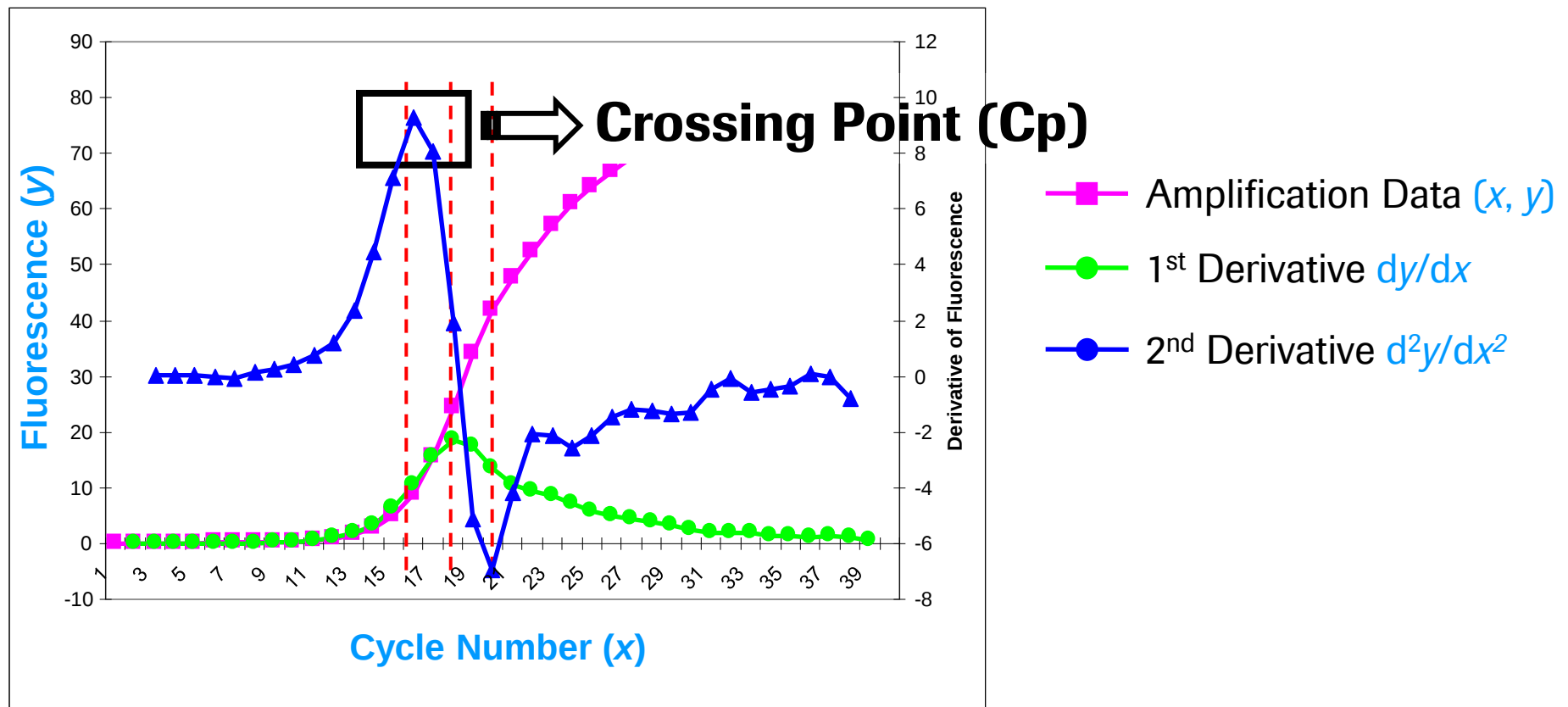
Crossing Point Determination

The Fit Points Method



Crossing Point Determination

The Second Derivative Maximum

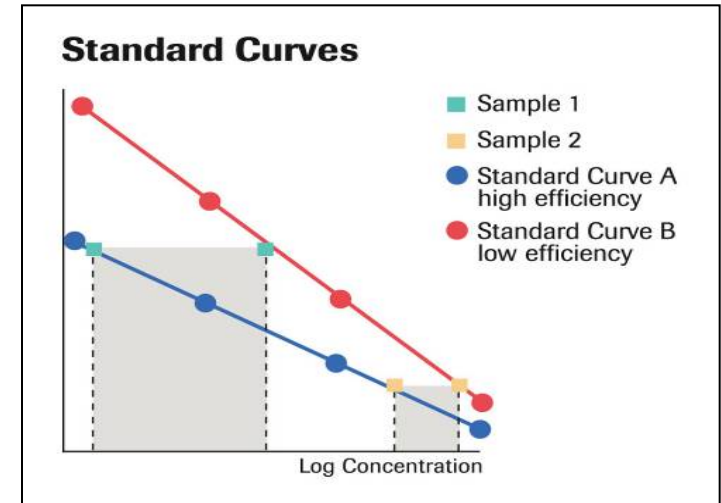


LightCycler® 480 System

Software Innovations(2)



•PCR efficiency consideration



•Calibrator-normalized PCR

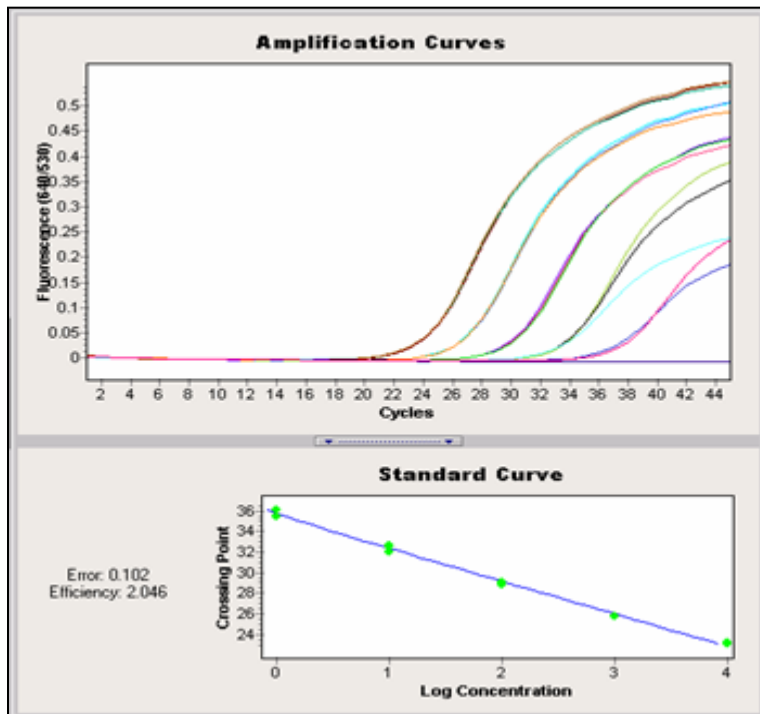
$$\text{normalized ratio} = \frac{\text{target/reference (unknown sample)}}{\text{target/reference (run calibrator)}}$$

LightCycler® 480 System

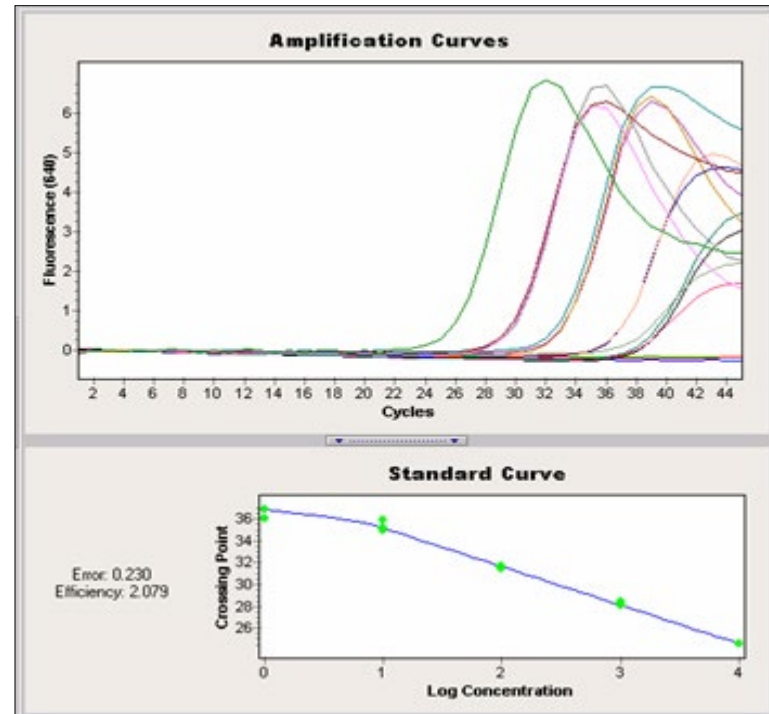
Software Innovations(3)



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



linear fit



non-linear fit

LightCycler® 480 System

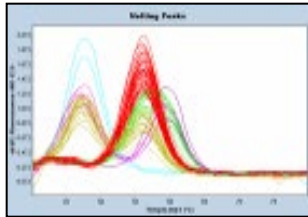
Software Innovations



	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

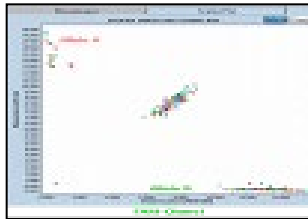
Flexible Analysis Options



Melting Curve Genotyping :SNP

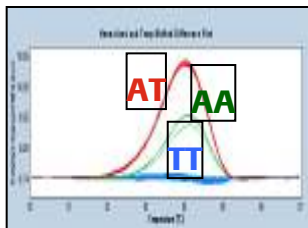
analysis with HybProbe probes

- Robust melting curve analysis
- Automated annotation
- Ideal for multiplex assays and haplotyping



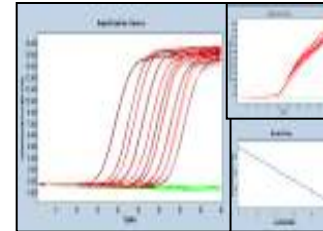
Endpoint Genotyping

- Using hydrolysis probes
- For known SNPs in known regions



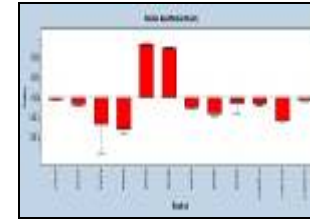
Gene Scanning-HRM

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



Absolute Quantification

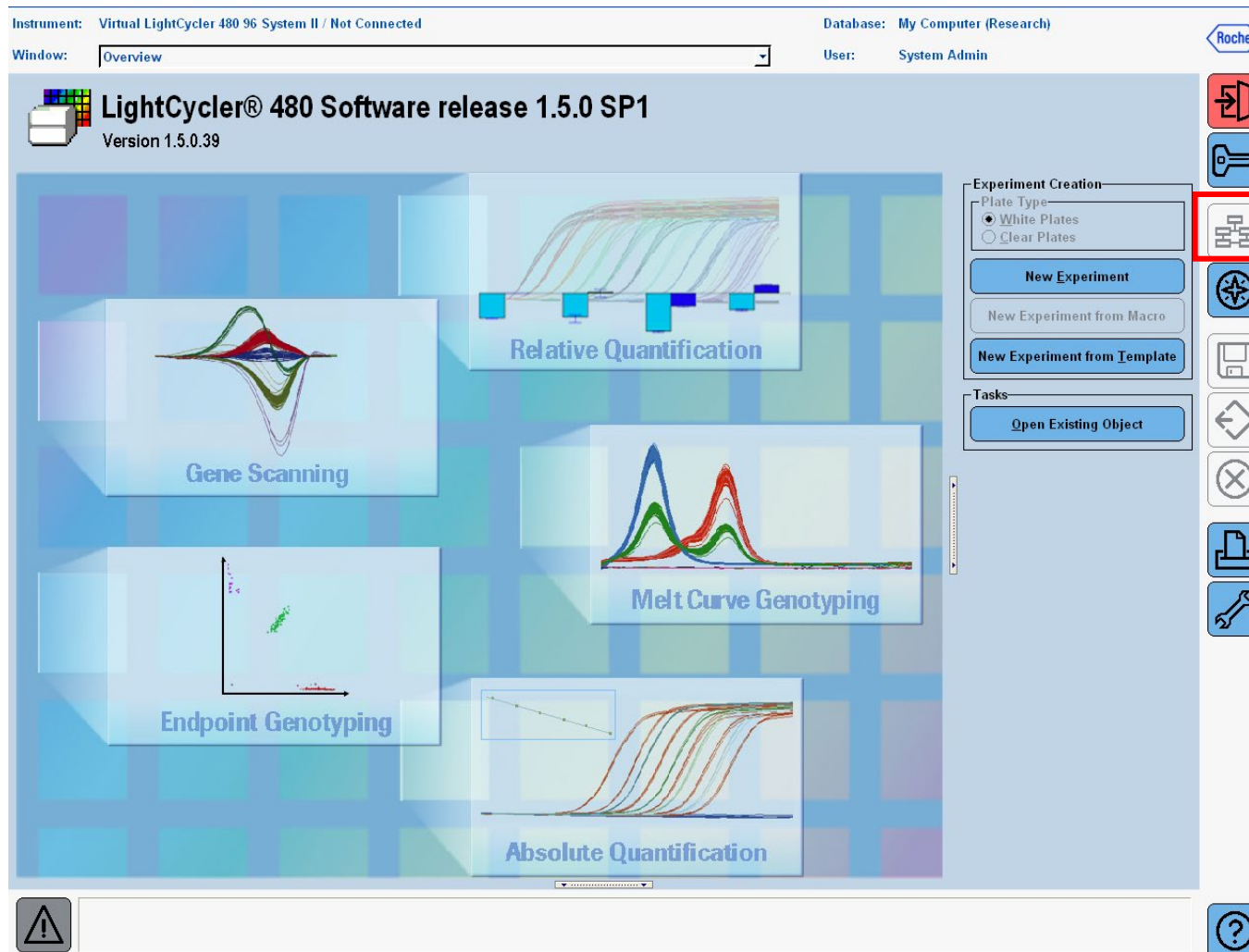
- Dynamic range: 1–10¹⁰ copies
- External standard curves



Relative Quantification

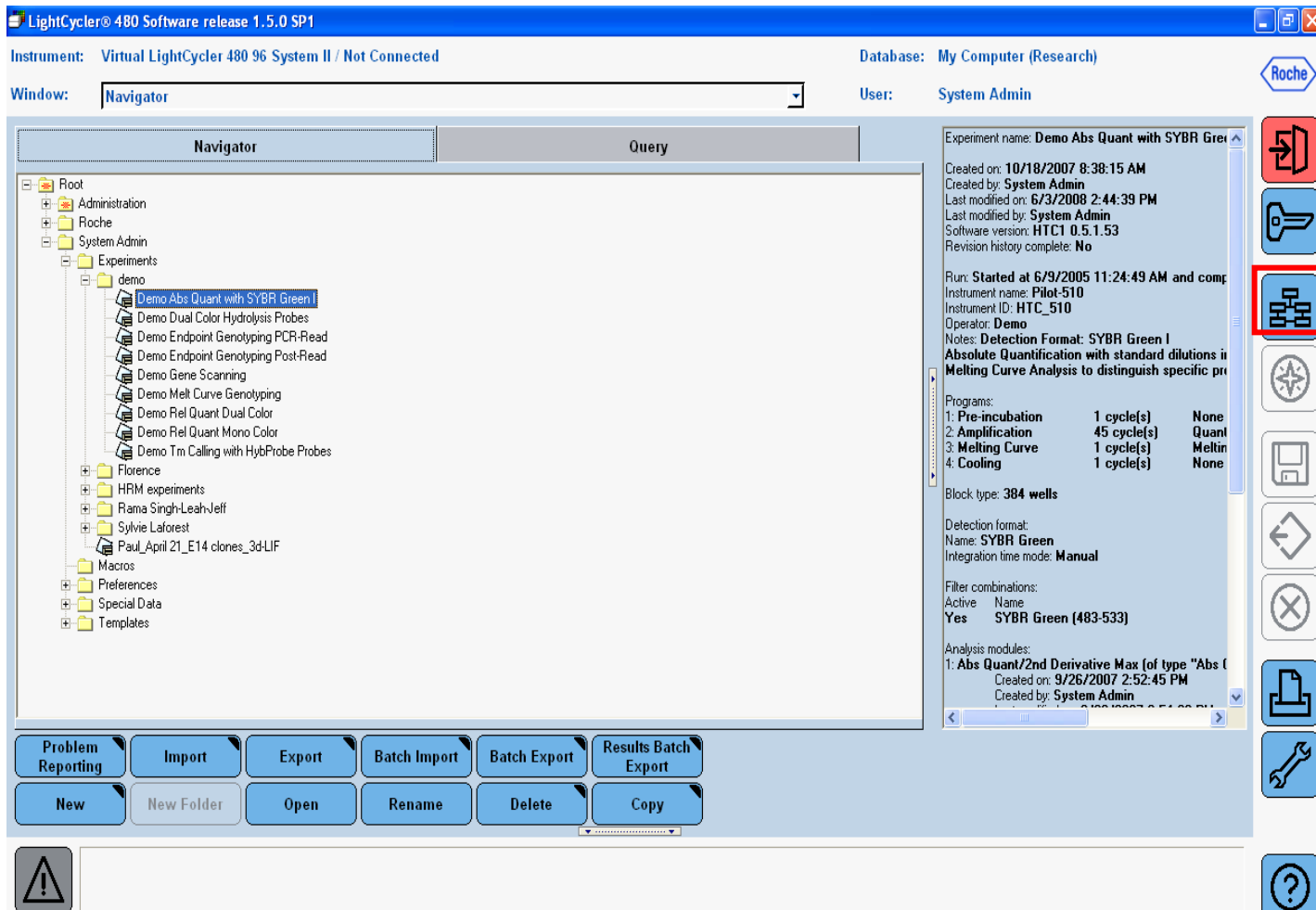
- Basic analysis: one click to result
- Advanced method for utmost accuracy
- Accurate results using efficiency correction
- Use of reference genes

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

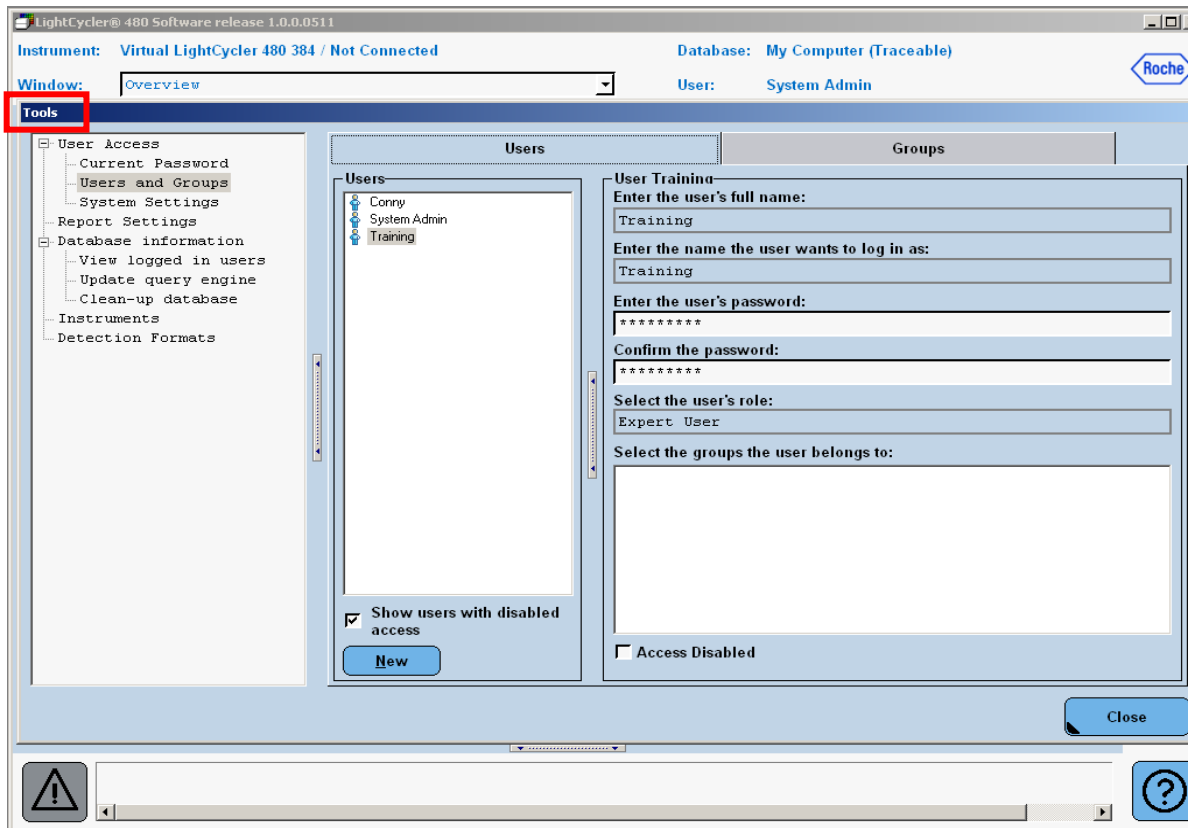
Navigator for data base entries

Query possibility to find information

Export and Import functions

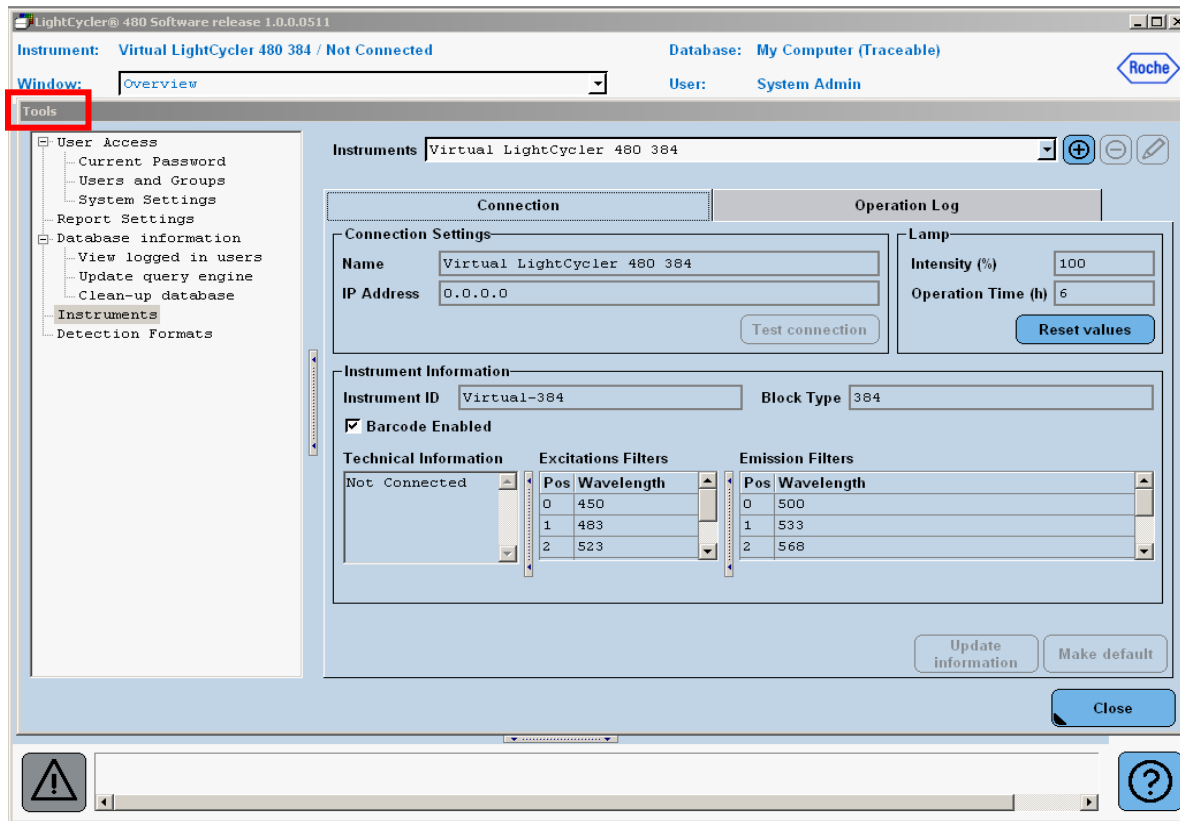
LightCycler® 480 Software

User Management



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

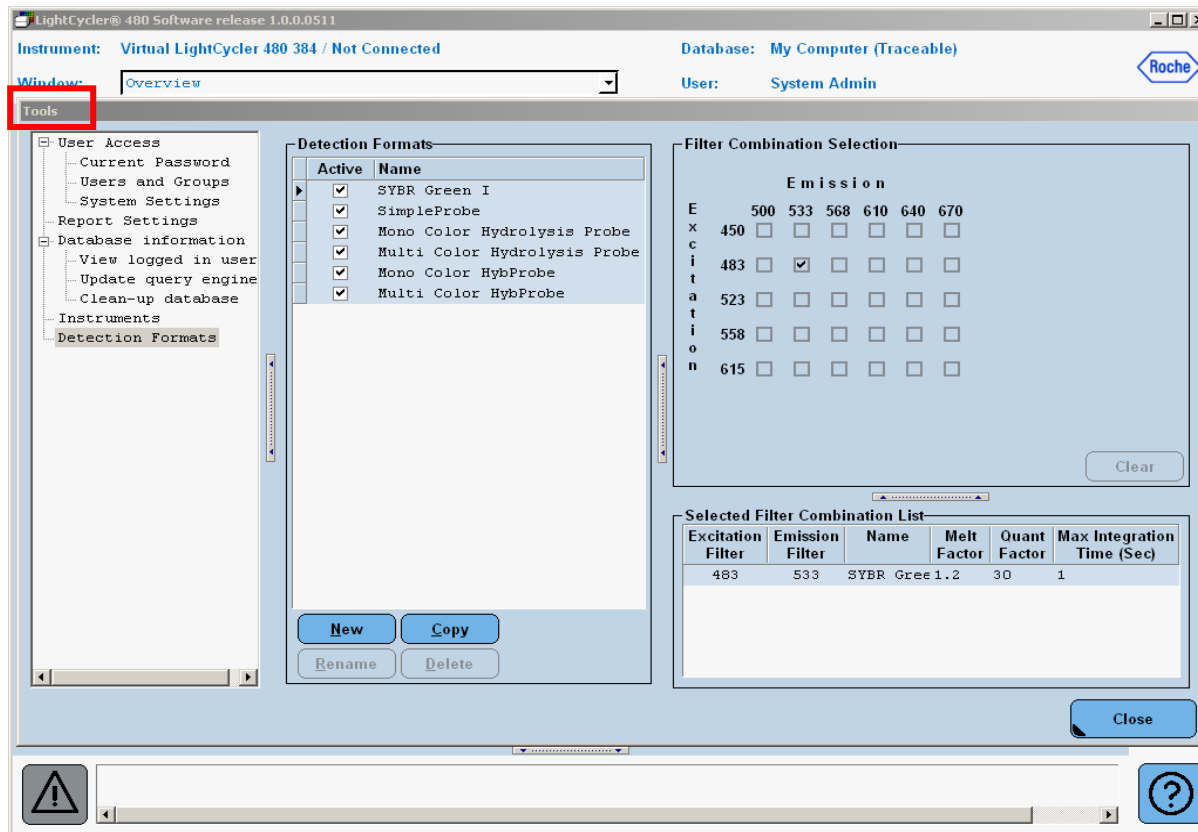
LightCycler® 480 Software *Instrument*



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

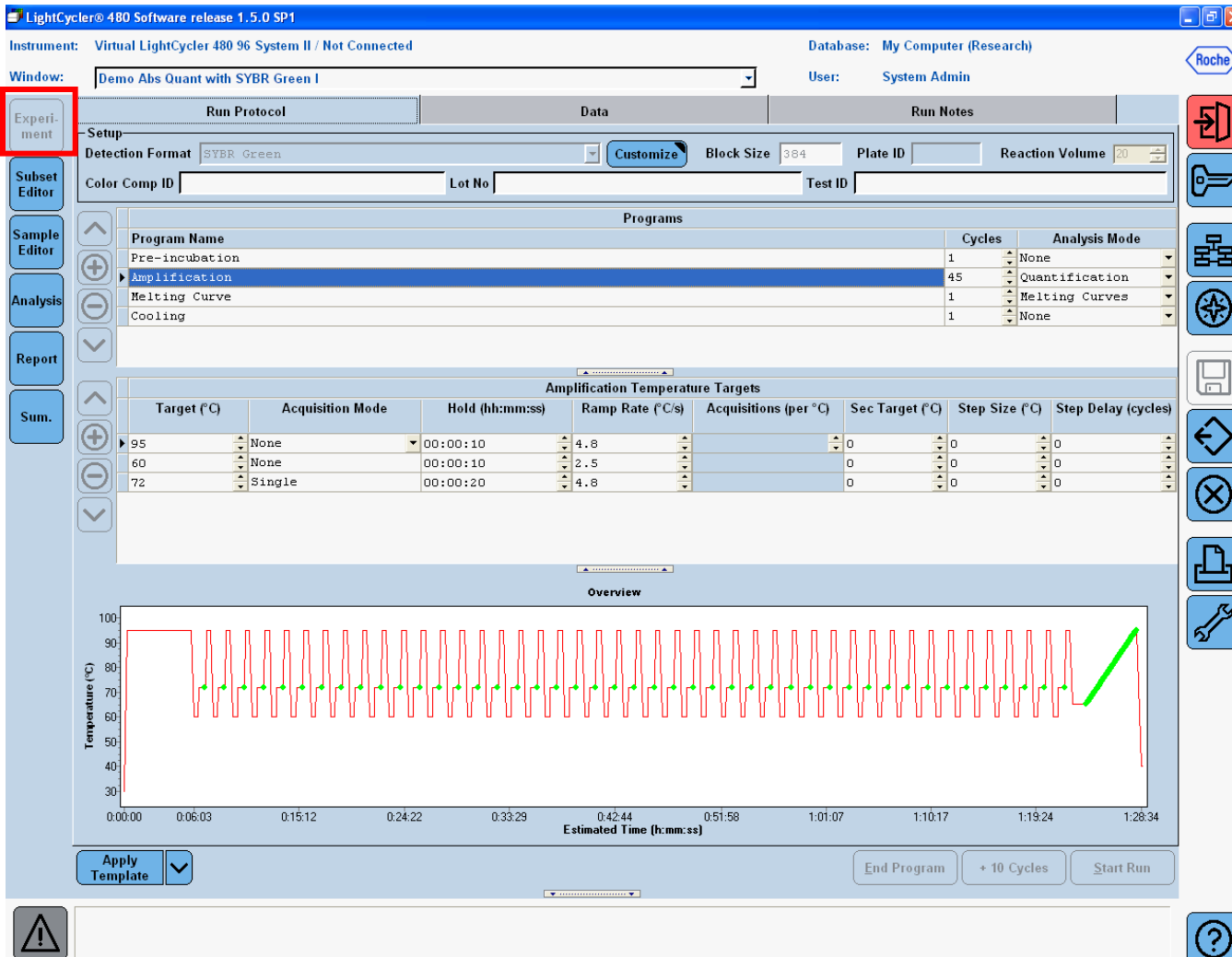
LightCycler® 480 Software

Detection Format



- **5 Excitation filters**
- **6 Detection filters**
- **Roche-defined detection formats**
- **User-defined detection formats possible**

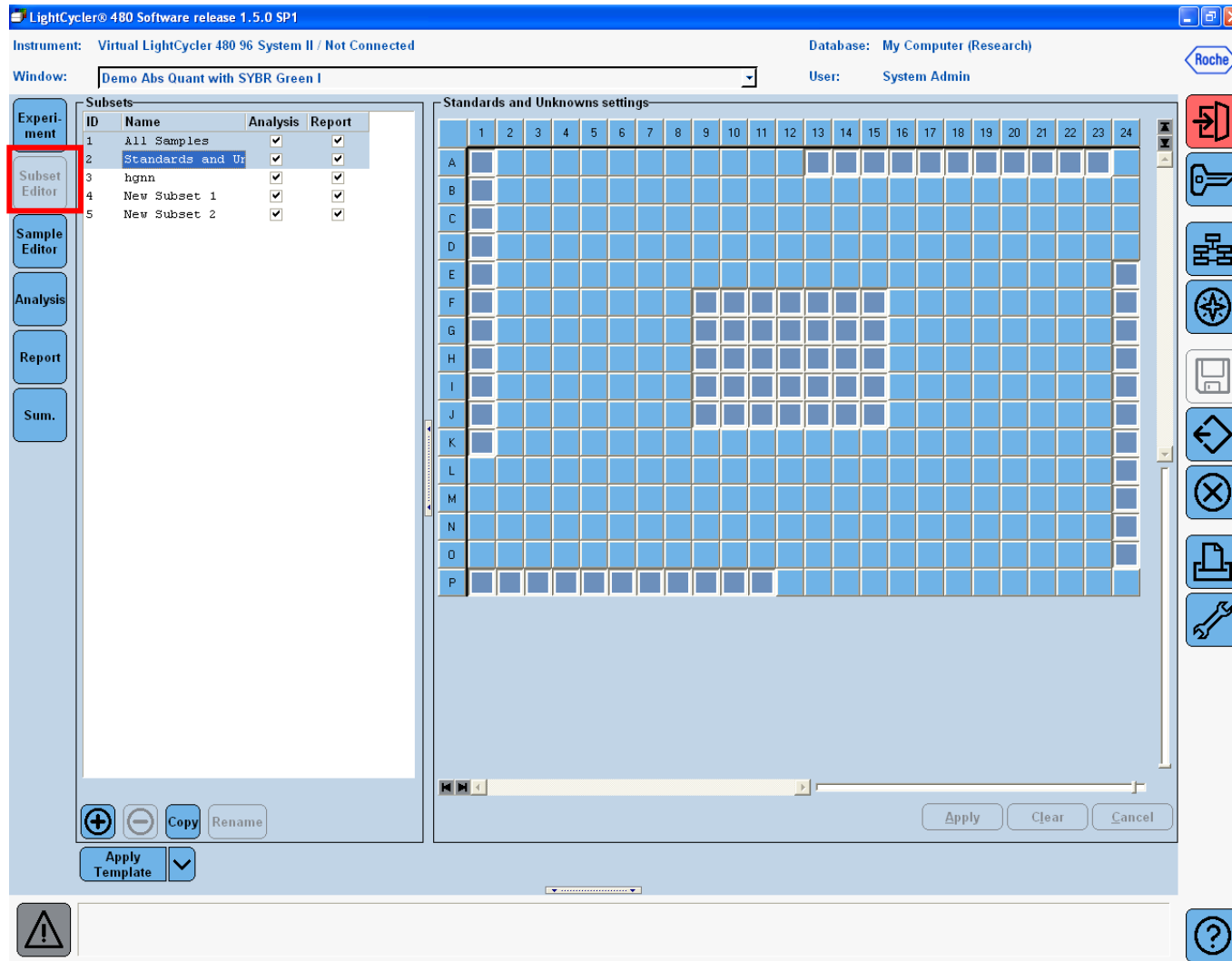
LightCycler® 480 Software Experiments



- 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*



LightCycler® 480 Software release 1.5.0 SP1

Instrument: Virtual LightCycler 480 96 System II / Not Connected Database: My Computer (Research) User: System Admin

Window: Demo Abs Quant with SYBR Green I

Step 1: Select Workflow
☐ Abs Quant ☒ Rel Quant ☐ Scanning ☐ Color Comp
☐ Tm ☐ Melt Geno ☐ Endpt Geno

Step 2: Select Samples
 Subset: Standards and Unknowns
 [Grid view showing sample layout]

Step 3: Edit Rel Quant Properties
 Sample Name: [Text field]
 Sample Type: ☐ Unknown ☐ Negative Control ☐ Positive Control/Calibrator ☐ Standard
 Concentration: [Text field] Auto Std Curve
 Gene target: Target name: Target 1 Eff: 2.00
☐ Target ☐ Reference ☒ Unassigned
 Make Replicates: [Dropdown]

Table:

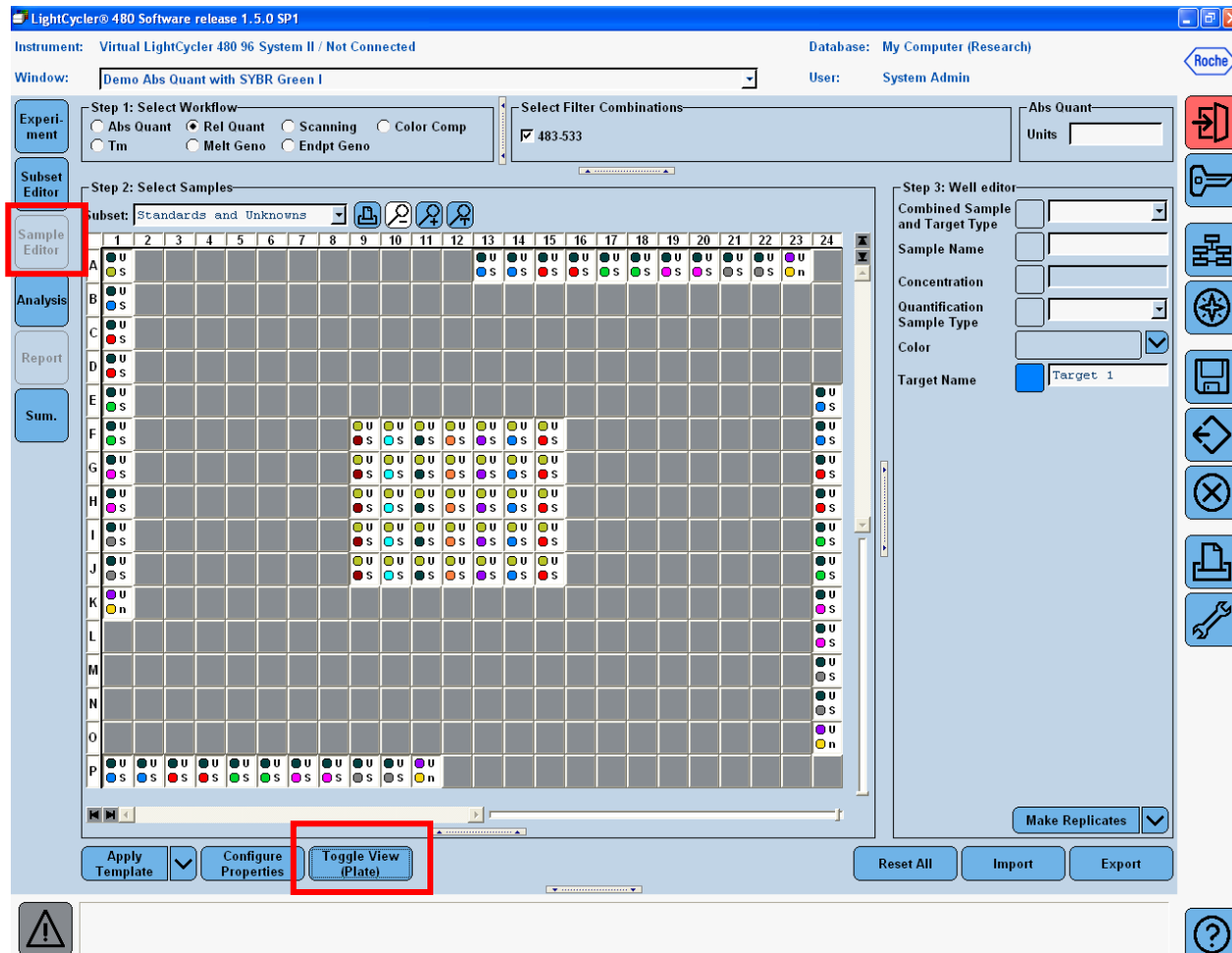
Pos	Color	Repl Of	Sample Name	Quantification Sample Type	Combined Sample and Target Type	Concentration	Target Name
A1		A1	Sample 1	Unknown	Unassigned Unknown		Target 1
A13		A1	Sample 1	Unknown	Unassigned Unknown		Target 1
A14		A1	Sample 1	Unknown	Unassigned Unknown		Target 1
A15		C1	Sample 2	Unknown	Unassigned Unknown		Target 1
A16		C1	Sample 2	Unknown	Unassigned Unknown		Target 1
A17		E1	Sample 3	Unknown	Unassigned Unknown		Target 1
A18		E1	Sample 3	Unknown	Unassigned Unknown		Target 1
A19		G1	Sample 4	Unknown	Unassigned Unknown		Target 1
A20		G1	Sample 4	Unknown	Unassigned Unknown		Target 1
A21		I1	Sample 5	Unknown	Unassigned Unknown		Target 1
A22		I1	Sample 5	Unknown	Unassigned Unknown		Target 1
A23		K1	no template	Negative Con	Unassigned Neg		Target 1
B1		A1	Sample 1	Unknown	Unassigned Unknown		Target 1
C1		C1	Sample 2	Unknown	Unassigned Unknown		Target 1
D1		C1	Sample 2	Unknown	Unassigned Unknown		Target 1
E1		E1	Sample 3	Unknown	Unassigned Unknown		Target 1
E24		A1	Sample 1	Unknown	Unassigned Unknown		Target 1
F1		E1	Sample 3	Unknown	Unassigned Unknown		Target 1
F9		F9	Standard 1	Standard	Unassigned Std	1.00E6	Target 1
F10		F10	Standard 2	Standard	Unassigned Std	1.00E5	Target 1
F11		F11	Standard 3	Standard	Unassigned Std	1.00E4	Target 1
F12		F12	Standard 4	Standard	Unassigned Std	1.00E3	Target 1
F13		F13	Standard 5	Standard	Unassigned Std	1.00E2	Target 1
F14		F14	Standard 6	Standard	Unassigned Std	1.00E1	Target 1
F15		F15	Standard 7	Standard	Unassigned Std	1.00E0	Target 1
F24		A1	Sample 1	Unknown	Unassigned Unknown		Target 1
G1		G1	Sample 4	Unknown	Unassigned Unknown		Target 1
G9		F9	Standard 1	Standard	Unassigned Std	1.00E6	Target 1
G10		F10	Standard 2	Standard	Unassigned Std	1.00E5	Target 1
G11		F11	Standard 3	Standard	Unassigned Std	1.00E4	Target 1
G12		F12	Standard 4	Standard	Unassigned Std	1.00E3	Target 1
G13		F13	Standard 5	Standard	Unassigned Std	1.00E2	Target 1

Buttons: Apply Template, Configure Properties, Toggle View (Table), Reset All, Import, Export

- Subset Editor
- **Sample Editor**
- Plate View
- Create or apply Template

LightCycler® 480 Software

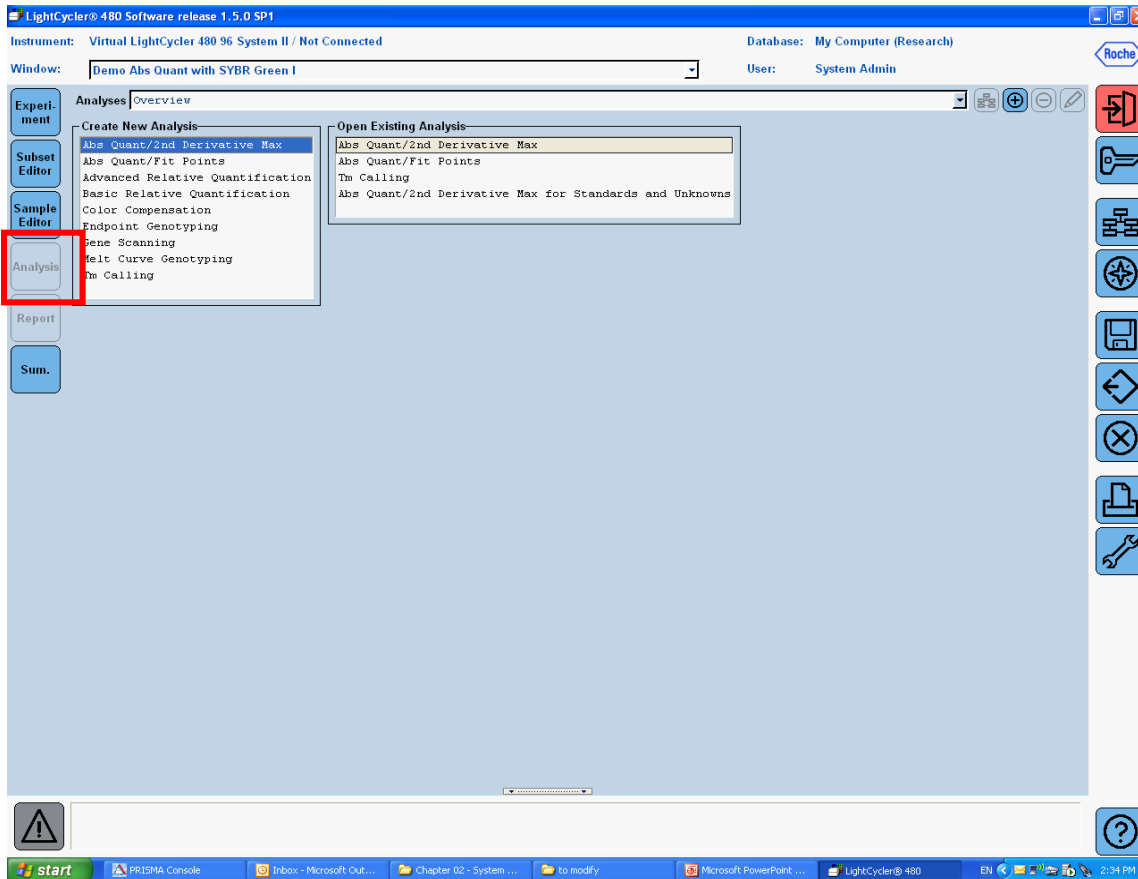
Sample Management (3): *Plate View*



- Subset Editor
- Sample Editor
- Plate view
- Create or apply Template

LightCycler® 480 Software

Analysis Modules



Analysis Modules

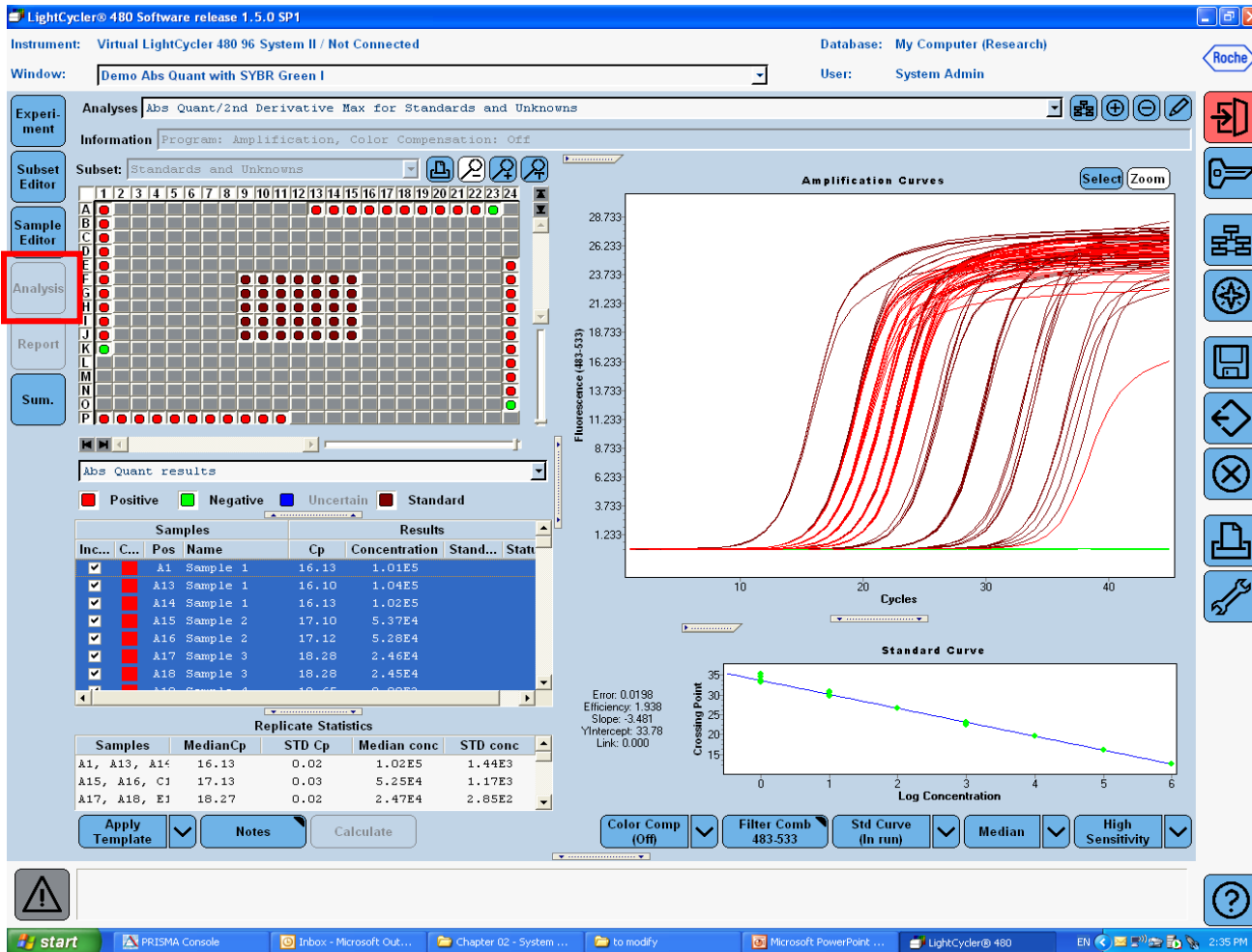
- Abs Quant/2nd Derivative Max
- Abs Quant/Fit Points
- Advanced Relative Quantification
- Basic Relative Quantification
- Color Compensation
- Endpoint Genotyping
- Gene Scanning
- Melt Curve Genotyping
- Tm Calling

Optional Modules

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

LightCycler® 480 Software

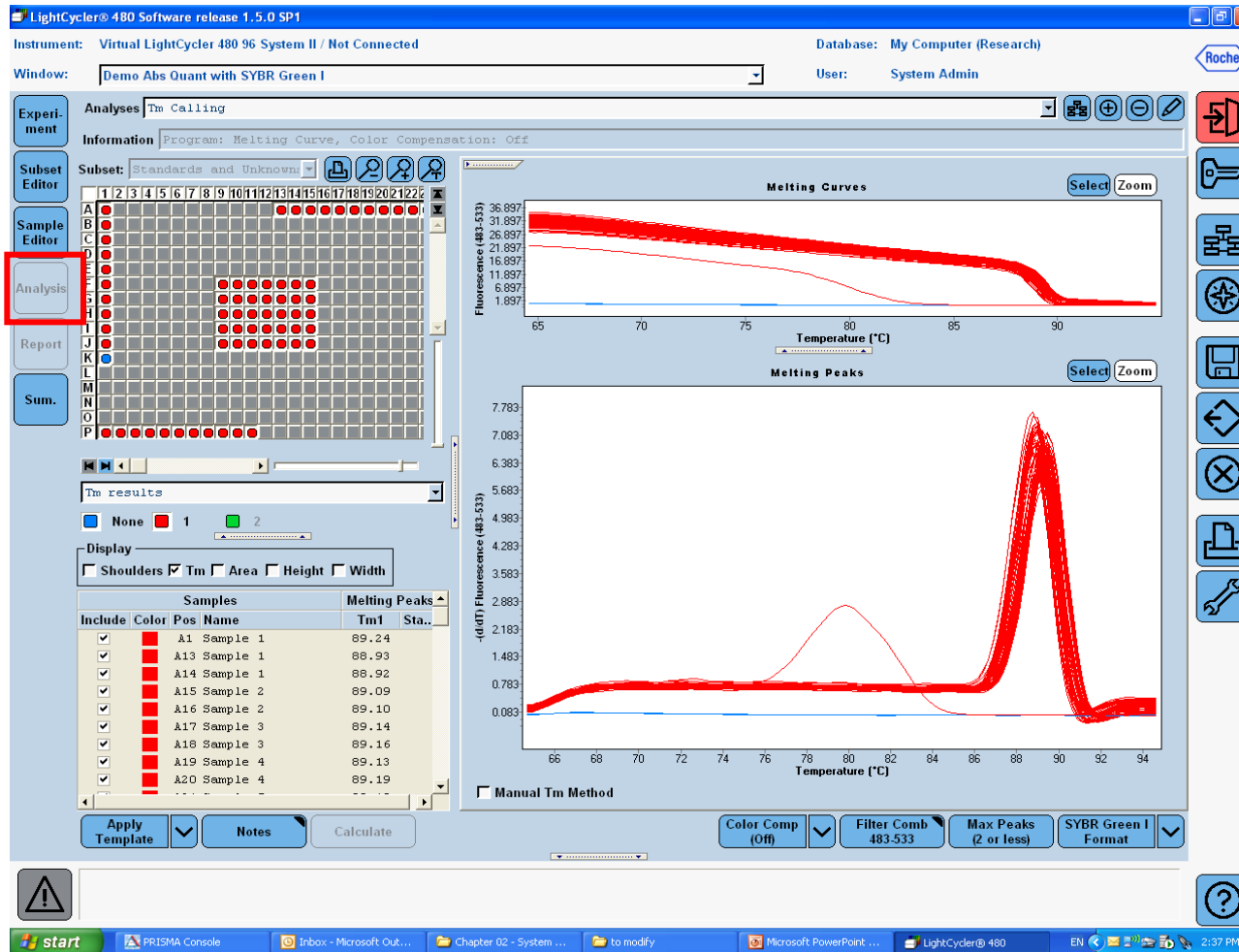
Analysis Module: Absolute Quantification



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

LightCycler® 480 Software

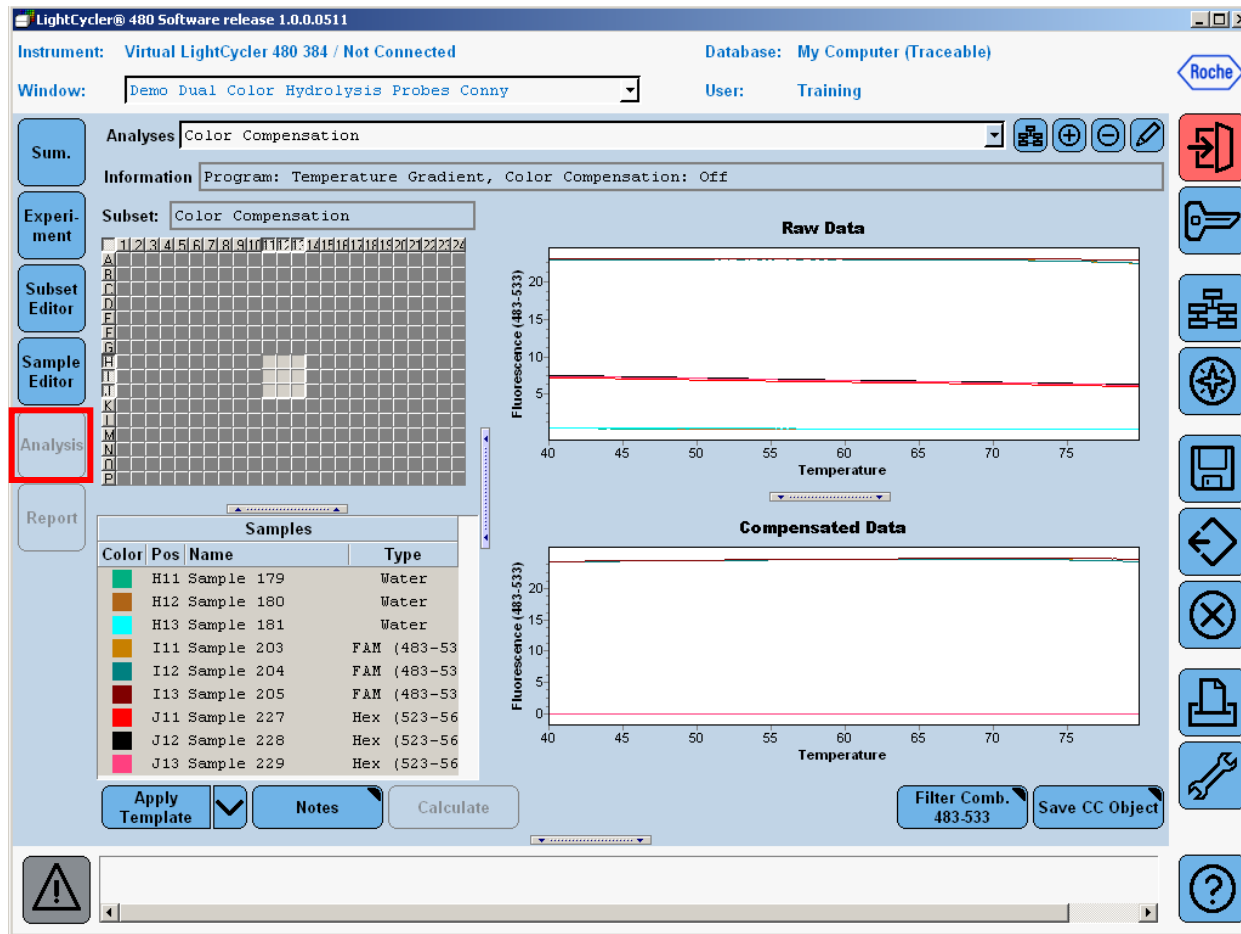
Analysis Module: *T_m* Calling



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

LightCycler® 480 Software

Analysis Module: Color Compensation



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

Create new analysis

Abs Quant Type

- ☒ Abs Quant/2nd Derivative Max Sensitivity
 - ☒ High Sensitivity ☐ High Confidence
- ☐ Abs Quant/Fit Points

Subordinate Abs Quant Analysis

- ☒ Create by Target Name
 - Create one analysis for each target name
- ☐ Create by Filter Combination
 - Create one analysis for each filter combination

Reference Analysis

- ☒ Create In-Run ☐ Select External

Pairing Rule

- ☐ One To One ☐ All To All
- ☒ All To Mean ☐ Mean To All

Default Standard Curve Settings

When there are no In-Run standards for a target name:

- ☒ always use efficiency
- ☐ allow external standards with matching target name

✓ ✕

LightCycler® 480 Software release 1.5.0 SP1

Instrument: Virtual LightCycler 480 96 System II / Not Connected Database: My Computer (Research)

Window: Demo Rel Quant Mono Color User: System Admin

Analyses: Advanced Rel Quant all-to-mean, ext Standards

Information: Subset: Samples+Calibrator, Program: amplification, References: In-Run, Abs Quant Type: Abs Quant/2nd Derivative Max

Sample Editor

Bar	Pairing	Sample Name	Target Name	References	Target Mean Cp	Reference Mean Cp	TargetRef	Normalized	Status
✓		calibrator	Target 1	Reference 1; Refere	22.43	24.14	1.064	1.000	
✓	A1/D1	sample 1	Target 1	Reference 1; Refere	23.76	26.31	1.874	1.762	
✓	A2/D2	sample 2	Target 1	Reference 1; Refere	21.34	24.58	2.978	2.799	
✓	A3/D3	sample 3	Target 1	Reference 1; Refere	24.30	26.18	1.199	1.127	
✓	A4/D4	sample 4	Target 1	Reference 1; Refere	23.56	25.27	1.060	0.9963	
✓	A5/D5	sample 5	Target 1	Reference 1; Refere	25.11	23.90	0.1528	0.1437	
✓		calibrator	Target 2	Reference 1; Refere	28.49	24.14	3.17E-2	1.000	
✓	A7/D1	sample 1	Target 2	Reference 1; Refere	20.21	26.31	43.02	1357	
✓	A8/D2	sample 2	Target 2	Reference 1; Refere	28.84	24.58	3.33E-2	1.049	
✓	A9/D3	sample 3	Target 2	Reference 1; Refere	33.98	26.18	2.09E-3	6.59E-2	
✓	A10/D4	sample 4	Target 2	Reference 1; Refere	31.32	25.27	8.51E-3	0.2683	
✓	A11/D5	sample 5	Target 2	Reference 1; Refere	33.66	23.90	5.87E-4	1.85E-2	

Sample View

Bar Chart

Relative Quantification Results

Apply Template Notes Calculate Color Comp (Off) Settings Show Abs Quant

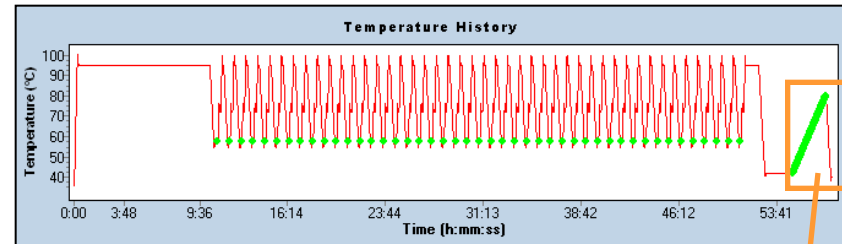
LightCycler® 480 Software

Analysis Module: Melting Curve Genotyping



Detection of known variants

Genotyping with HybProbe-/Simple Probe probes



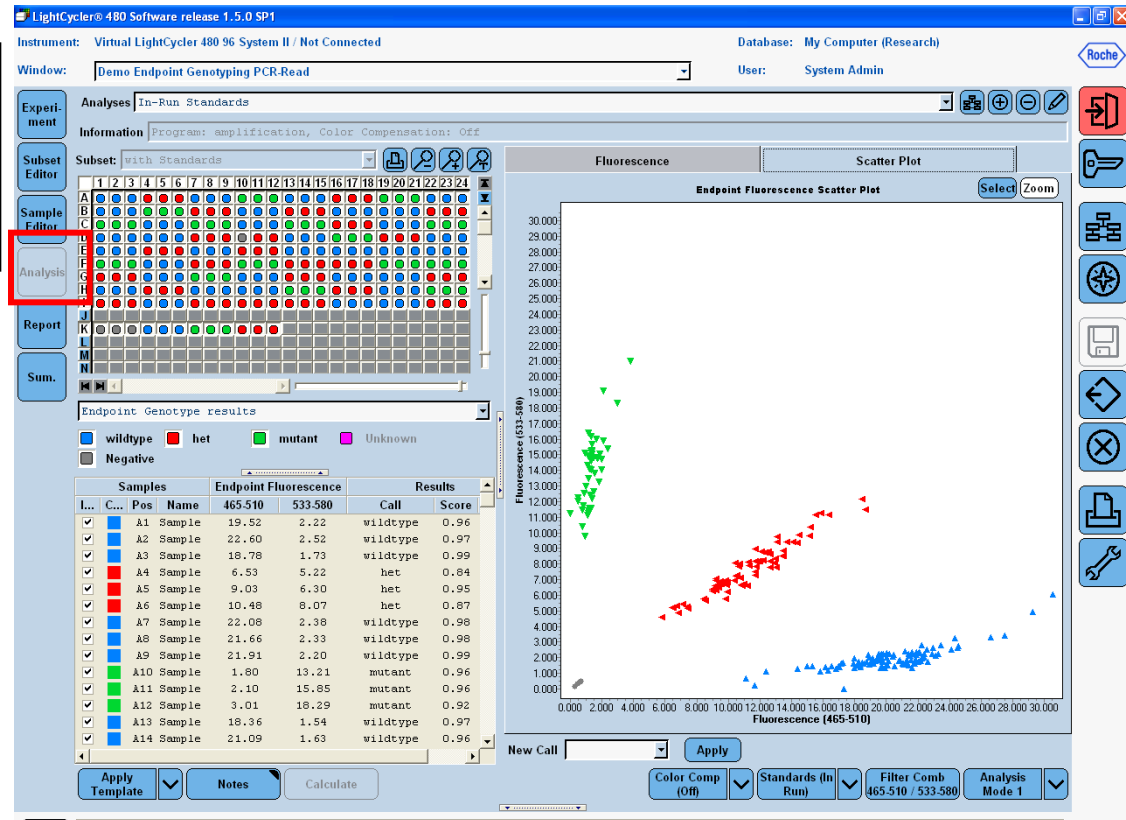
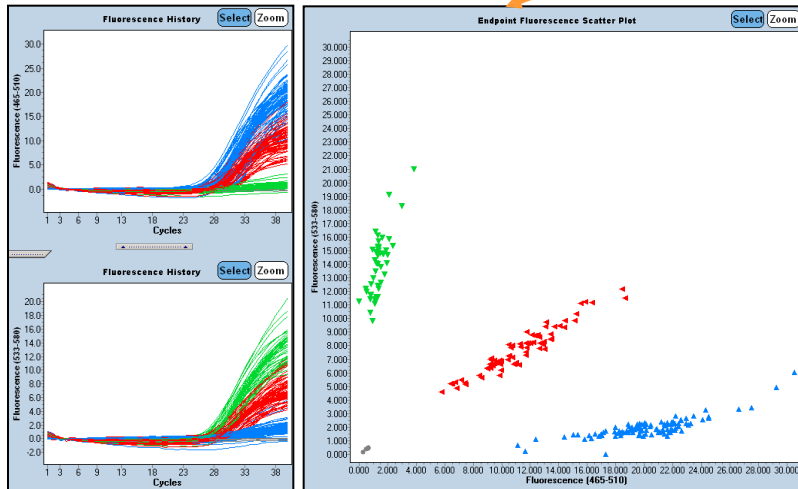
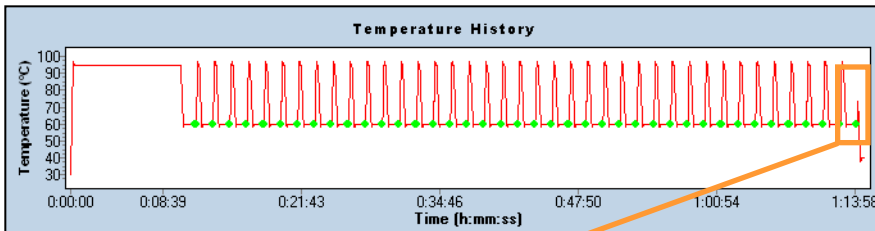
LightCycler® 480 Software

Analysis Module: Endpoint Genotyping



Detection of known variants

Genotyping with Hydrolysis probes

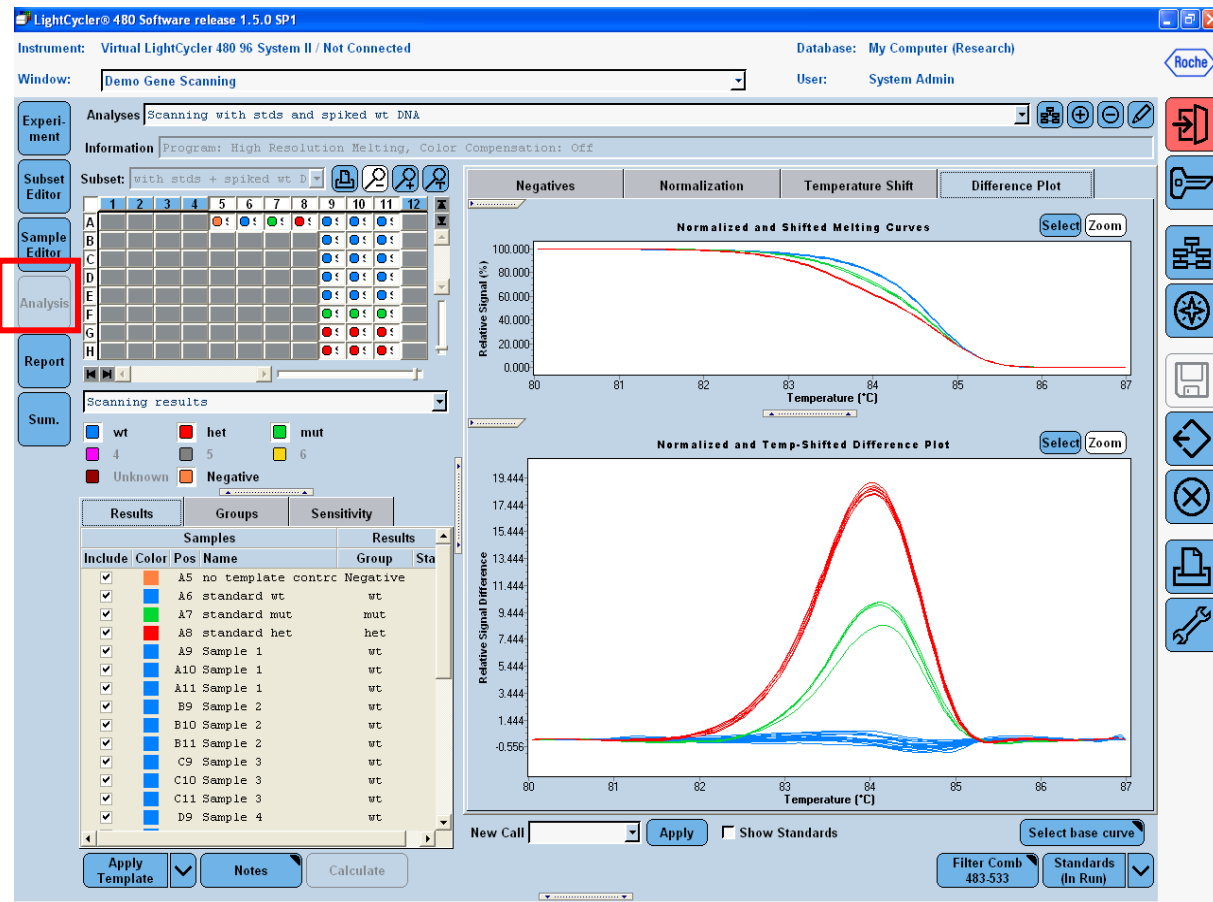
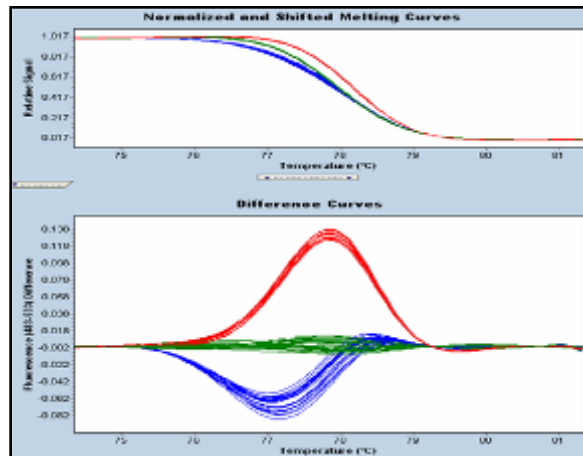
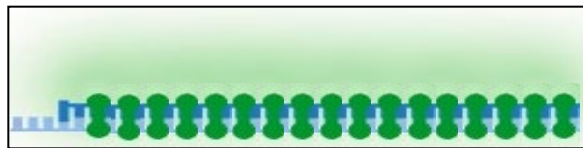
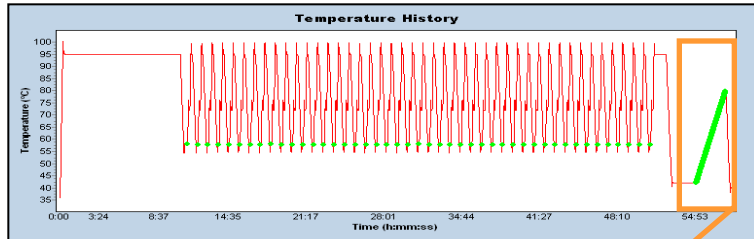


LightCycler® 480 Software

Analysis Module: Gene Scanning



Detection of any variants



LightCycler® 480 Software Report



Report Settings

Subset: All Samples

General | **Detailed**

- ☒ M107-RelQuant
 - ☒ Experiment
 - ☒ Protocol
 - ☐ Samples
 - ☐ Instrument
 - ☐ Revision History
- ☒ Basic Relative Quantification for All Samples (Relative Quantification)
 - ☒ Settings
 - ☐ Calibrators
 - ☐ Target Names
 - ☐ Pairings
 - ☒ Results
 - ☒ Results Bar Chart
 - ☒ Analysis Notes
- ☒ Subordinate for Target 1\465-510\All Samples (Abs Quant/Fit Points)
 - ☒ Settings
 - ☐ Results
 - ☐ Statistics
 - ☐ Amplification Curves
 - ☒ Noiseband Amplification Curves
 - ☐ Analysis Amplification Curves
 - ☐ Standard Curve
 - ☐ Analysis Notes
- ☒ Subordinate for Target 2\465-510\All Samples (Abs Quant/Fit Points)
- ☒ Subordinate for Reference 1\465-510\All Samples (Abs Quant/Fit Points)
- ☒ Subordinate for Reference 2\465-510\All Samples (Abs Quant/Fit Points)
- ☒ Advanced Relative Quantification for Samples+Calibrator (Relative Quantification)
 - ☒ Settings
 - ☐ Calibrators
 - ☐ Target Names
 - ☐ Pairings
 - ☒ Results
 - ☒ Results Bar Chart
 - ☐ Analysis Notes

Default Settings

Apply Template ▼ Generate

View Report

Zoom (Fit Width) | Page 1 of 6 | PDF

LightCycler® 480 Software

Report

M107-Rel Quant Experiment

Creation Date: 15.10.2007 15:25:07	Last Modified Date: 06.11.2007 16:18:31
Operator: Demo	Owner: Expert
Start Time: 15.10.2007 16:47:45	End Time: 15.10.2007 17:28:07
Run State: Completed	Software Version: LC480 1.4.5.115
Macro:	Macro Owner:
Macro Status:	
Template: Mono Color Hydrolysis Probe - UPL Probe 95-II	Plate ID: 00153252
Test ID:	Lot ID:
Color Comp ID:	

Run Notes: Mono color Realtime Quantification experiment. Two target genes and two reference genes are detected with Universal ProbeLibrary probes, all labeled with 5'-FAM.

Programs:

Program Name: pre-incubation							
Cycles: 1		Analysis Mode: None					
Target (°C)	Acquisition Mode	Hold (minutes)	Ramp Rate (°C/s)	Acquisition (per °C)	Sec Target (°C)	Step size (°C)	Step Delay (cycles)
95	None	00:10:00	4.40		0	0	0

Program Name: amplification							
Cycles: 45		Analysis Mode: Quantification					
Target (°C)	Acquisition Mode	Hold (minutes)	Ramp Rate (°C/s)	Acquisition (per °C)	Sec Target (°C)	Step size (°C)	Step Delay (cycles)
95	None	00:00:10	4.40		0	0	0
60	Single	00:00:30	2.20		0	0	0
72	None	00:00:01	4.40		0	0	0

Program Name: cooling							
Cycles: 1		Analysis Mode: None					
Target (°C)	Acquisition Mode	Hold (minutes)	Ramp Rate (°C/s)	Acquisition (per °C)	Sec Target (°C)	Step size (°C)	Step Delay (cycles)
40	None	00:00:30	2.20		0	0	0

Basic Relative Quantification for All Samples (Relative Quantification)

M107-Rel Quant 06.11.2007 Page 1 of 6

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

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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 connectivity.
- 21 CFR part 11 compliance data protection.
- Premium **customer support and instrument service**

LightCycler® 480 System Support and Service



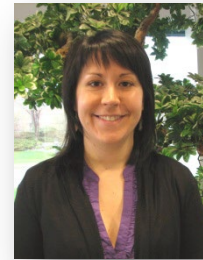
- Roche Diagnostics head office based in Laval, Quebec.
- Ontario Service Team (Instruments and Applications) for Life Sciences Research and Molecular Diagnostics:



Roche Care Centre

Technical Support Specialists

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



Charles Marseille

Genevieve Major

Sandra Pittet

Local Application Support

Field Application Specialists



Steven van Konynenburg

Mena Farag

Sandra Jeftic

Local Instrument Support

Field Support Representatives



Kenneth Brace

Steve Ruffell

Doing now what patients need next