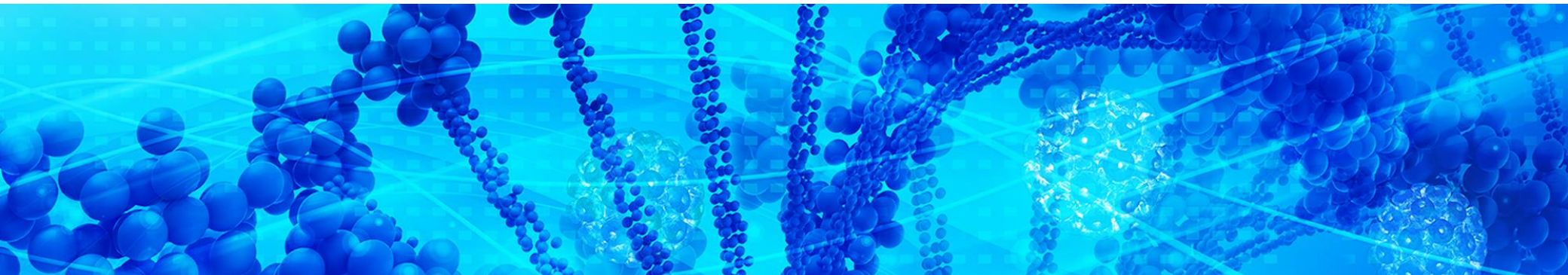


REAL TIME PCR PRINCIPLE AND APPLICATIONS ***ACCURACY. VERSATILITY. CONVENIENCE***

又鑫生物科技有限公司

YU SHING Bio-Tech Co., Ltd

Product Manager Sophia Lin





Assay Formats and Cp Calculation

Absolute/Relative Quantification

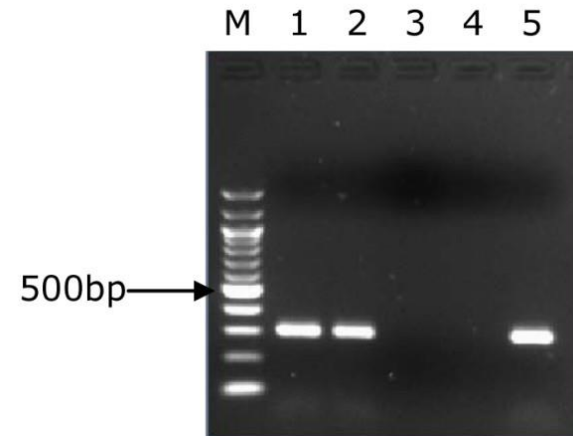
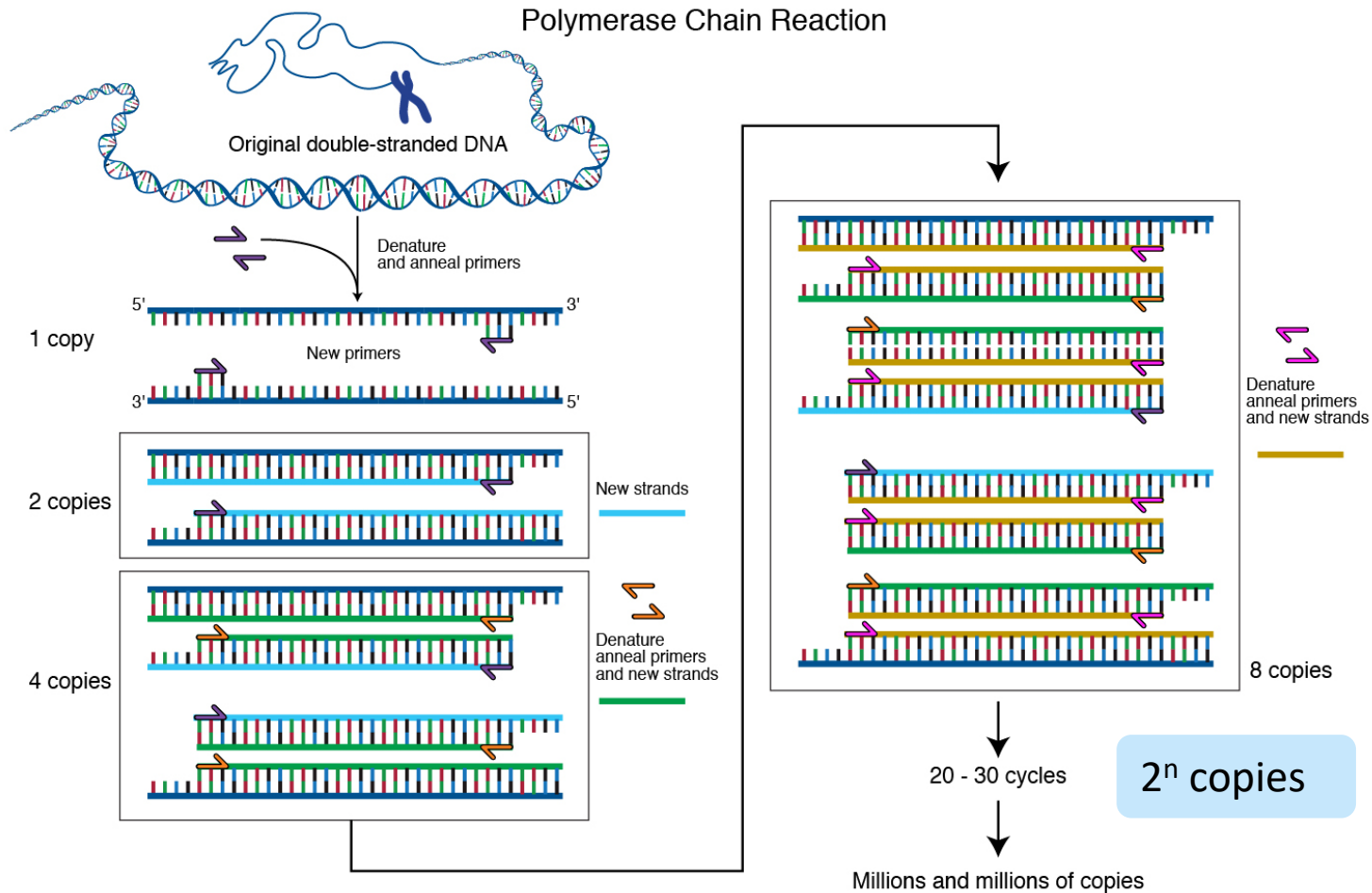
Hardware Overview for LightCycler® Systems

System Operation Procedures

LC 96/LC 480 QC Report

Q&A

Polymerase Chain Reaction (PCR)



Denature

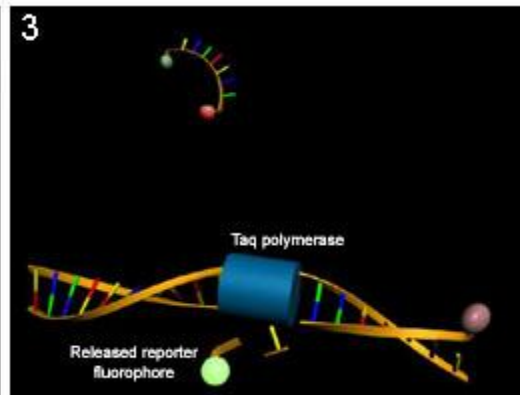
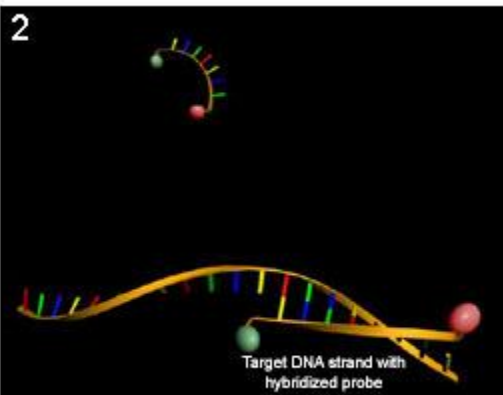
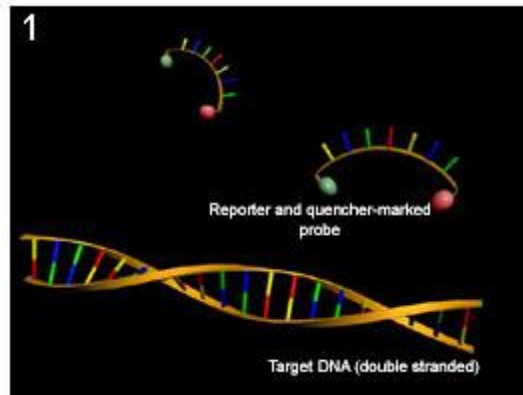
Annealing

Extension

Real-Time Polymerase Chain Reaction (real-time PCR)

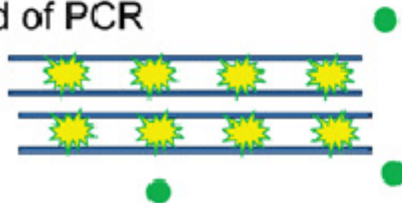
a SYBR Green assay

DNA



● Bound SYBR Green I

End of PCR

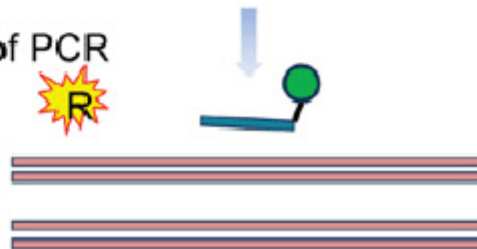


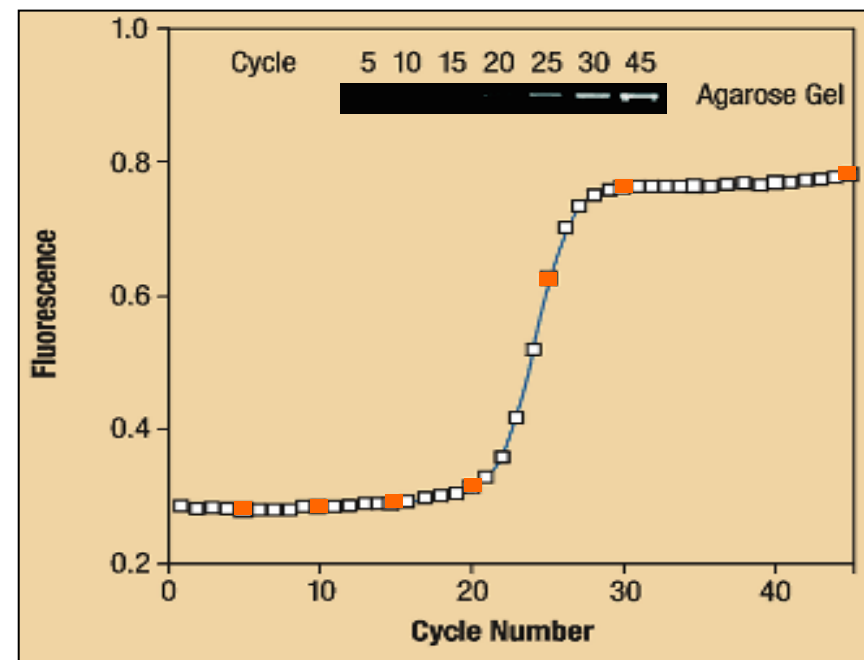
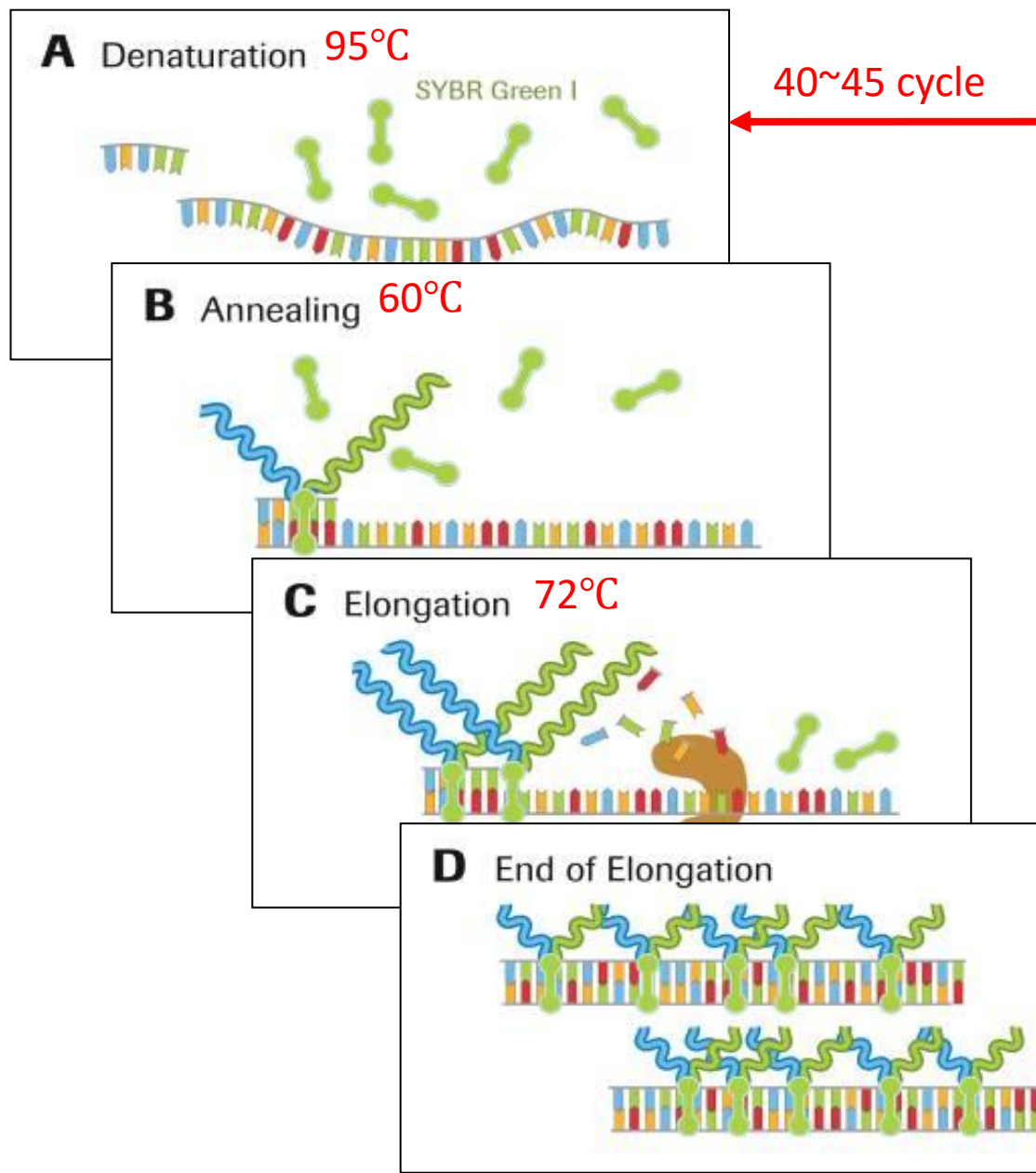
b TaqMan assay

DNA

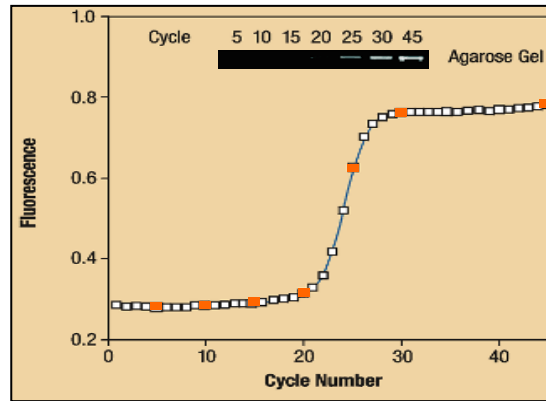


End of PCR



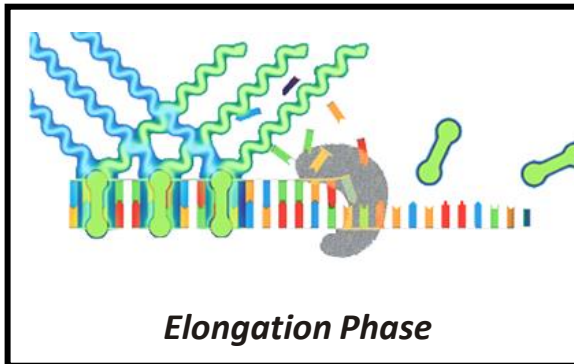


Detection Formats



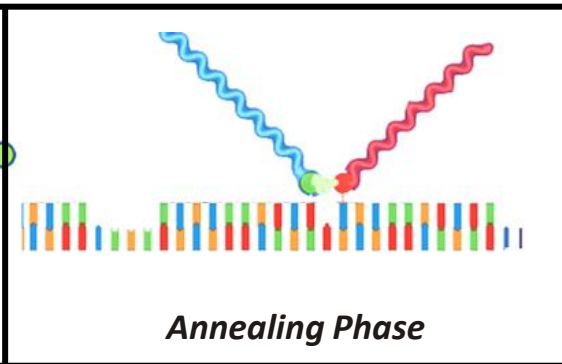
Non-specific binding dye

SYBR Green I

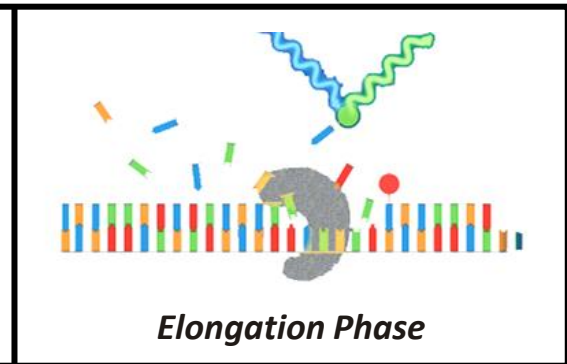


Specific labeled probes

Hybridization Probe

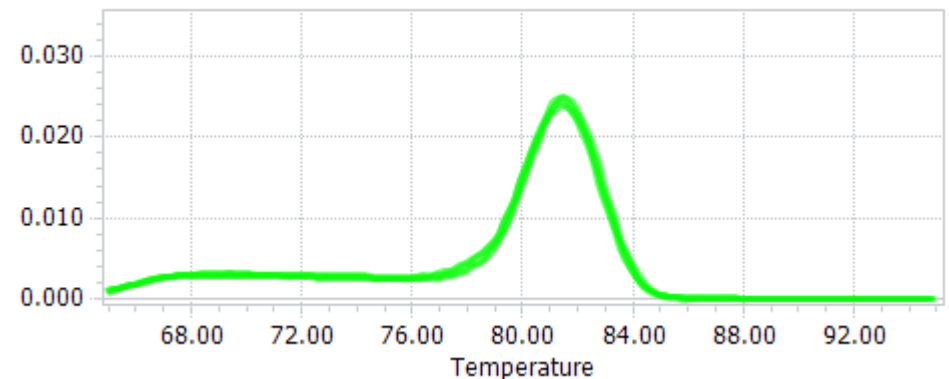
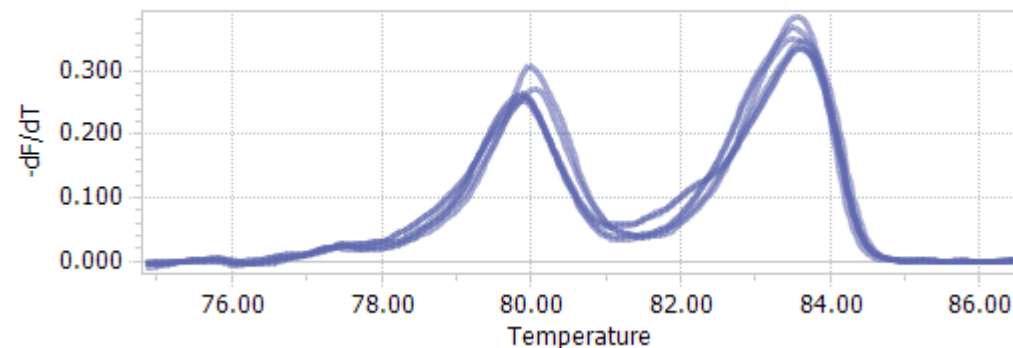
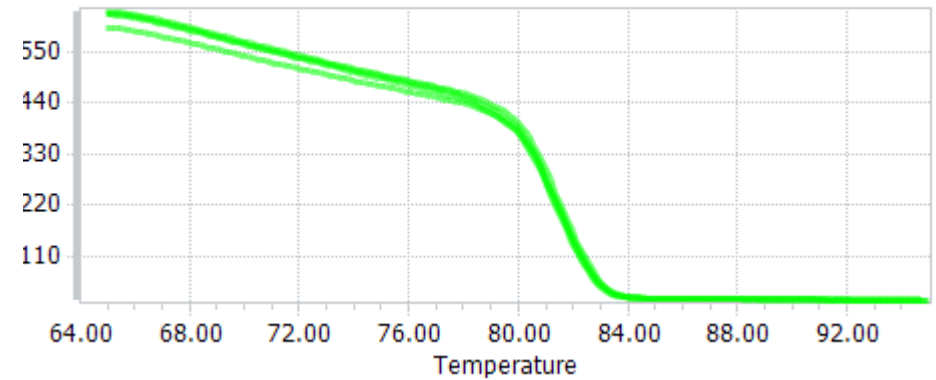
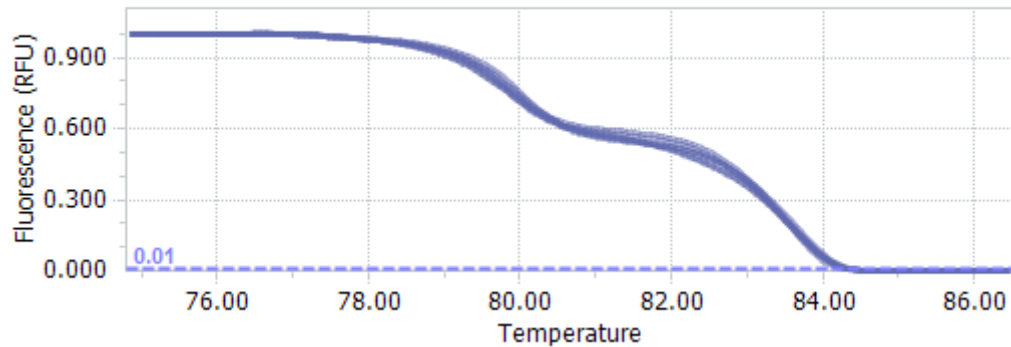
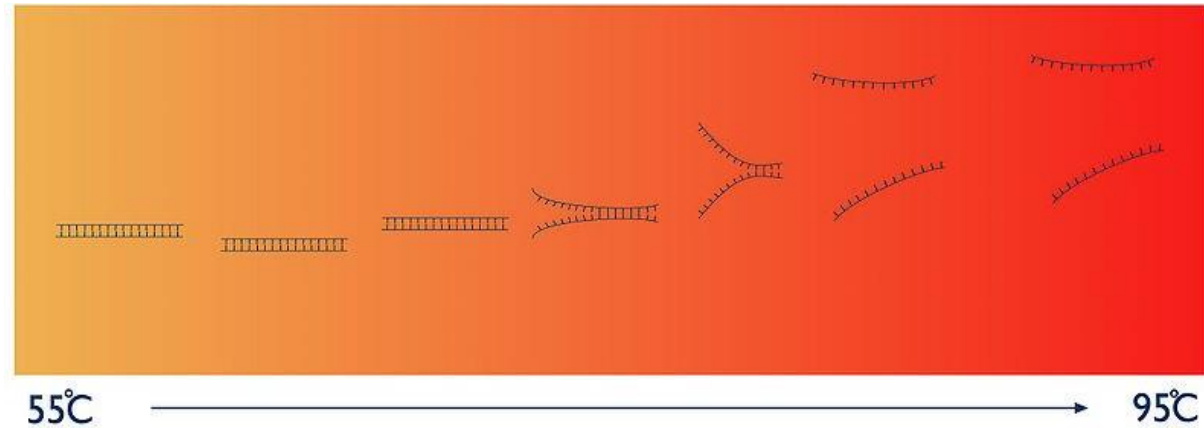
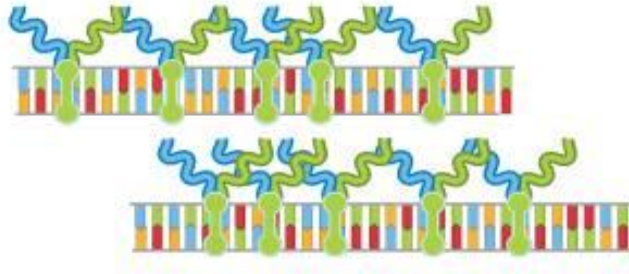


Hydrolysis Probe (TaqMan)

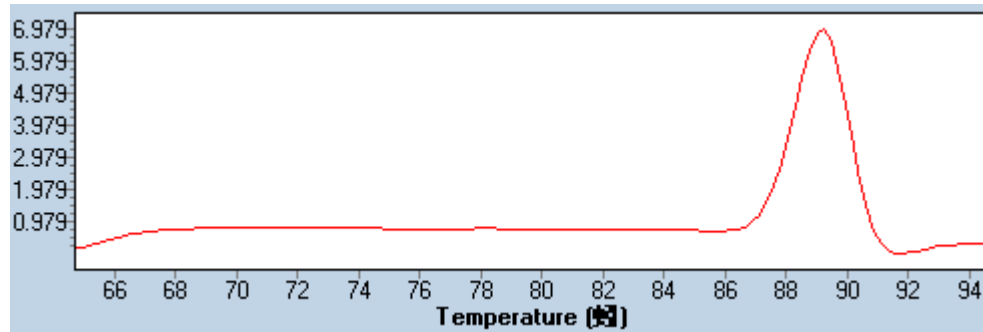


Analysis Module **T_m Calling/Melting Curve**

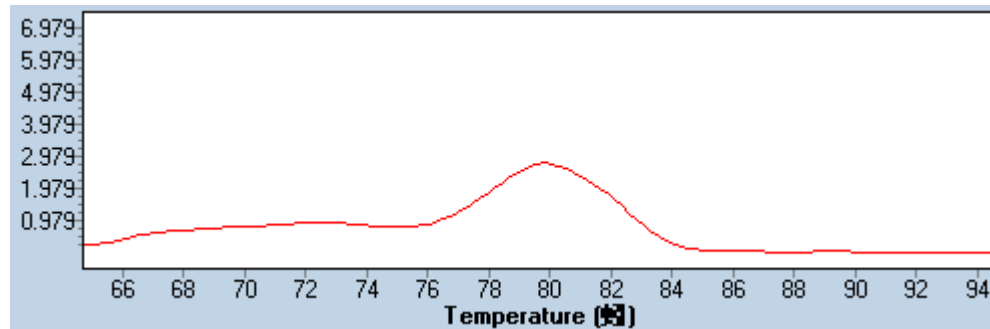
D End of Elongation



NTC(No Template Control)



Positive Sample Melting curve

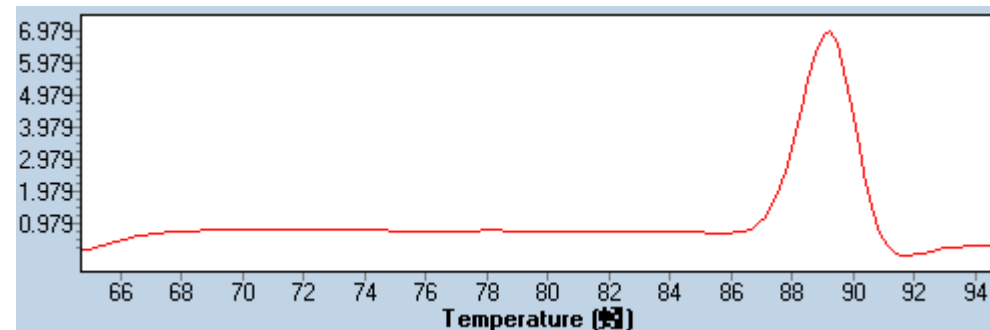


1.Primer dimer :

Primer conc. ↓

Primer design

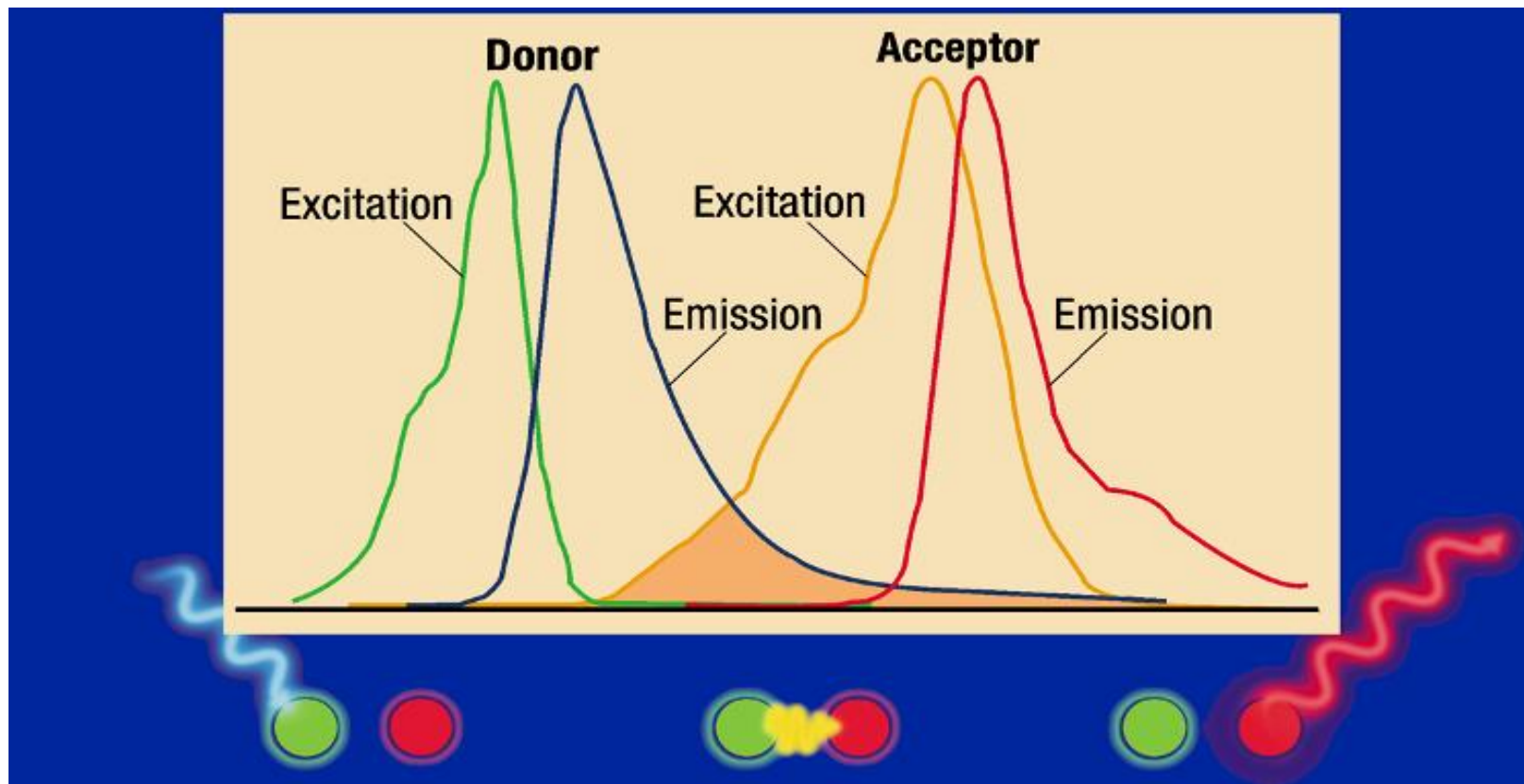
Amplicon length : 100bps↑



2.Contamination

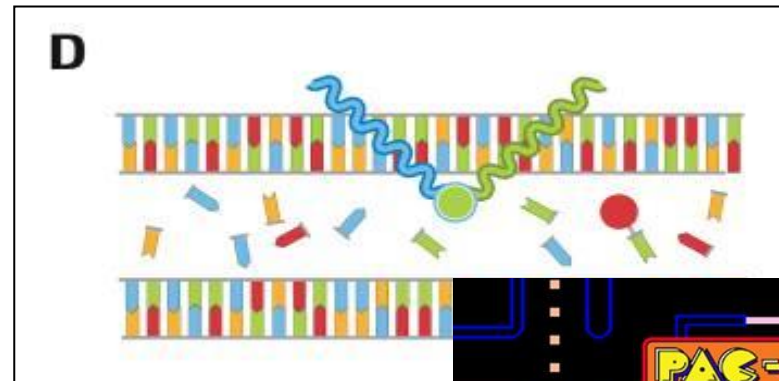
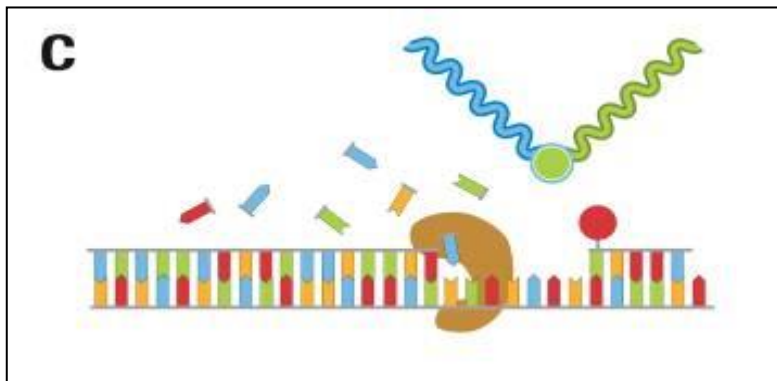
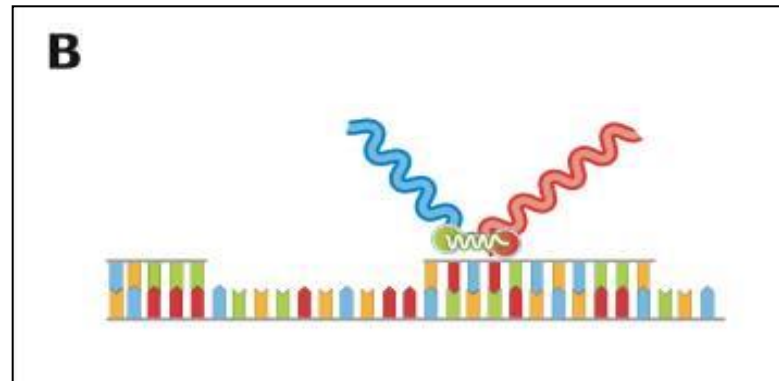
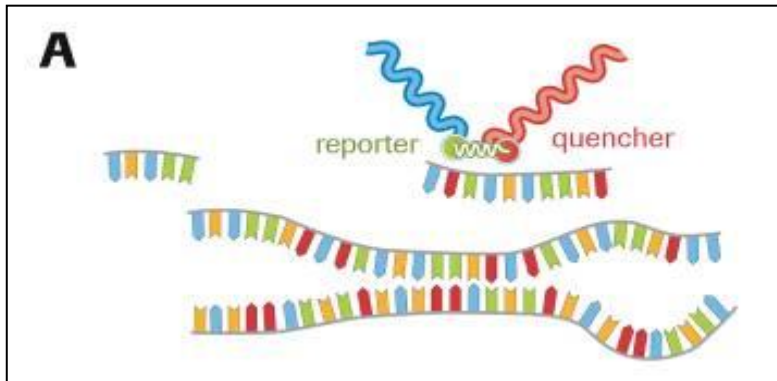
LightCycler® Assay Formats

Fluorescence Resonance Energy Transfer (FRET)



LightCycler® Assay Formats

TaqMan Probe

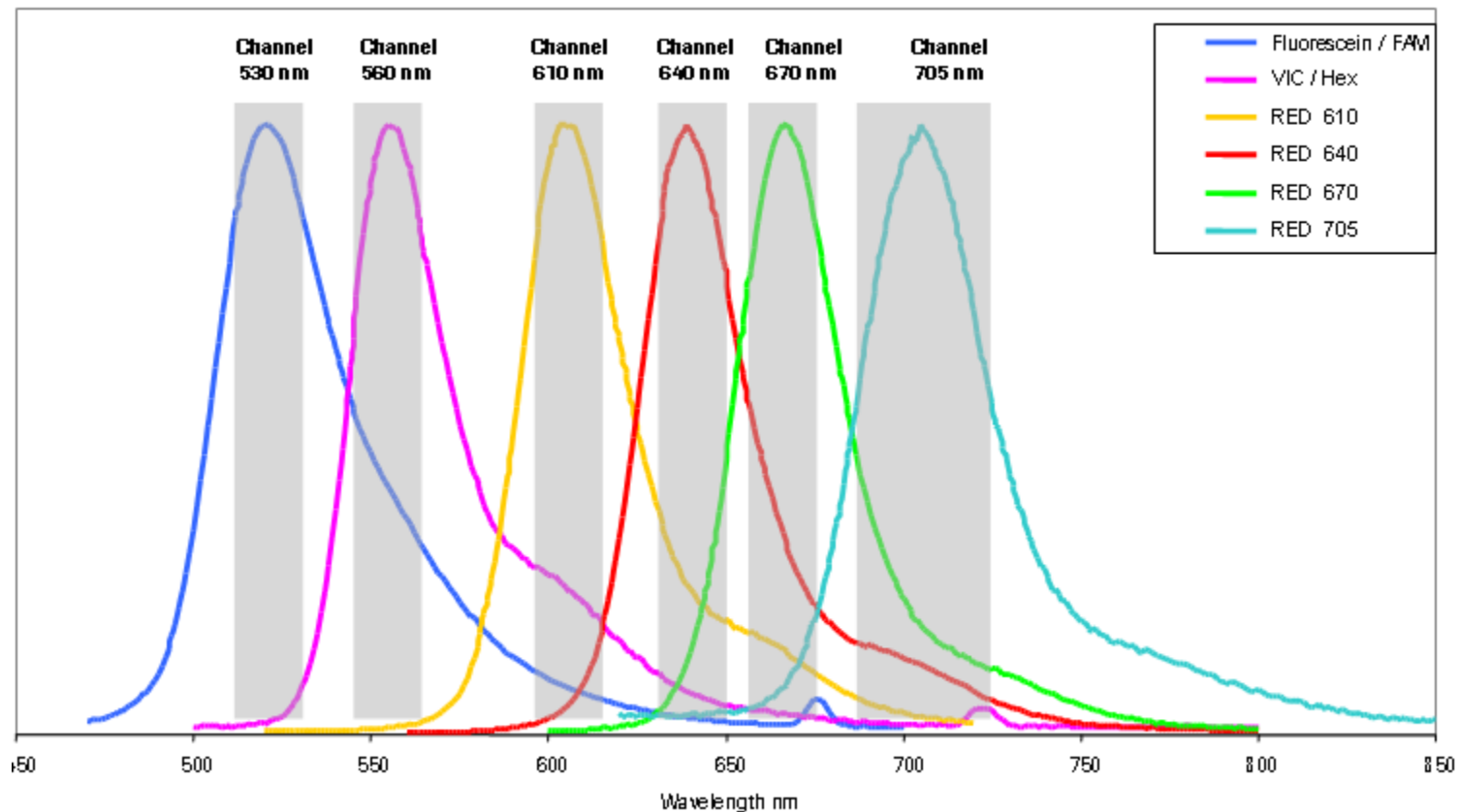


1. 多色Multiplex
2. 不需melting curve



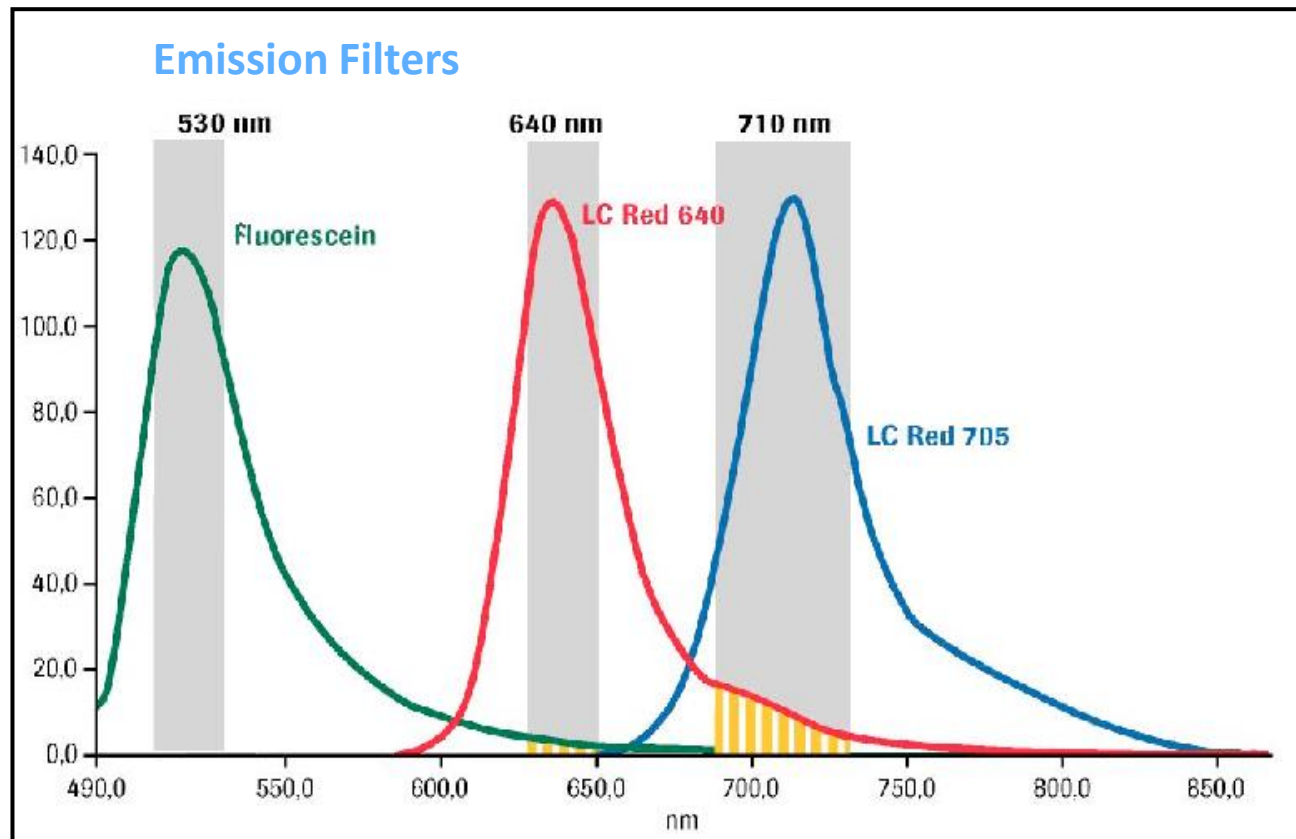
LightCycler® Assay Formats

Dye Emission Spectra



LightCycler® Assay Formats

Dye Emission Spectra and Crosstalk



Tricolor Hydrolysis Probe - Example

Application with Spectral Crosstalk

FAM

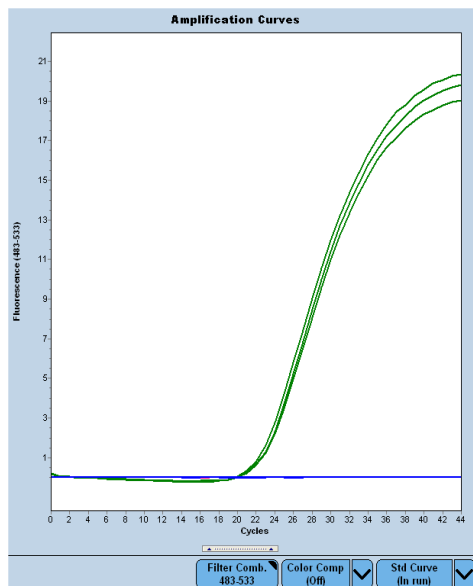
—

Red 610

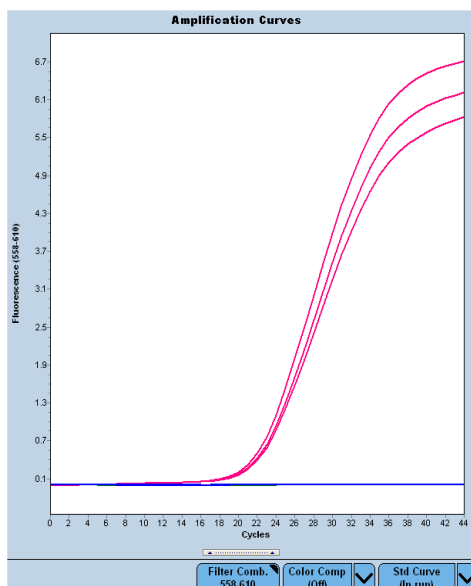
—

Cy 5 (PBGD + G6PDH + CyP2 C9)

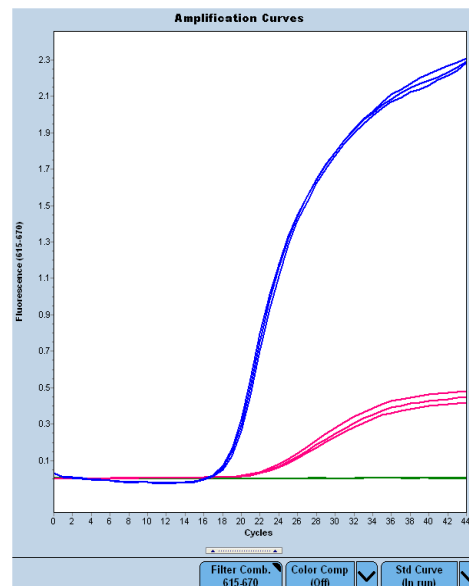
Crosstalk of Cy 5 into channel 610 is reliably compensated



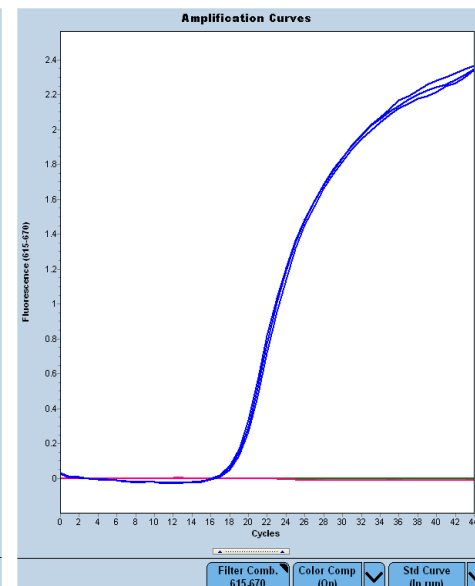
533



610



670 no CC

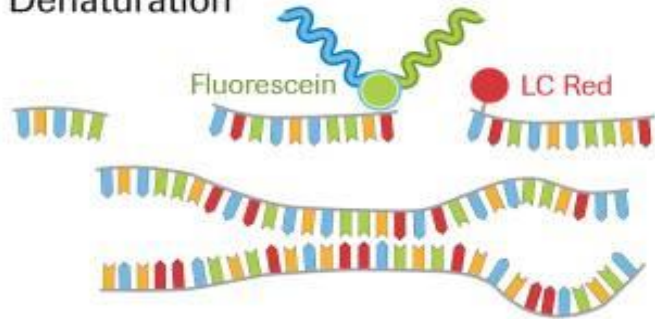
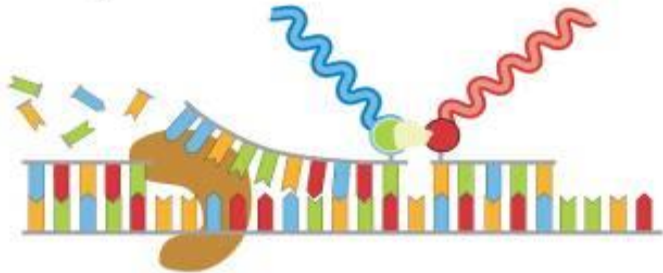


670 with CC

LightCycler® Assay Formats

Hybridization Probes

HybProbe

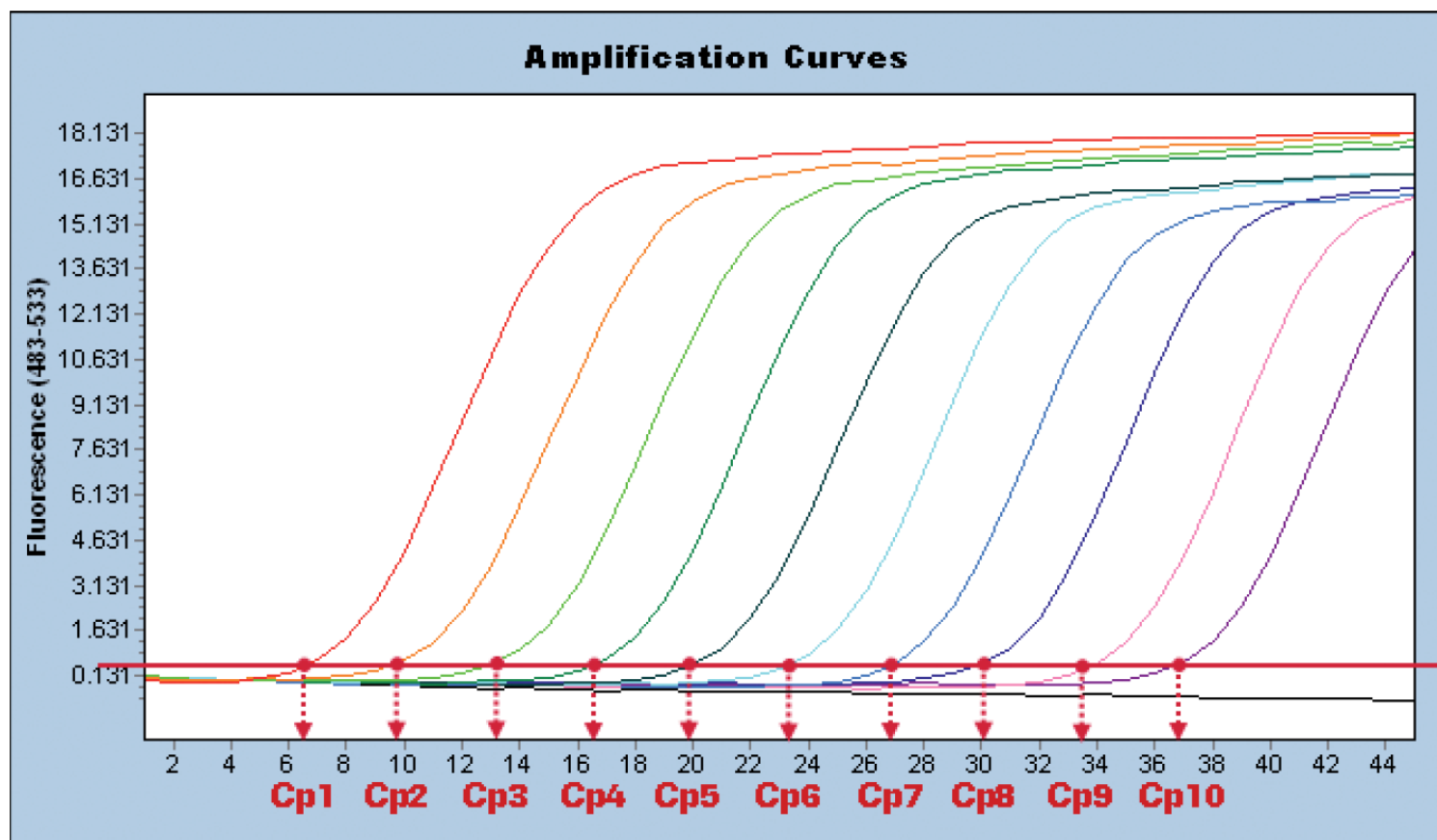
A Denaturation**B** Annealing**C** Elongation**D** End of Elongation

No primer-dimer or side-products' disturbance ! – **High specificity**

Don't need to run the Melting curve – **High efficiency,**

There is a correlation between Cp and concentration

*The **higher concentration** of target nucleic acid in the starting material, the sooner a significant increase in fluorescent signal will be observed, yielding a **lower Cycle no.***



Cp(Cross Point)

Ct(Threshold Cycle)

Cq(Quantification Cycle)

Calculation

Nomenclature

TaqMan Probe-Hydrolysis Probe

The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments

Stephen A. Bustin,^{1*} Vladimir Benes,² Jeremy A. Garson,^{3,4} Jan Helleman,⁵ Jim Huggett,⁶
Mikael Kubista,^{7,8} Reinhold Mueller,⁹ Tania Nolan,¹⁰ Michael W. Pfaffl,¹¹ Gregory L. Shipley,¹²
Jo Vandesompele,⁵ and Carl T. Wittwer^{13,14}

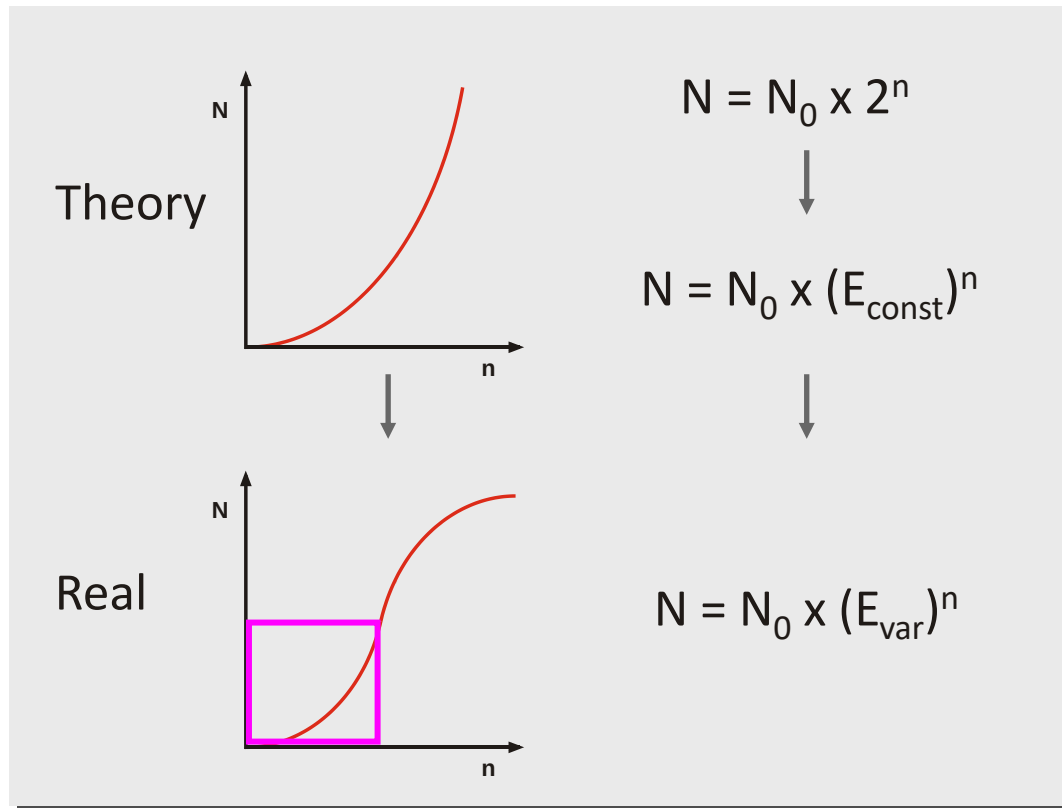
1. Nomenclature

A few terms require standardization to ensure clarification:

- 1.1 We propose that the abbreviation qPCR be used for **quantitative real-time PCR** and that RT-qPCR be used for reverse transcription–qPCR. Applying the abbreviation RT-PCR to qPCR causes confusion.
- 1.2 Genes used for normalization should be referred to as **reference genes**, not as *housekeeping genes*.
- 1.3 TaqMan probes should be referred to as **hydrolysis probes**.
- 1.4 The term *FRET probe* (fluorescence resonance energy transfer probe) refers to a generic mechanism in which emission/quenching relies on the interaction between the electron-excitation states of 2 fluorescent dye molecules. LightCycler-type probes should be referred to as *dual hybridization probes*.
- 1.5 The *Oxford English Dictionary* lists only *quantification*, not *quantitation*; therefore, the former is the proper word.
- 1.6 The nomenclature describing the fractional PCR cycle used for quantification is inconsistent, with *threshold cycle* (C_t), *crossing point* (C_p), and *take-off point* (TOP) currently used in the literature. These terms all refer to the same value from the real-time instrument and were coined by competing manufacturers of real-time instruments for reasons of product differentiation, not scientific accuracy or clarity. We propose the use of **quantification cycle** (C_q), according to the RDML (Real-Time PCR Data Markup Language) data standard (<http://www.rdml.org>) (27).

PCR Quantification

Theoretical and Practical Aspects



log-phase-PCR



end-point-PCR

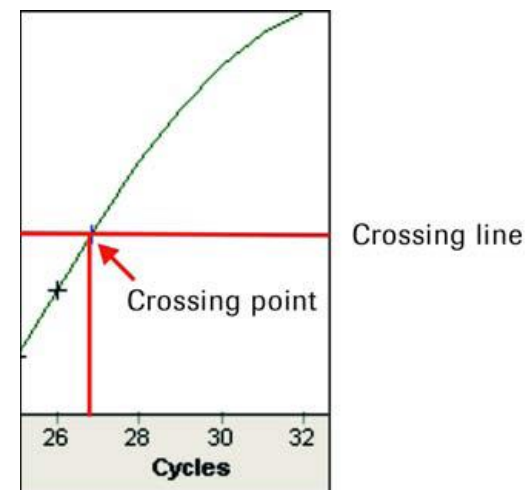
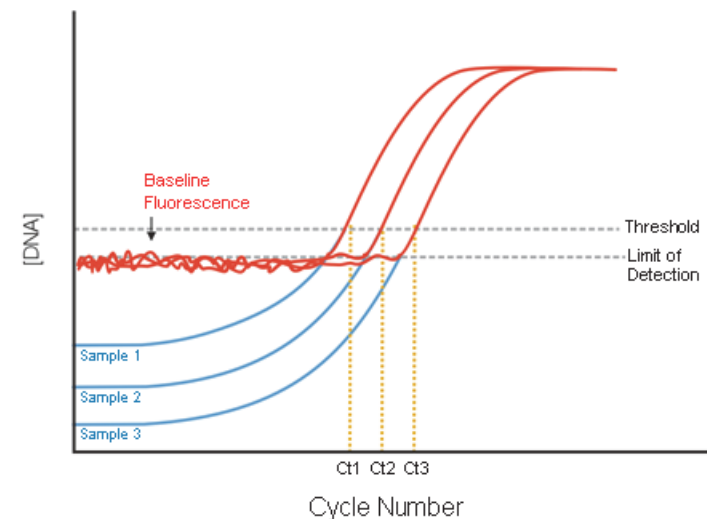
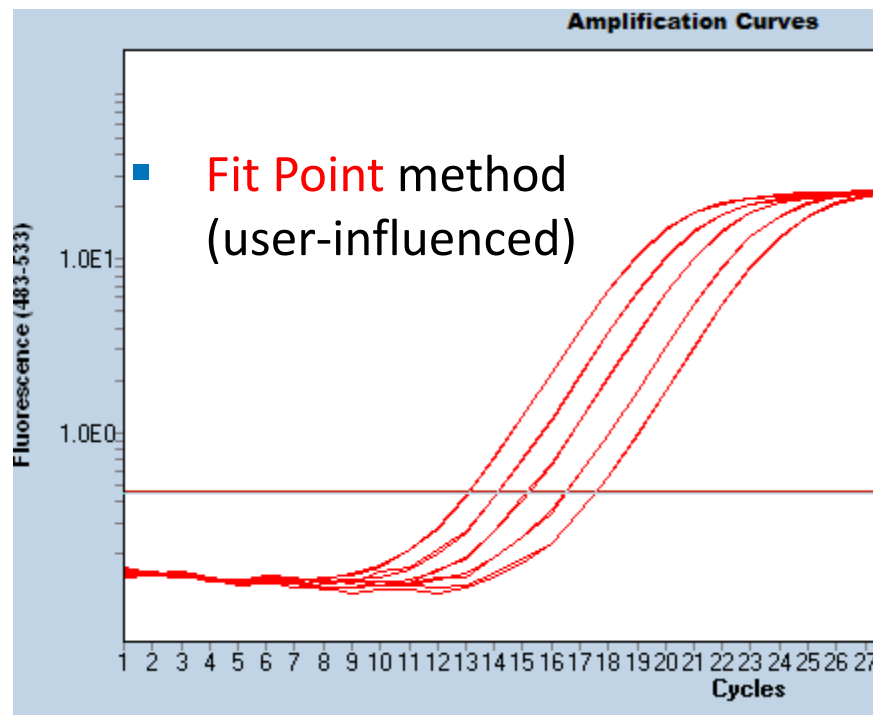
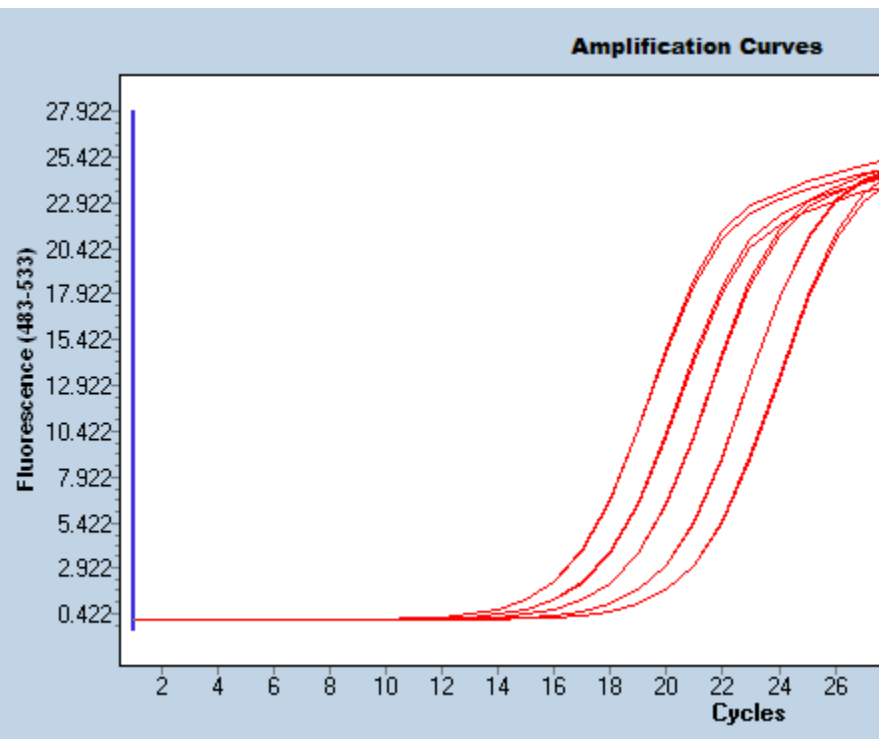
N: number of amplified molecules

N_0 : initial number of molecules

n: number of amplification cycles

E: amplification efficiency

How to calculate the Ct/Cp/Cq value





Assay Formats and Cp Calculation

Absolute/Relative Quantification

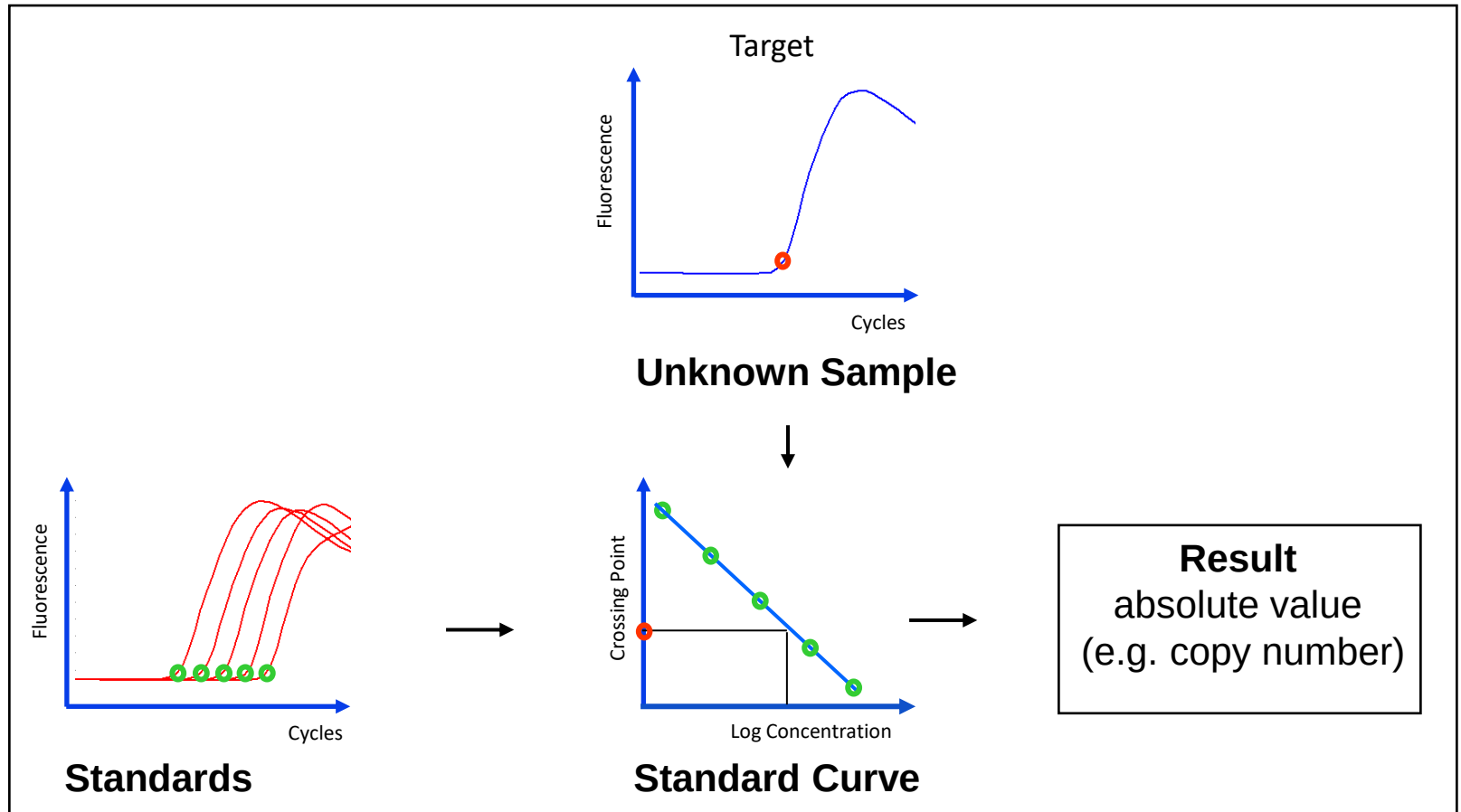
Hardware Overview for LightCycler® Systems

System Operation Procedures

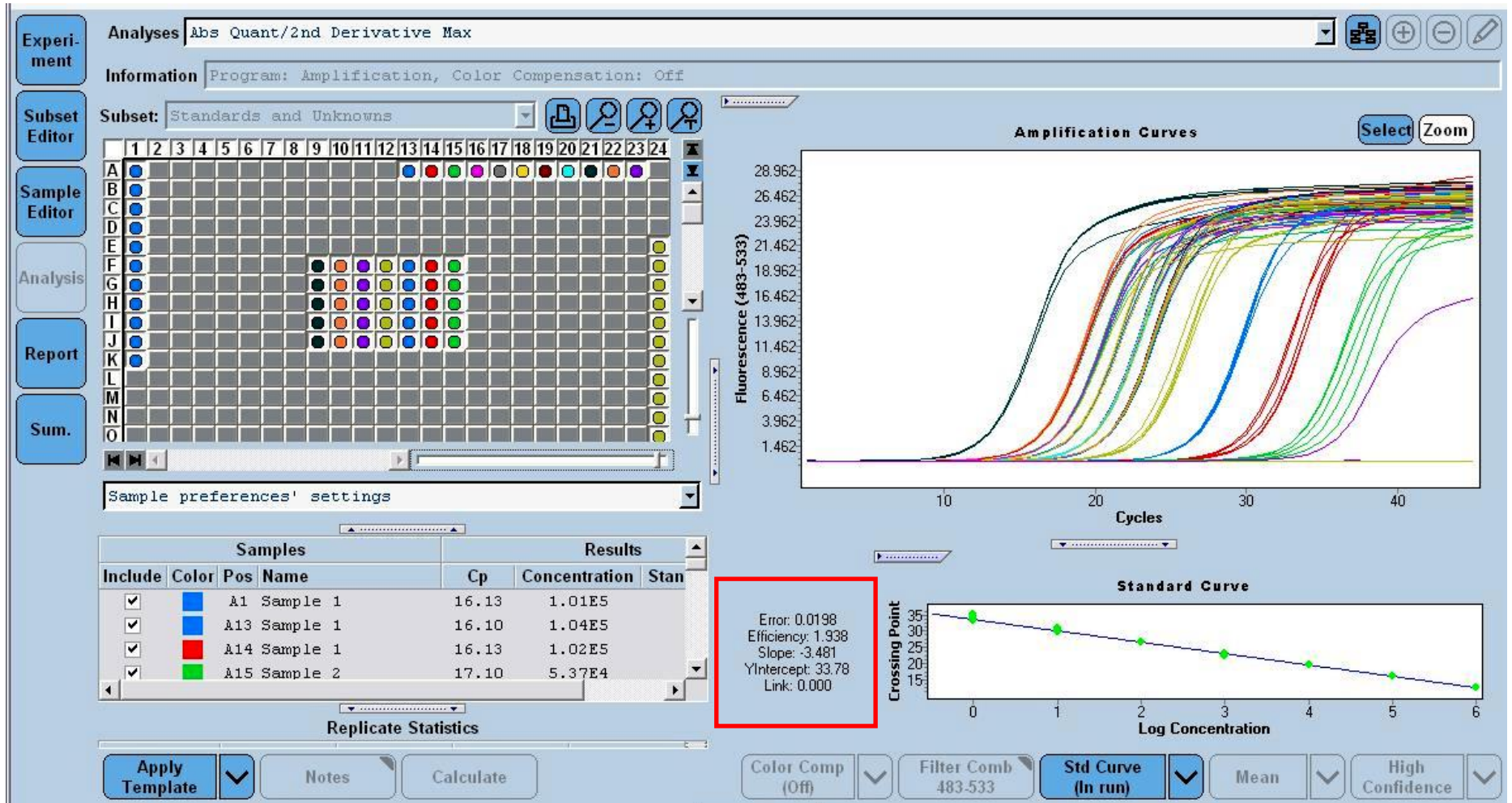
LC 96/LC 480 QC Report

Q&A

Absolute Quantification with External Standards: Principle



Absolute Quantification with External Standards: Example



Relative Quantification *Scheme*

Wanted:

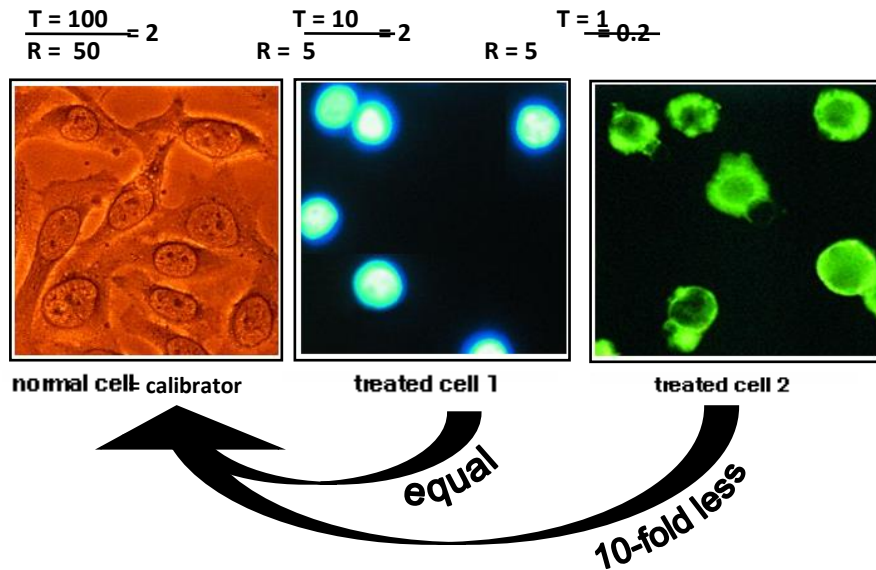
Target gene expression level

Required:

Target gene concentration

Reference gene concentration

Calibrator sample (optional)



Relative Quantification - Without Efficiency correction

$$\text{Relative Ratio} = \frac{\text{concentration of target}}{\text{concentration of reference}}$$

Type	gene	Cp
Sample	Target	22
	Reference	21

$$\frac{2^2}{2^1} \rightarrow \frac{2^{22}}{2^{21}}$$

$$= 2^{22-21} = 2^1 \rightarrow = 2^{-1} = 1/2$$

$$= 2^{\Delta Ct} \rightarrow = 2^{-\Delta Ct}$$

Type	gene	Cp
Calibrator (control)	Target	25
	Reference	20

$$= 2^{25-20} = 2^5 \rightarrow = 2^{-5}$$

$$= 2^{\Delta Ct} \rightarrow = 2^{-\Delta Ct} = 1/32$$

Relative Quantification - Without Efficiency correction

$$\text{Calibrator Normalized Ratio} = \frac{\frac{\text{concentration of target (sample)}}{\text{concentration of reference (sample)}}}{\frac{\text{concentration of target (calibrator)}}{\text{concentration of reference (calibrator)}}}$$

Type	gene	Cp
Sample	Target	22
	Reference	21

$$= 2^{22-21} = 2^1 \rightarrow = 2^{-1} = 1/2$$

$$= 2^{\Delta Ct} \rightarrow = 2^{-\Delta Ct}$$

Type	gene	Cp
Calibrator (control)	Target	25
	Reference	20

$$= 2^{25-20} = 2^5 \rightarrow = 2^{-5} = 1/32$$

$$= 2^{\Delta Ct} \rightarrow = 2^{-\Delta Ct}$$

$$= 2^{-1-(-5)} = 2^4 = 16$$

$$= 2^{-\Delta\Delta Ct}$$

Example for Relative Quantification

Type	gene	Cp
Calibrator	Target	25
	Reference	20
Sample	Target	22
	Reference	21

I. Without efficiency correction

$$\begin{aligned}
 \text{Relative amount} &= 2^{-\Delta\Delta CT} \\
 &= 2^{-[(22-21)-(25-20)]} = 2^{-[1-5]} = 2^4 = 16
 \end{aligned}$$

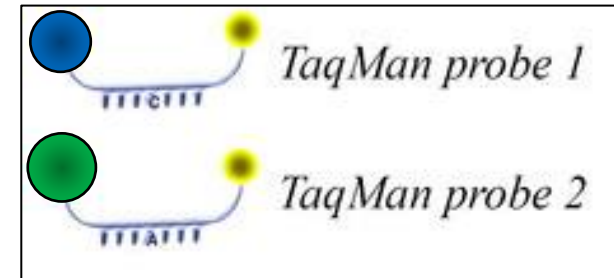
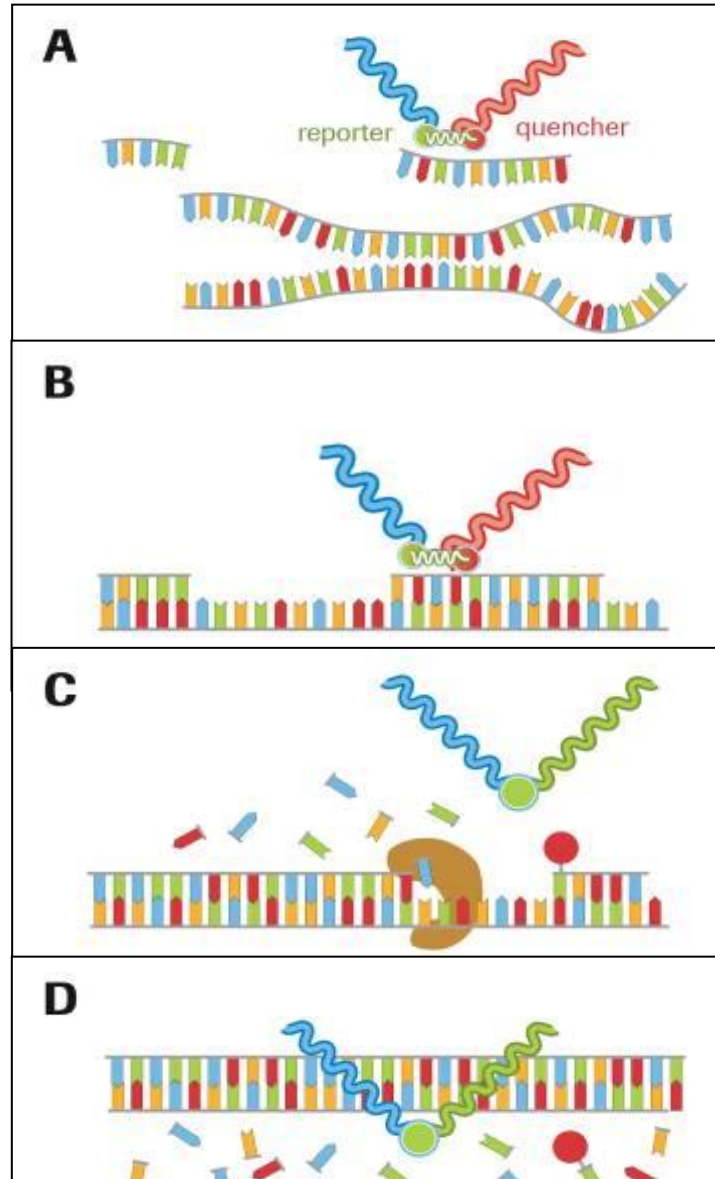
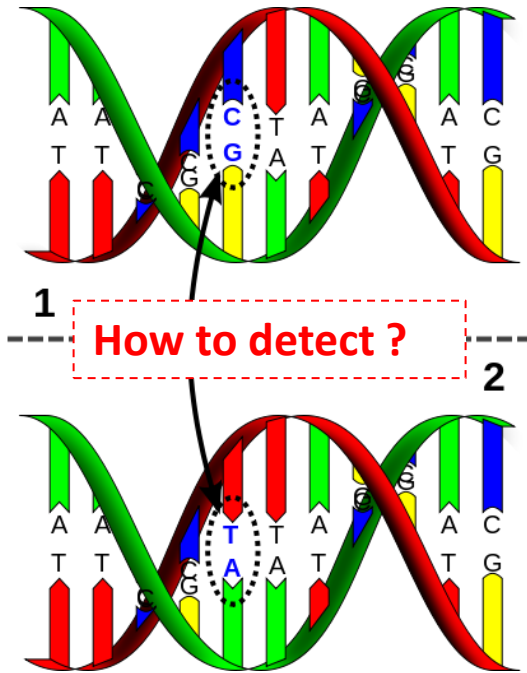
II. With efficiency correction

$$\begin{aligned}
 &E_T = 1.6 \\
 \longrightarrow &E_R = 1.8
 \end{aligned}$$

$$\begin{aligned}
 \text{Relative amount} &= E_T^{CpT(C) - CpT(S)} \times E_R^{CpR(S) - CpR(C)} \\
 &= 1.6^{(25-22)} \times 1.8^{(21-20)} = 1.6^3 \times 1.8^1 = 7.37
 \end{aligned}$$

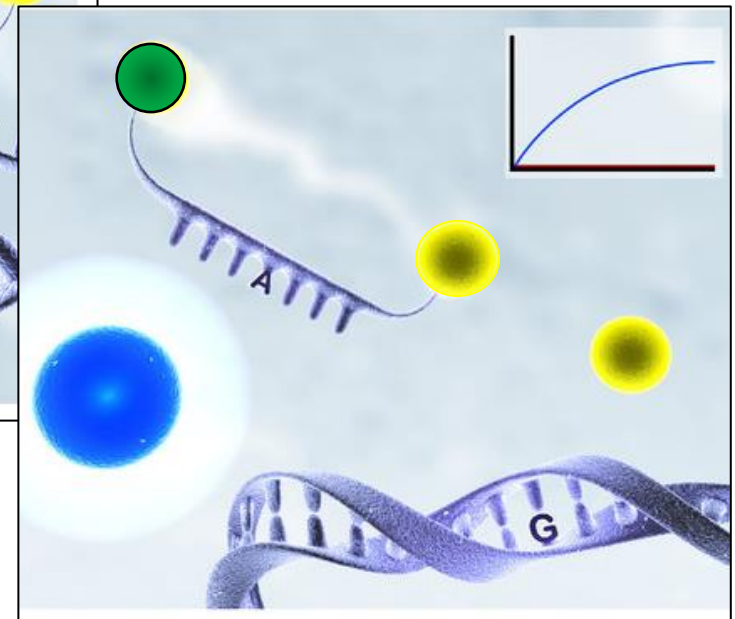
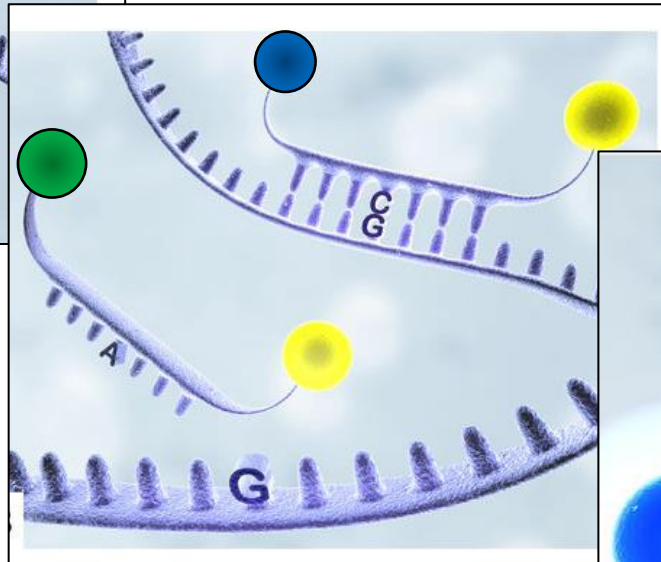
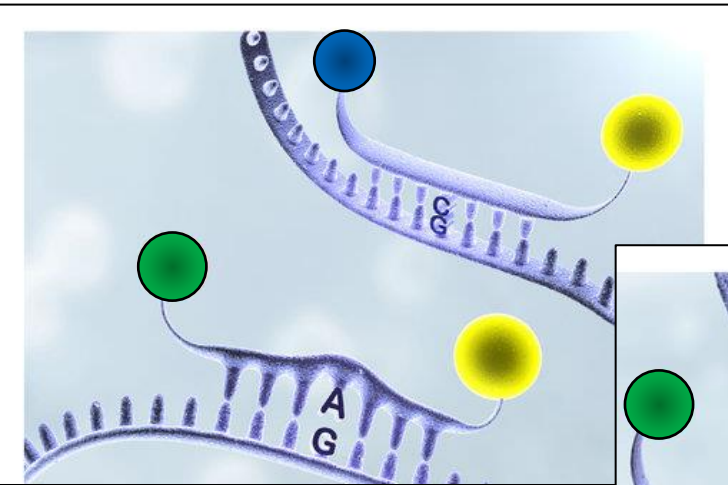
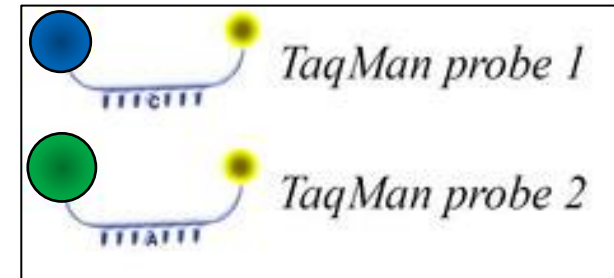
Endpoint Genotyping (allelic discrimination)

Principle



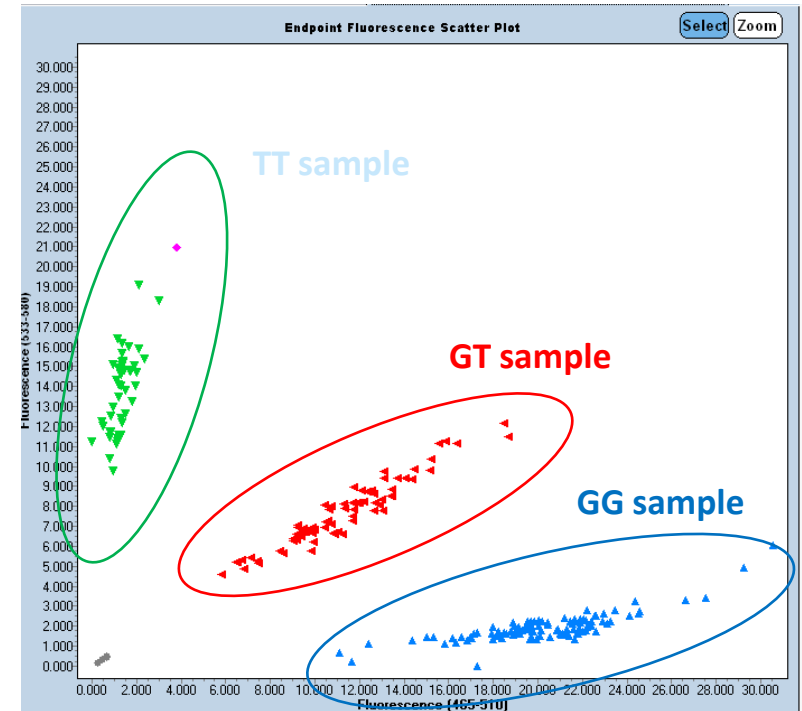
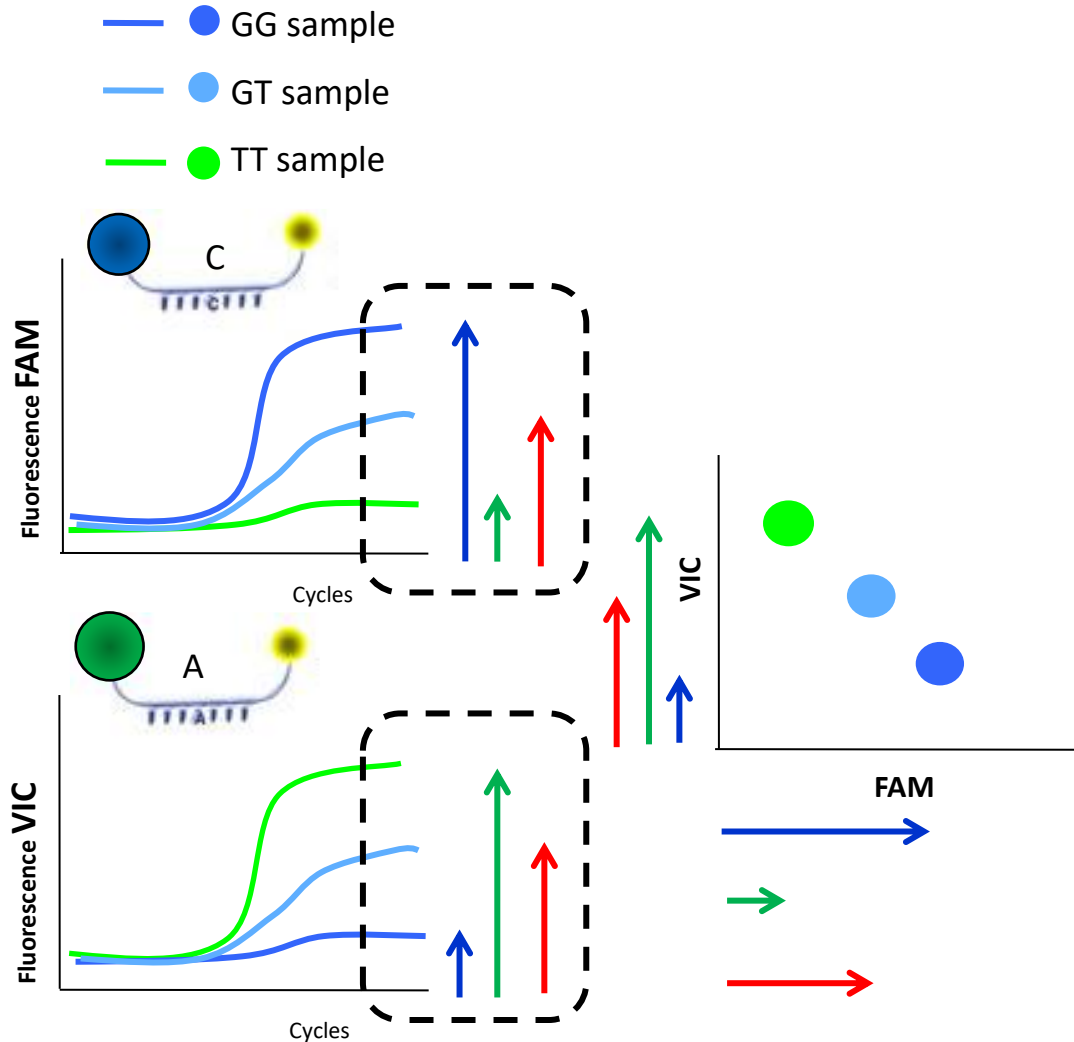
Endpoint Genotyping (allelic discrimination)

Principle



Endpoint Genotyping (allelic discrimination)

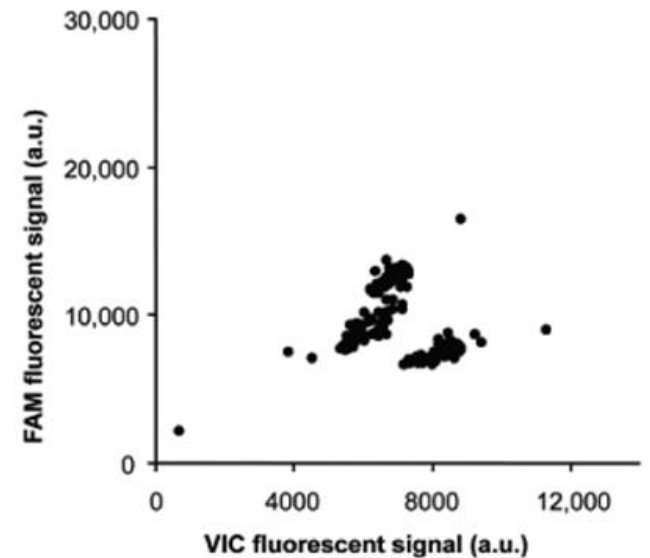
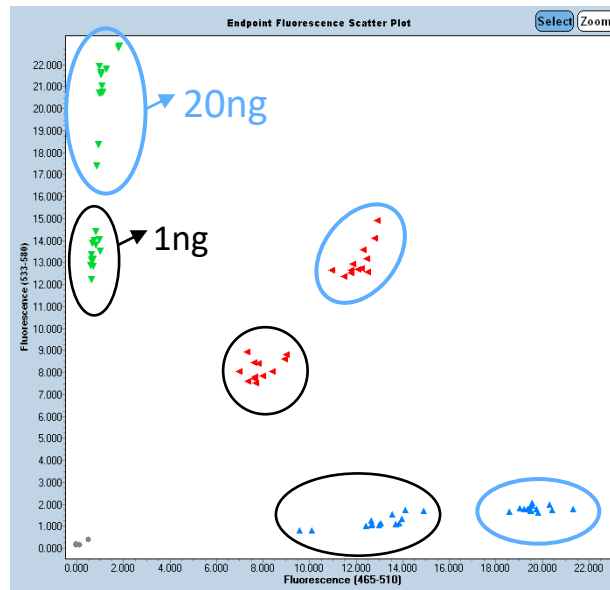
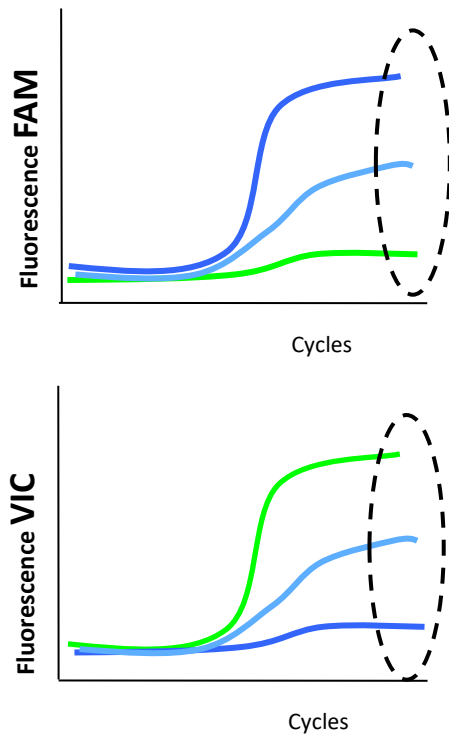
Principle



Endpoint Genotyping Data

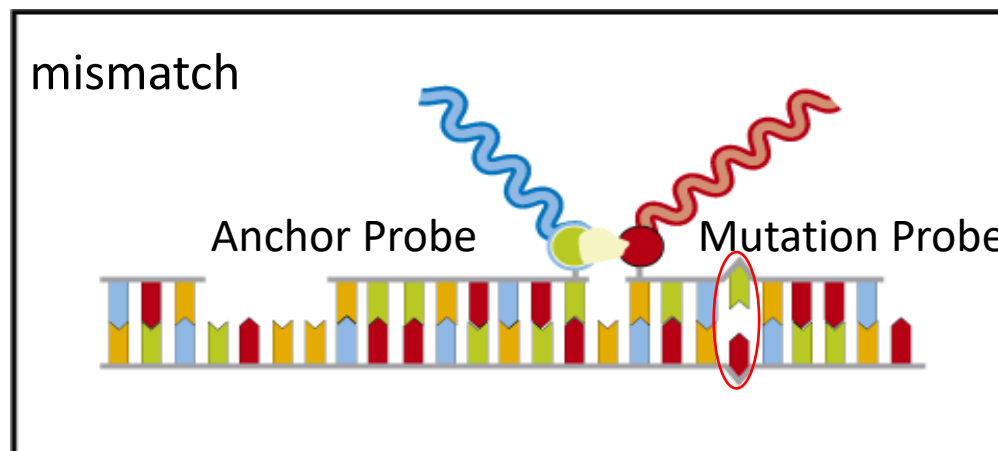
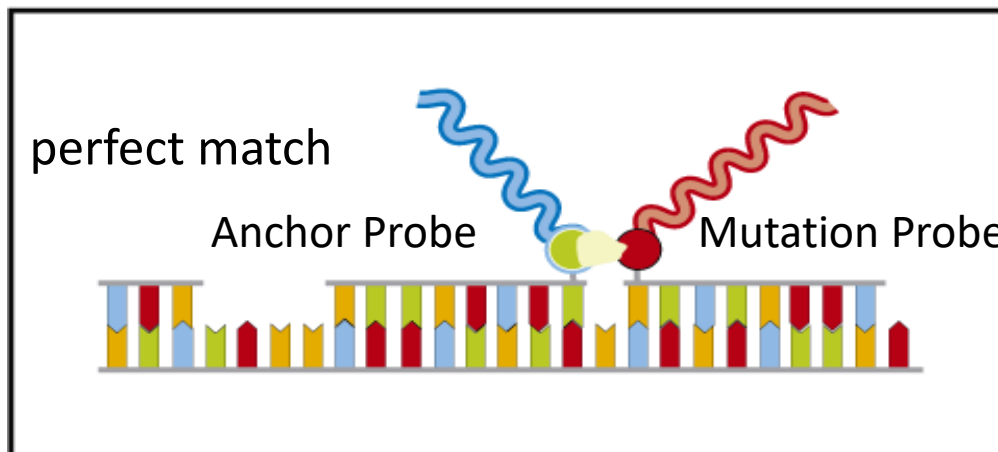
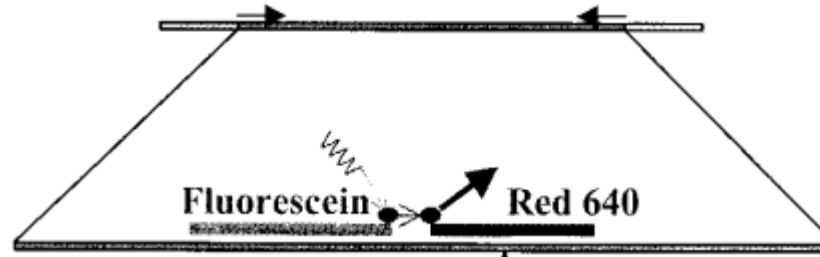
Influence of DNA Concentration

1 and 20 ng DNA



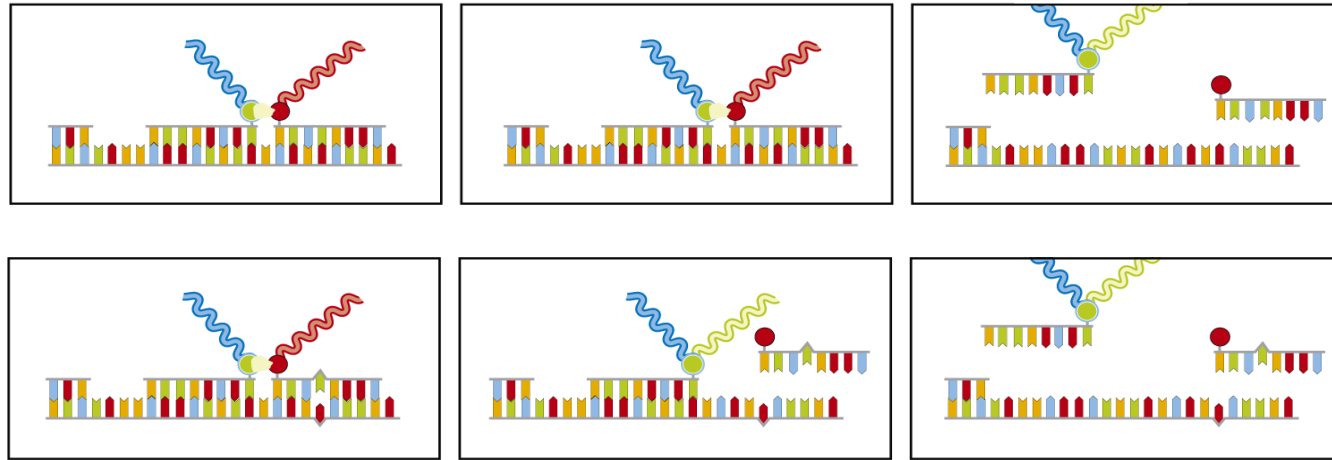
Melting Curve Genotyping

Principle



Melting Curve Genotyping

Principle

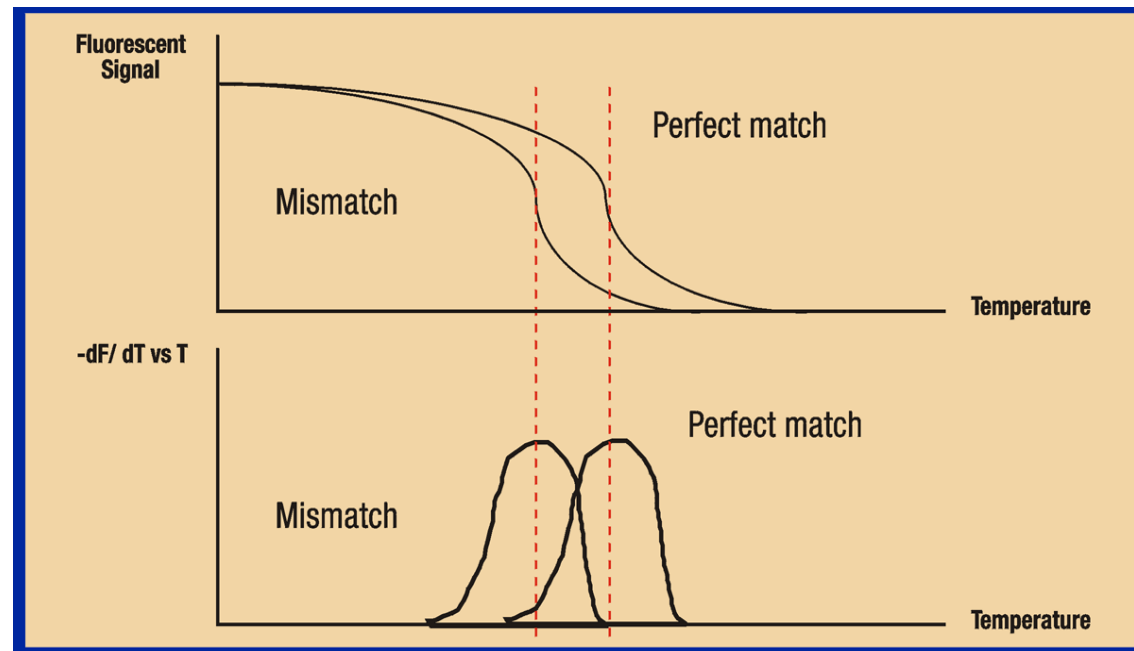


Low

Medium

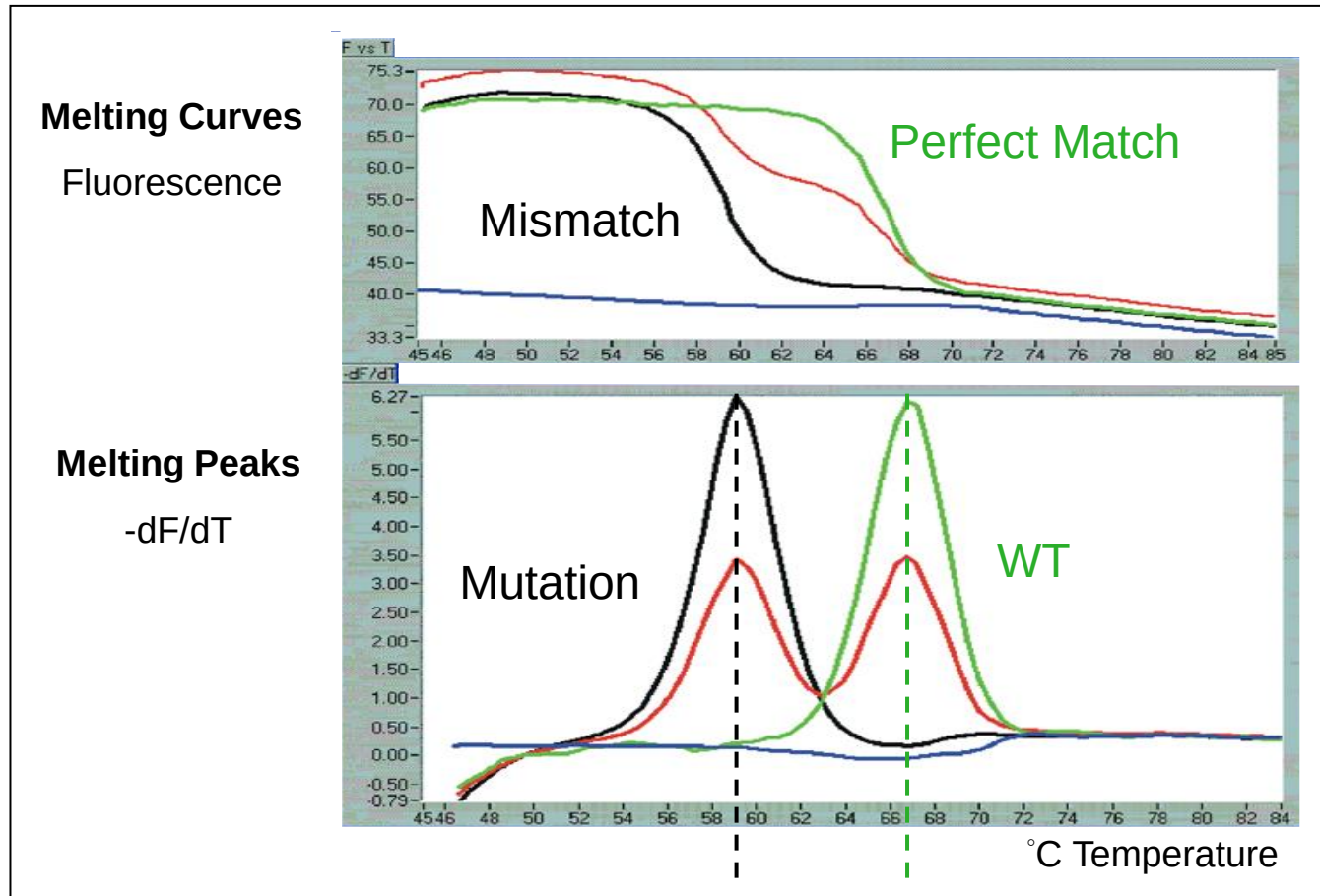
High

Temperature



Melting Curve Based Genotyping

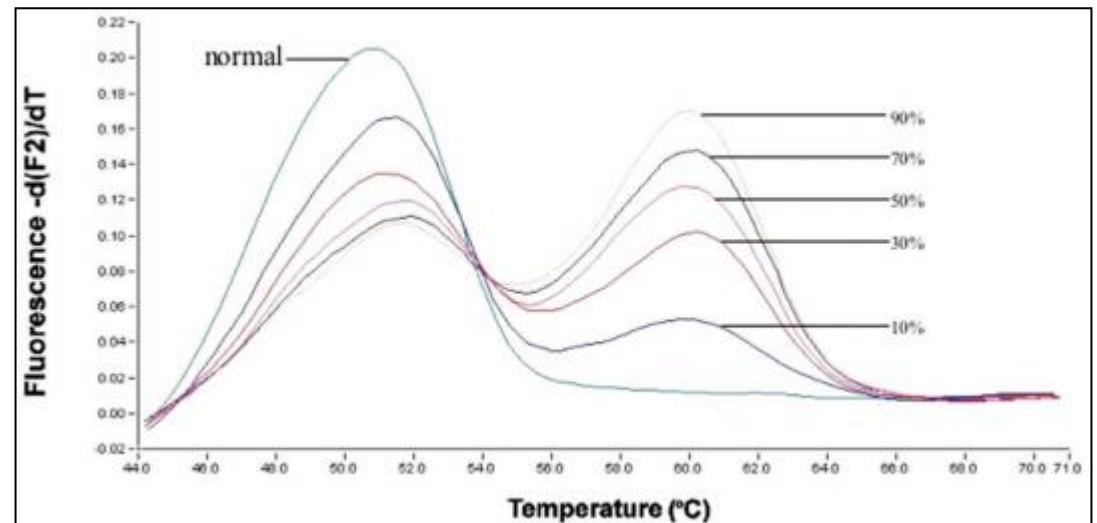
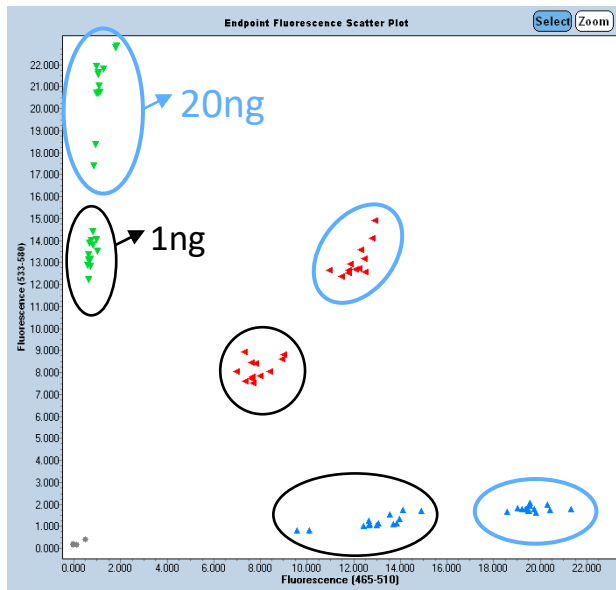
Genotyping of Single Point Mutation



Endpoint & Melting Genotyping Data

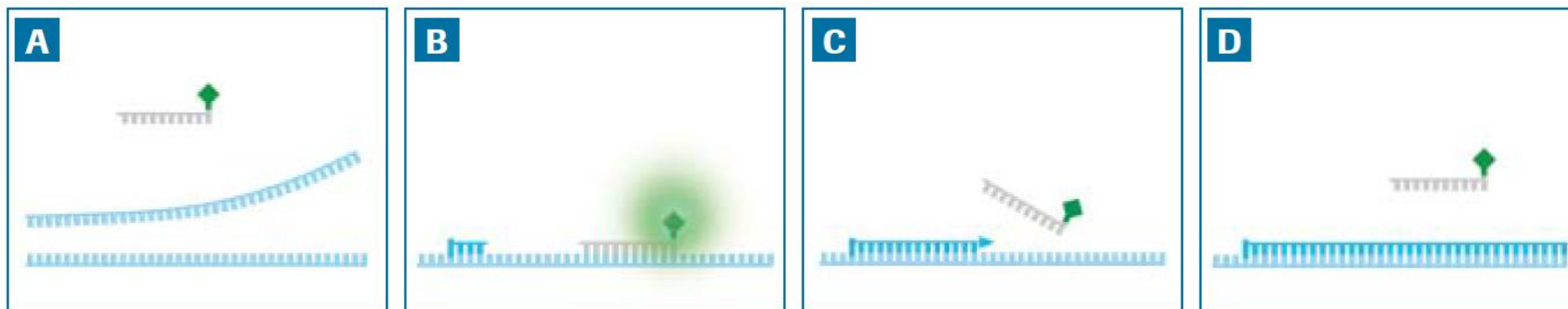
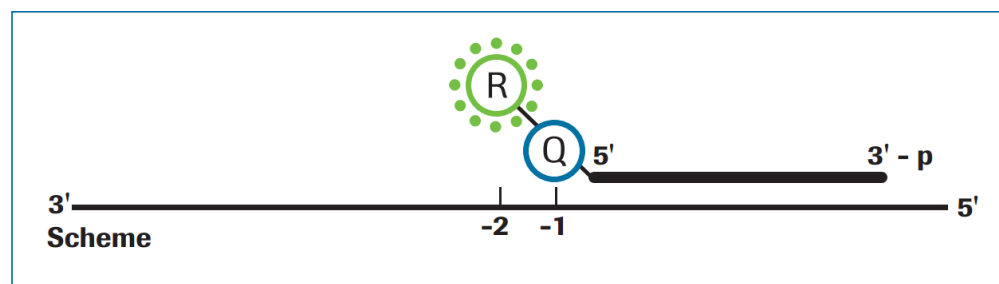
Influence of DNA Concentration

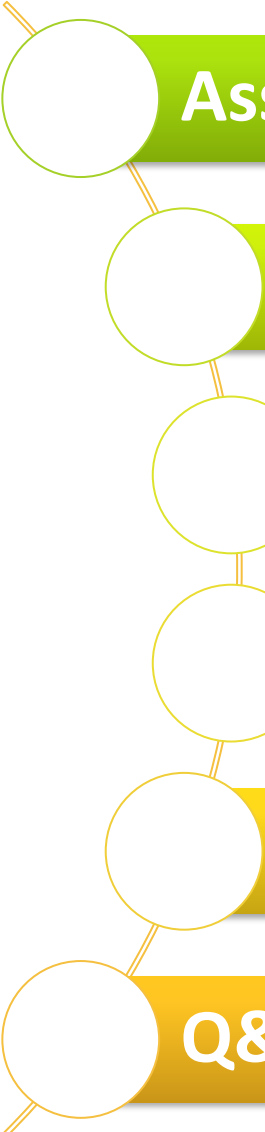
1 and 20 ng DNA



Melting Genotyping Data

SimpleProbe





Assay Formats and Cp Calculation

Absolute/Relative Quantification

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Hardware Overview for LightCycler® Systems



LightCycler 2.0



LightCycler 96

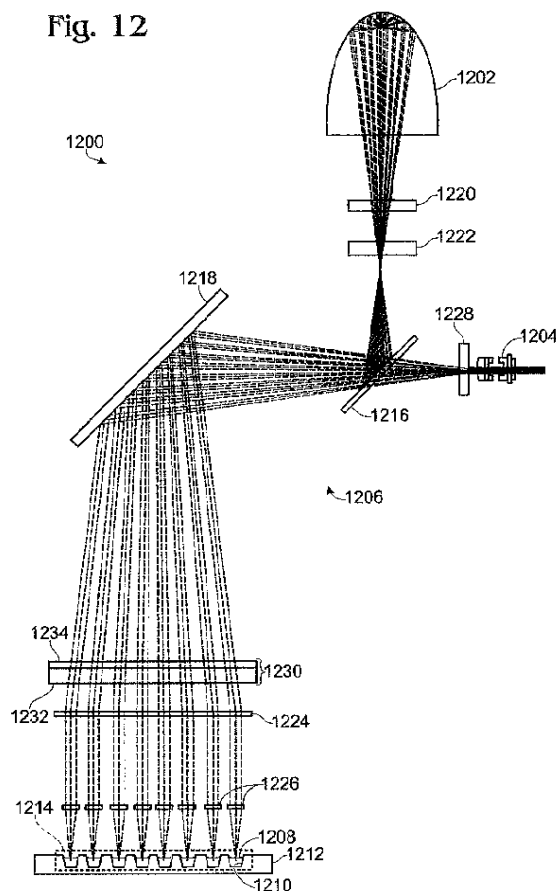


LightCycler 480

Cobas® z 480 analyzer

Data Capture (Other Brand)

Fig. 12



Report signal : SYBR Green 1

Reference signal : ROX

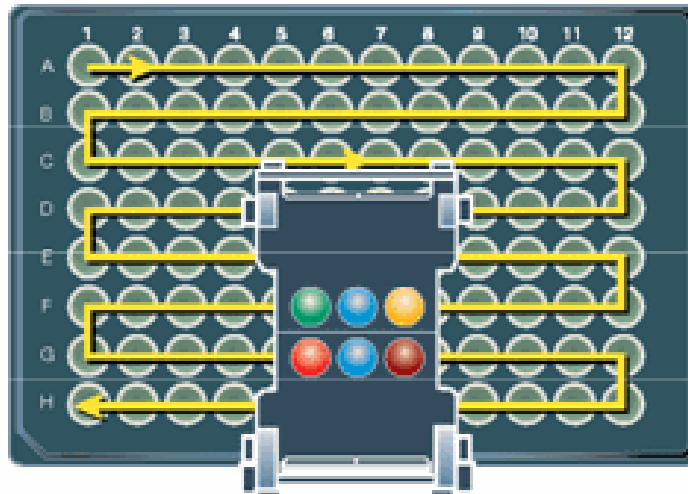
• 校正

(MeltDoctor™ HRM Calibration Plates)

Data Capture (Other Brand)



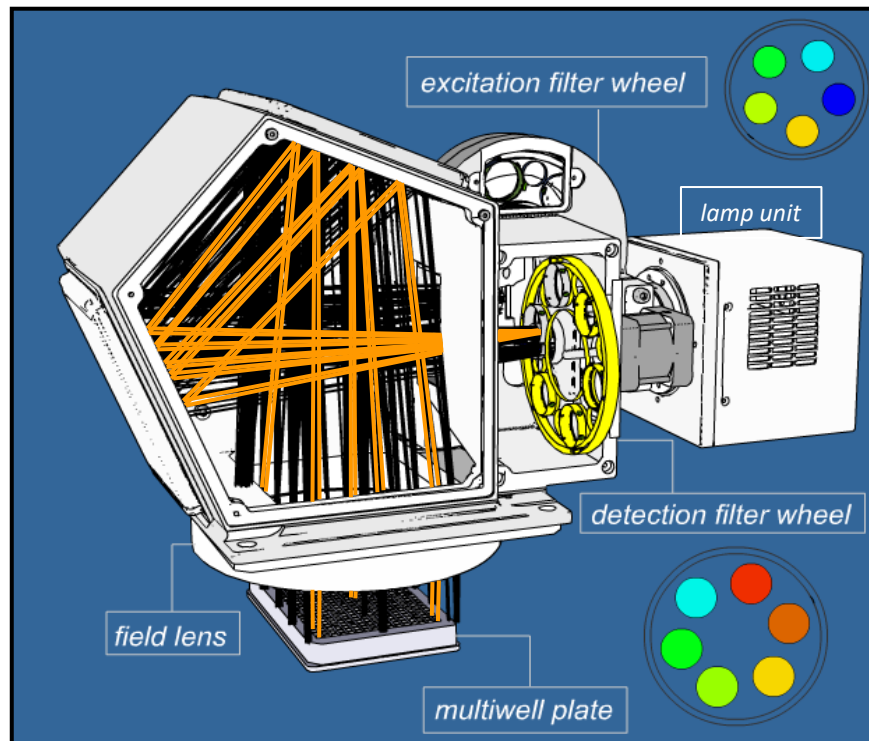
- 非同步
- 時間長



- 校正
(Melt Calibration Kit)

LightCycler® 480 System

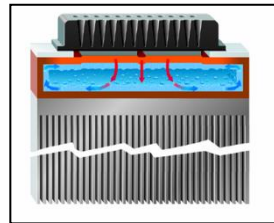
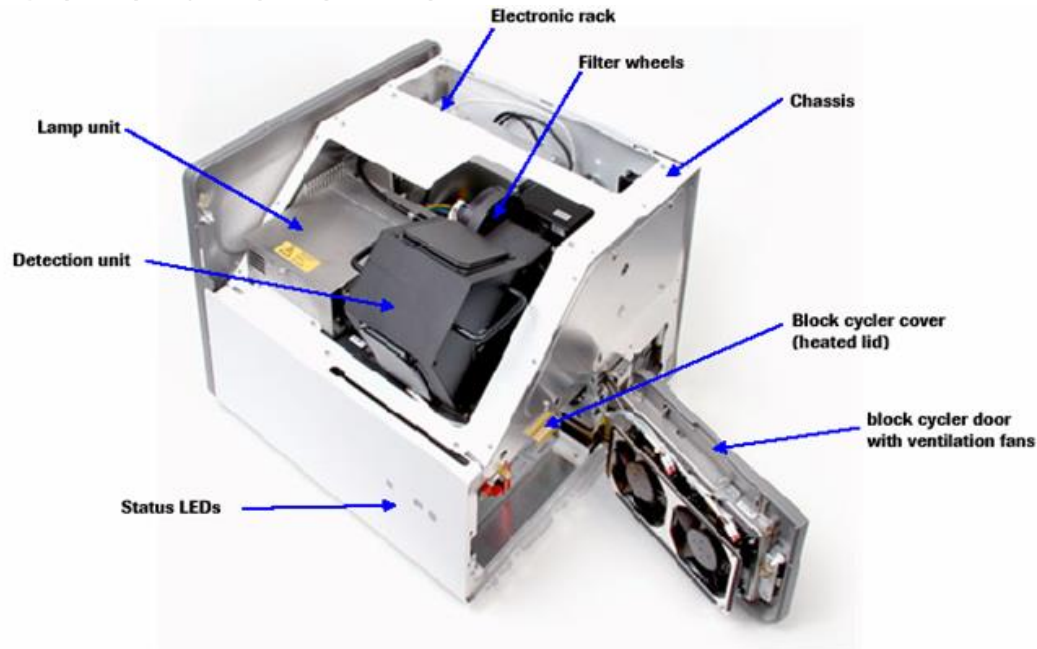
Optical unit and lightpath



- **Xenon lamp/LED***
 - high intensity
 - broad dynamic range
 - lifetime
 - for **Xenon**: approx. 500-1,000 hrs
 - for **LED**: approx. 10,000 hrs
- **CCD camera**
- **Five excitation filters**
- **Six detection filters**
- **Optimized arrangement of optical components**
- **Homogeneous excitation and fluorescence detection**

LightCycler® 480 System

General overview



First version: LightCycler® 480 Instrument I

Xenon Lamp, Filter Set I

Current version: LightCycler® 480 Instrument II

Xenon or LED, Filter Set II

Detection formats:

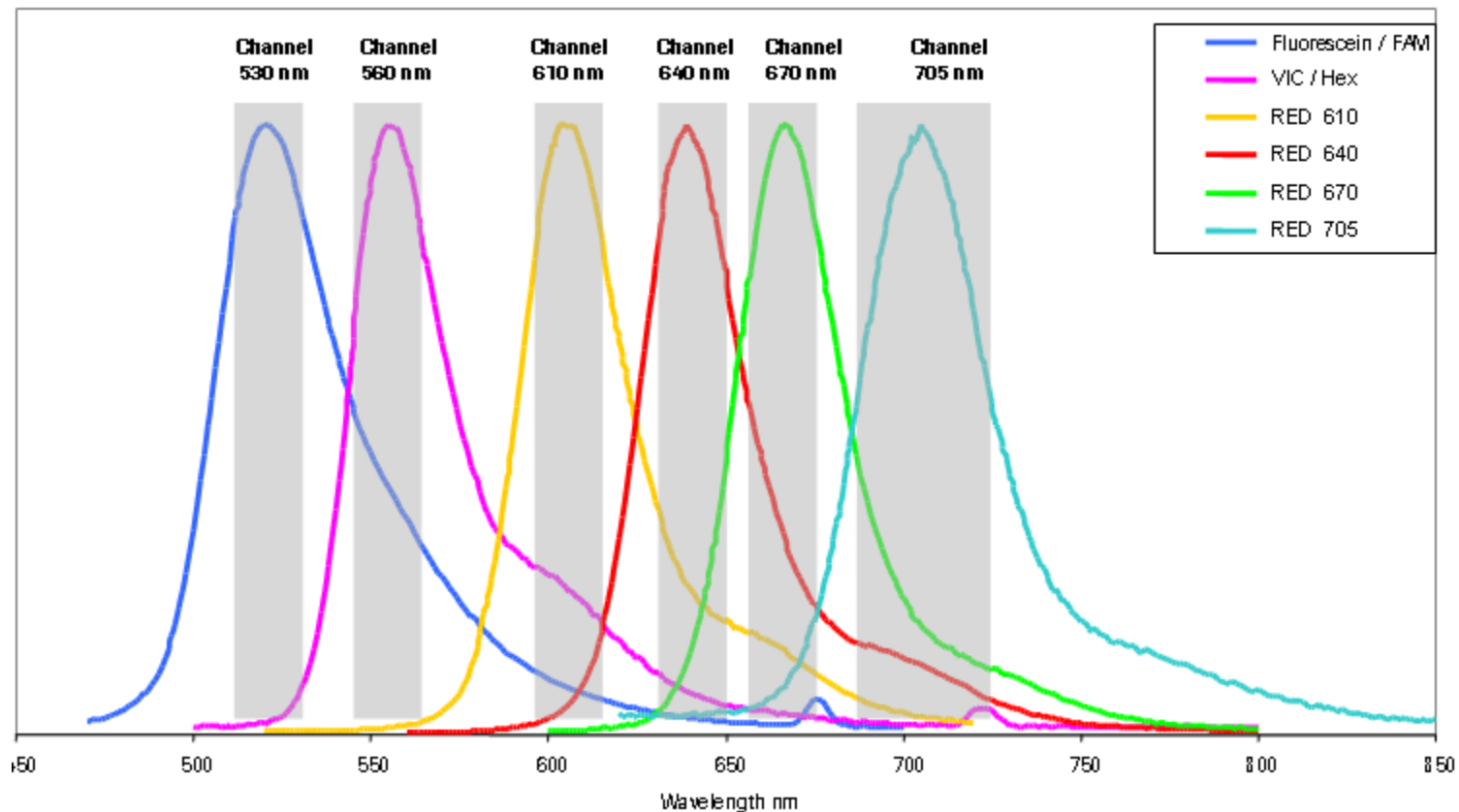
- Intercalating green dyes (e.g., SYBR Green I)
- Hydrolysis / UPL probes
- HybProbe Probes

Exchangeable Block Cycler Unit:

- 96 well
- 384 well

LightCycler® Assay Formats

Dye Emission Spectra



Instrument Introduction

cobas[®] z 480 analyzer Components

LightCycler[®] 480

Excitation	Emission
dark	500/20
450/30	533/20
483/35	568/20
523/20	610/20
558/30	640/20
615/30	670/20

LightCycler[®] 480 II

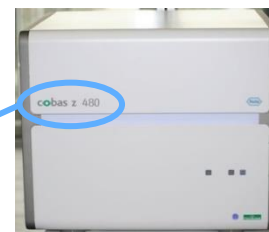
Excitation	Emission
dark	488/20
440/35	510/20
465/25	580/20
498/40	610/20
533/25	640/20
618/35	660 LP

Cobas[®] z 480 analyzer

Excitation	Emission
dark	510/20
465/25	580/20
498/40	610/20
540/10	645/20
610/20	670/20
680/10	700 LP



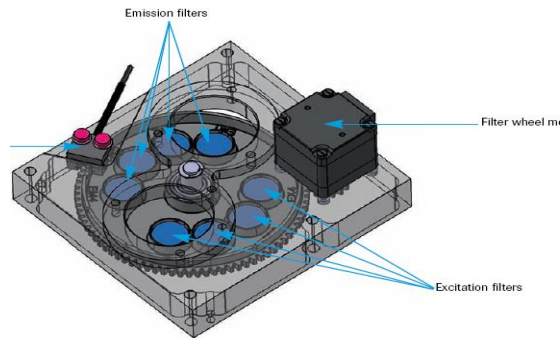
cobas z 480



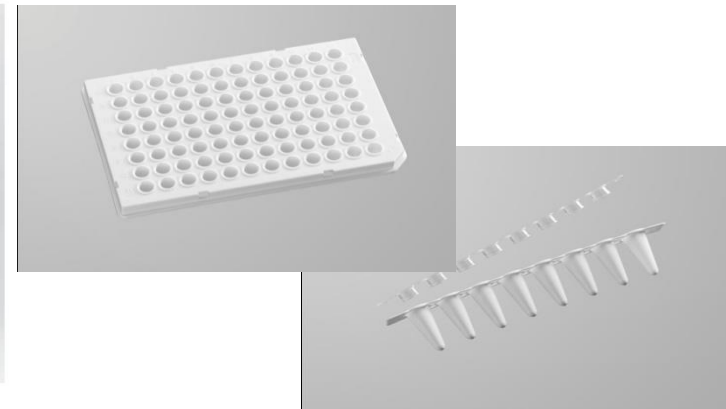
- Xenon lamp
- 96-well version only

LightCycler® 96 System

Overview

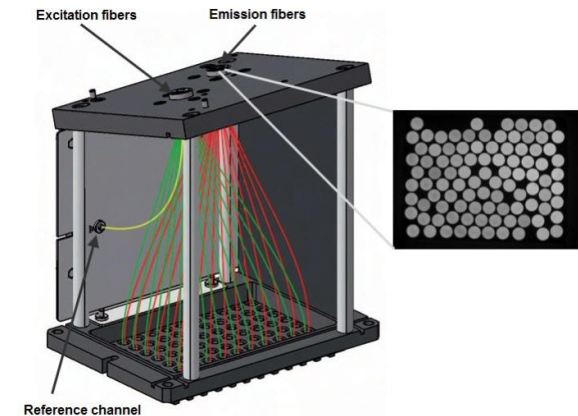
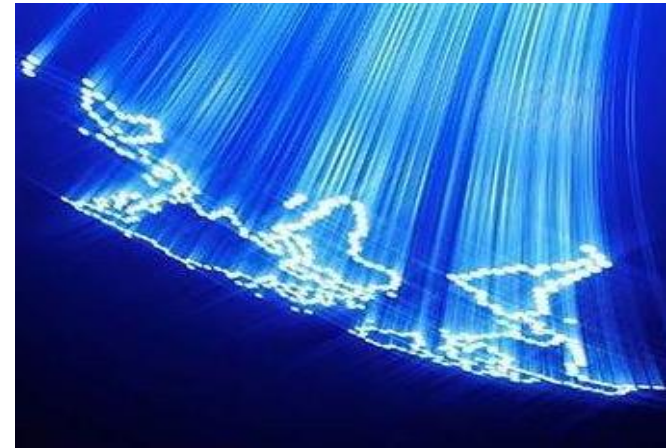


Fluorophore	Excitation filter	Emission filter	Detection format
SYBR Green I ResoLight Dye FAM	470	514	Intercalating dye
VIC HEX Yellow555	533	572	Hydrolysis probes/ Universal ProbeLibrary probes
Red610 Texas Red	577	620	Hydrolysis probes
Cy5	645	697.5	Hydrolysis probes



LightCycler® 96 System

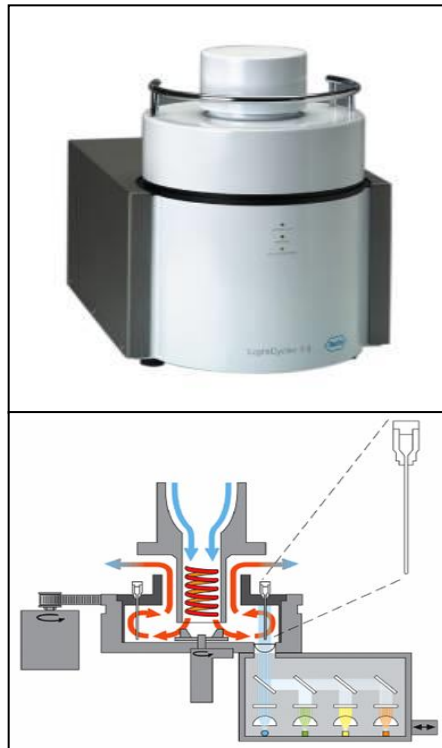
Optical unit and lightpath



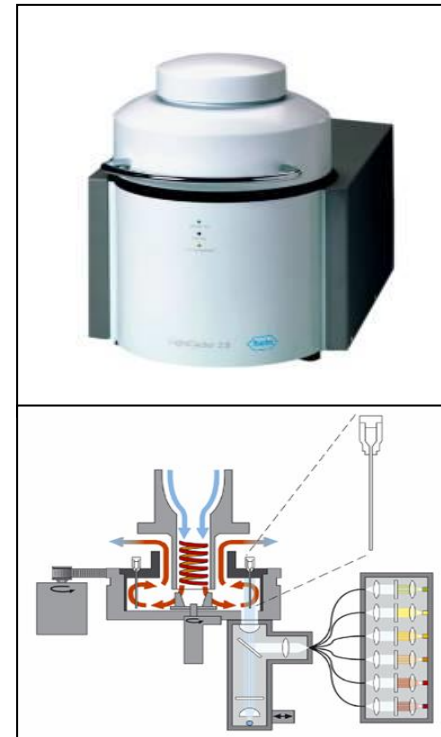
LightCycler® 2.0 and 1.5

Optical unit and lightpath

- LightCycler® 1.5 Instrument



- LightCycler® 2.0 Instrument





Assay Formats and Cp Calculation

Absolute/Relative Quantification

Hardware Overview for LightCycler® Systems

System Operation Procedures

LC 96/LC 480 QC Report

Q&A

LightCycler[®] 480 Real-Time PCR System



System Start-Up



左 警 示 燈	右 警 示 燈	機 器 狀 態
橘 *閃爍*	橘 *閃爍*	正 在 初 始 化
綠	橘	機 器 啟 動 完 成 ， 96/384 孔 盤 還 未 放 入
綠	橘 *閃爍*	96/384 孔 盤 正 在 放 入 中
綠	綠	機 器 啟 動 完 成 ， 96/384 孔 盤 已 經 放 入
綠 *閃爍*	綠 *閃爍*	實 驗 進 行 中

System Start-Up

- ▶ To load the prepared multiwell plate into the LightCycler[®] 480 Instrument, press the push button on the front of the instrument (located next to the instrument status LEDs):



Place the multiwell plate into the loading frame of the loader with the flat edge pointing towards the instrument. (The short plate edge with beveled corners points away from the instrument.)



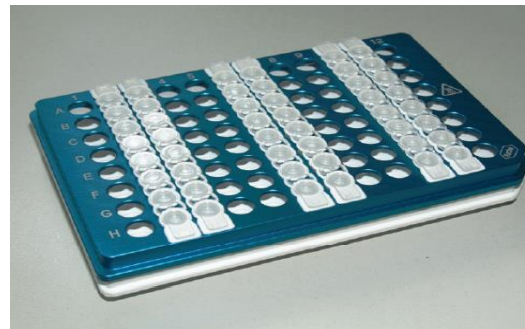
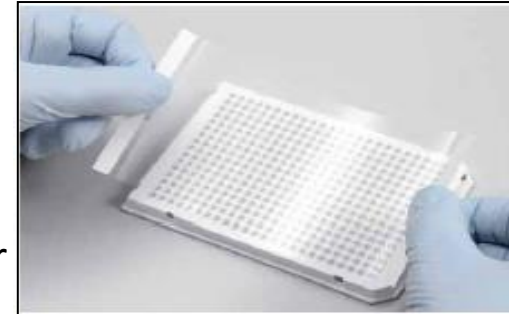
Press the plate loading push button again to retract the loader with the inserted multiwell plate into the instrument. You are now ready to start the run.

LightCycler® 480 System

Disposables

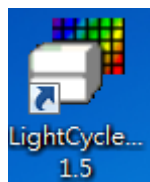


96-well plate for
10–100 µl



LightCycler® 8-Tube Strips (white and clear)

Open the software and Login to the data base



Instrument: No active instrument Database:

Window: [dropdown] User:

For PC login
ID: operator
PW: LC480

Login

User name: * [input field] ← admin

Password: * [input field] ← LightCycler480

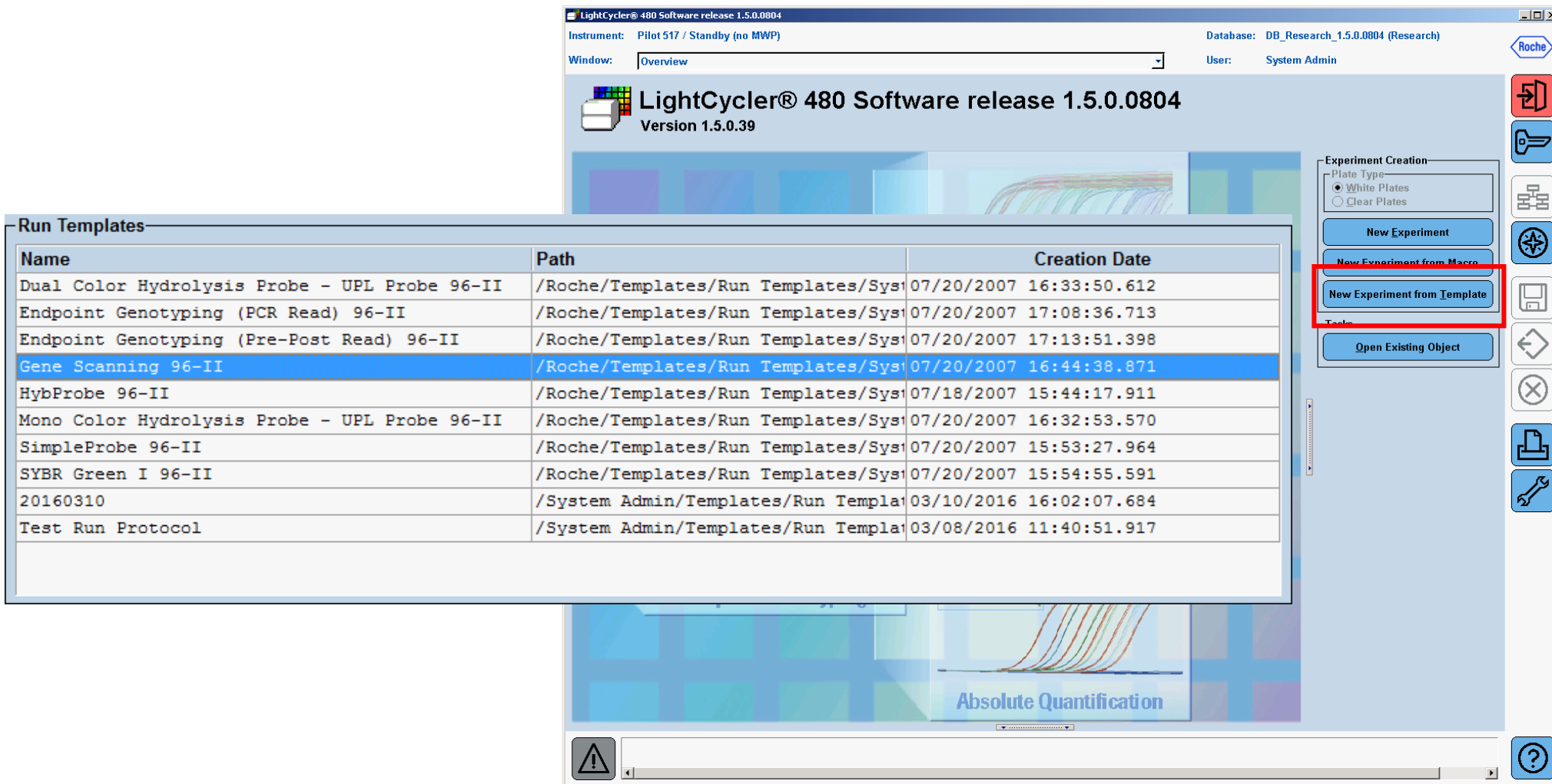
Log on to: * [dropdown menu: Research Database]

[icon] Options >> [checkmark] [X]

53

LightCycler® 480 Software Version 1.5

Front screen: New experiment from template



The screenshot displays the LightCycler® 480 Software release 1.5.0.0804 interface. The main window shows the 'Run Templates' list, which is a table of templates. The 'Gene Scanning 96-II' template is selected. On the right side, the 'Experiment Creation' panel is visible, showing options for 'Plate Type' (White Plates, Clear Plates) and buttons for 'New Experiment', 'New Experiment from Macro', 'New Experiment from Template' (highlighted with a red box), and 'Open Existing Object'.

Name	Path	Creation Date
Dual Color Hydrolysis Probe - UPL Probe 96-II	/Roche/Templates/Run Templates/Sys	07/20/2007 16:33:50.612
Endpoint Genotyping (PCR Read) 96-II	/Roche/Templates/Run Templates/Sys	07/20/2007 17:08:36.713
Endpoint Genotyping (Pre-Post Read) 96-II	/Roche/Templates/Run Templates/Sys	07/20/2007 17:13:51.398
Gene Scanning 96-II	/Roche/Templates/Run Templates/Sys	07/20/2007 16:44:38.871
HybProbe 96-II	/Roche/Templates/Run Templates/Sys	07/18/2007 15:44:17.911
Mono Color Hydrolysis Probe - UPL Probe 96-II	/Roche/Templates/Run Templates/Sys	07/20/2007 16:32:53.570
SimpleProbe 96-II	/Roche/Templates/Run Templates/Sys	07/20/2007 15:53:27.964
SYBR Green I 96-II	/Roche/Templates/Run Templates/Sys	07/20/2007 15:54:55.591
20160310	/System Admin/Templates/Run Templa	03/10/2016 16:02:07.684
Test Run Protocol	/System Admin/Templates/Run Templa	03/08/2016 11:40:51.917

The 'Experiment Creation' panel on the right includes the following options:

- Plate Type:
 - ☒ White Plates
 - ☐ Clear Plates
- New Experiment
- New Experiment from Macro
- New Experiment from Template** (highlighted)
- Open Existing Object

Start Run



Experiment

Subset Editor

Sample Editor

Analysis

Report

Run Protocol	Data	Run Notes																
Setup Detection Format SYBR Green I / HI Customize Block Size 96 Plate ID Reaction Volume 20 Color Comp ID Lot No Test ID 																		
Programs <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Program Name</th> <th>Cycles</th> <th>Analysis Mode</th> </tr> </thead> <tbody> <tr> <td>pre-incubation</td> <td>1</td> <td>None</td> </tr> <tr> <td>amplification</td> <td>45</td> <td>Quantification</td> </tr> <tr> <td>melting curve</td> <td>1</td> <td>Melting Curves</td> </tr> <tr> <td>cooling</td> <td>1</td> <td>None</td> </tr> </tbody> </table>			Program Name	Cycles	Analysis Mode	pre-incubation	1	None	amplification	45	Quantification	melting curve	1	Melting Curves	cooling	1	None	
Program Name	Cycles	Analysis Mode																
pre-incubation	1	None																
amplification	45	Quantification																
melting curve	1	Melting Curves																
cooling	1	None																
Temperature Targets <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Target (°C)</th> <th>Acquisition Mode</th> <th>Hold (hh:mm:ss)</th> <th>Ramp Rate (°C/s)</th> <th>Acquisitions (per °C)</th> <th>Sec Target (°C)</th> <th>Step Size (°C)</th> <th>Step Delay (cycles)</th> </tr> </thead> <tbody> <tr> <td>95</td> <td>None</td> <td>00:05:00</td> <td>4.4</td> <td></td> <td>0</td> <td>0</td> <td>0</td> </tr> </tbody> </table>			Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)	95	None	00:05:00	4.4		0	0	0
Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)											
95	None	00:05:00	4.4		0	0	0											
Overview																		
Apply Template Save As Template End Program + 10 Cycles Start Run																		

Start Run



Experiment

Subset Editor

Sample Editor

Analysis

Report

Run Protocol	Data	Run Notes																																
Setup Detection Format SYBR Green I / Customize Block Size 96 Plate ID Reaction Volume 20																																		
Color Comp ID Lot No Test ID																																		
Programs <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Program Name</th> <th>Cycles</th> <th>Analysis Mode</th> </tr> </thead> <tbody> <tr> <td>pre-incubation</td> <td>1</td> <td>None</td> </tr> <tr> <td>amplification</td> <td>45</td> <td>Quantification</td> </tr> <tr> <td>melting curve</td> <td>1</td> <td>Melting Curves</td> </tr> <tr> <td>cooling</td> <td>1</td> <td>None</td> </tr> </tbody> </table>			Program Name	Cycles	Analysis Mode	pre-incubation	1	None	amplification	45	Quantification	melting curve	1	Melting Curves	cooling	1	None																	
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amplification Temperature Targets <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Target (°C)</th> <th>Acquisition Mode</th> <th>Hold (hh:mm:ss)</th> <th>Ramp Rate (°C/s)</th> <th>Acquisitions (per °C)</th> <th>Sec Target (°C)</th> <th>Step Size (°C)</th> <th>Step Delay (cycles)</th> </tr> </thead> <tbody> <tr> <td>95</td> <td>None</td> <td>00:00:10</td> <td>4.4</td> <td></td> <td>0</td> <td>0</td> <td>0</td> </tr> <tr> <td>60</td> <td>None</td> <td>00:00:10</td> <td>2.2</td> <td></td> <td>0</td> <td>0</td> <td>0</td> </tr> <tr> <td>72</td> <td>Single</td> <td>00:00:10</td> <td>4.4</td> <td></td> <td>0</td> <td>0</td> <td>0</td> </tr> </tbody> </table>			Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)	95	None	00:00:10	4.4		0	0	0	60	None	00:00:10	2.2		0	0	0	72	Single	00:00:10	4.4		0	0	0
Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)																											
95	None	00:00:10	4.4		0	0	0																											
60	None	00:00:10	2.2		0	0	0																											
72	Single	00:00:10	4.4		0	0	0																											
Overview																																		
Apply Template End Program + 10 Cycles Start Run																																		

Start Run

Experiment

Subst Editor

Sample Editor

Analysis

Report

Run Protocol

Setup

Detection Format: SYBR Green I / HI **Customize** Block Size: 96 Plate ID: []

Color Comp ID: [] Lot No: [] Test I: []

Programs

Program Name
pre-incubation
amplification
melting curve
cooling

Temperature Targets

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)
95	None	00:05:00	4.4	

Overview

0:00:00 0:05:34 0:13:59 0:22:24 0:30:53 0:39:18

Estimated Time (h:mm:ss)

Save Template

123

- System Admin
 - Experiments
 - Macros
 - Preferences
 - Special Data
 - Templates
 - Analysis Templates
 - Report Templates
 - Run Templates**
- 0306
- 0409
- 111
- 123
- 20131001
- 20131024
- 20131108
- New Experiment Run Protocol
- Sample Templates

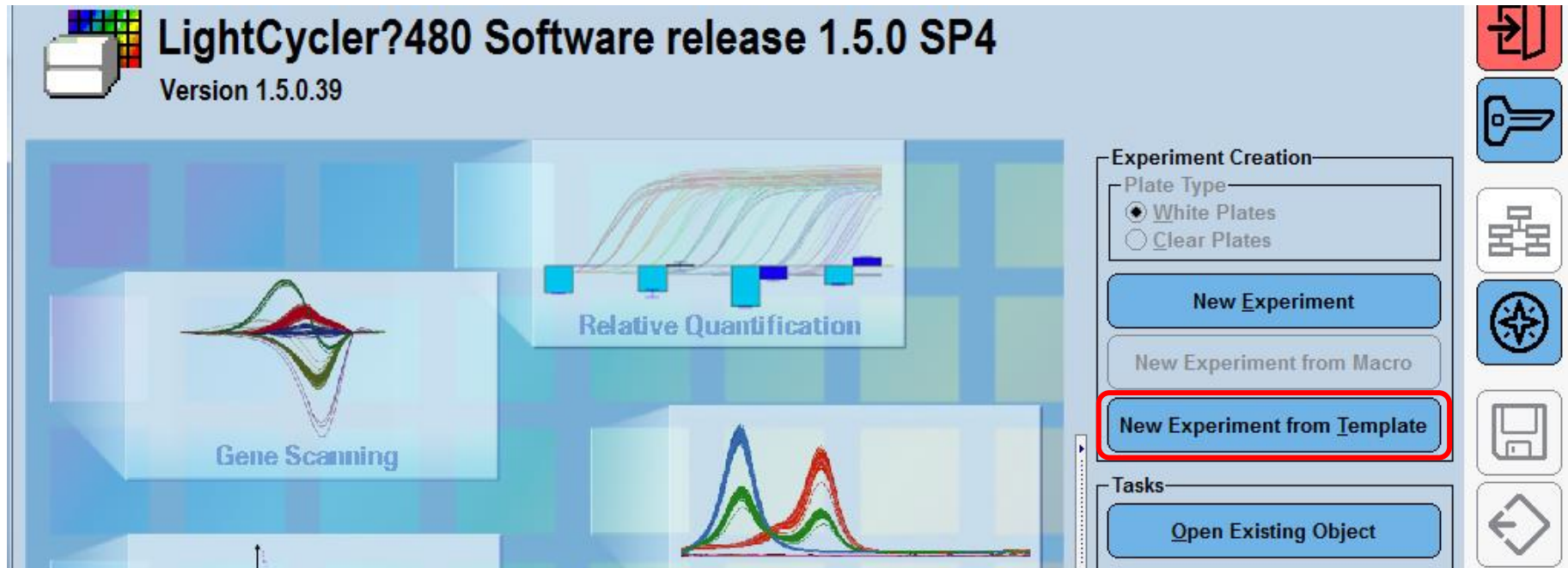
Name: Give me a name

Apply Template

Save As Template

End Program **+ 10 Cycles** **Start Run**

Start your experiment by your template



Subset



Experiment

Subset Editor

Sample Editor

Analysis

Report

Step 2

Subsets

ID	Name	Analysis	Report
1	All Samples	☒	☒

All Samples settings

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

+

-

Copy

Rename

Step 3

Apply

Clear

Cancel

Step 1

Sample Editor – AQ example

Experiment

Subset Editor

Sample Editor

Analysis

Report

Step 1: Select Workflow

☒ Abs Quant
 ☐ Rel Quant
 ☐ Scanning
 ☐ Color Comp
☐ Tm
 ☐ Melt Geno
 ☐ Endpt Geno

Step 2: Select Samples

Subset: All Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	U	U	U	U	U	U	U	U	U	U	U	U
B	U	U	U	U	U	U	U	U	U	U	U	U
C	U	U	U	U	U	U	U	U	U	U	U	U
D	U	U	U	U	U	U	U	U	U	U	U	U
E	U	U	U	U	U	U	U	U	U	U	U	U
F	U	U	U	U	U	U	U	U	U	U	U	U
G	U	U	U	U	U	U	U	U	U	U	U	U

Step 3: Edit Abs Quant Properties

Sample Name

Sample Type

☒ Unknown
 ☐ Negative Control
☐ Positive Control/Calibrator
☐ Standard Concentration

Auto Std Curve

Make Replicates

Auto Replicate

Clear Replicates

RQ example

Step 1: Select Workflow

☐ Abs Quant
 ☒ Rel Quant
 ☐ Scanning
 ☐ Color Comp
☐ Tm
 ☐ Melt Geno
 ☐ Endpt Geno

Step 2: Select Samples

Subset: All Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	U	U	U	U	U	U	U	U	U	U	U	U
B	U	U	U	U	U	U	U	U	U	U	U	U

Step 3: Edit Rel Quant Properties

Sample Name

Sample Type

☒ Unknown
 ☐ Negative Control
☐ Positive Control/Calibrator
☐ Standard Concentration

Auto Std Curve

Gene target

Target name

Eff 2.00

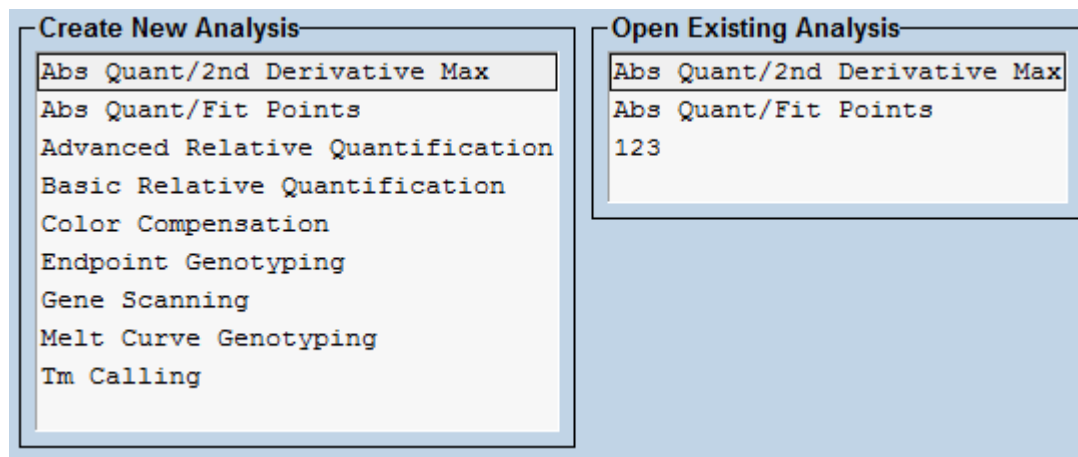
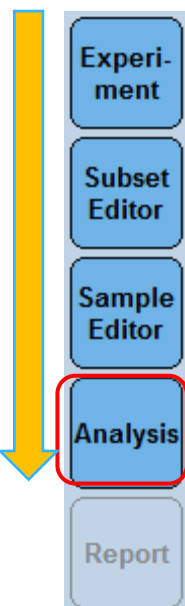
☐ Target
 ☐ Reference
 ☒ Unassigned

Make Replicates

Auto Replicate

Clear Replicates

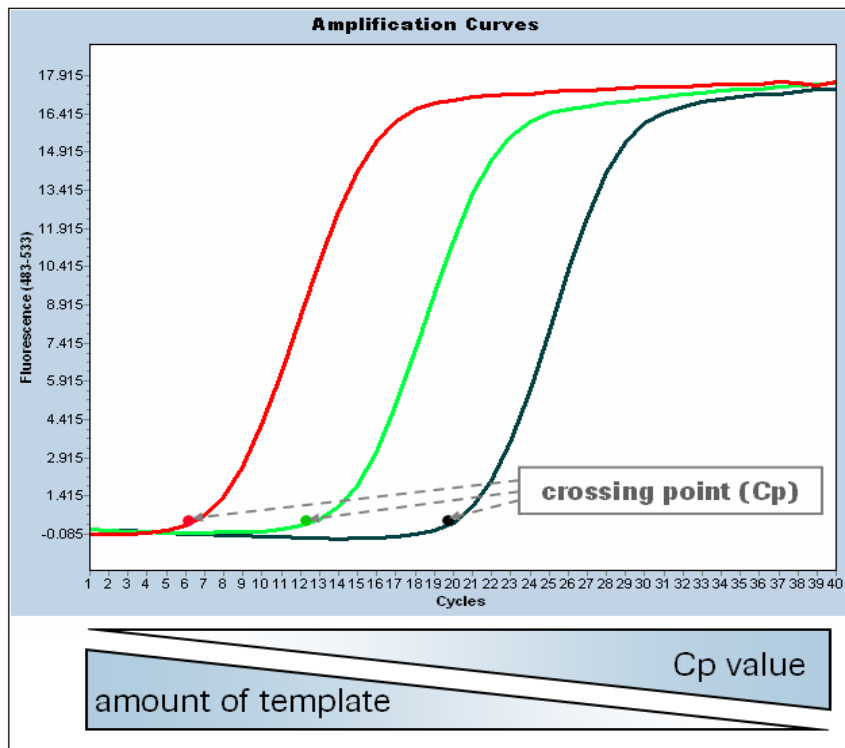
Analysis



分析模式簡要說明：

分析模式	說明
Abs Quant / 2nd Derivative Max	絕對定量 (2 nd Max)
Abs Quant / Fit Points	絕對定量 (Fit Point)
Advanced Relative Quantification	進階相對定量(2 nd Max or Fit Point)
Basic Relative Quantification	基礎相對定量 (Fit Point only)
Endpoint Genotyping	基因分型(適用於TaqMan probe)
Melt Curve Genotyping	基因分型(適用於Hyprobe)
Tm Calling	Tm分析

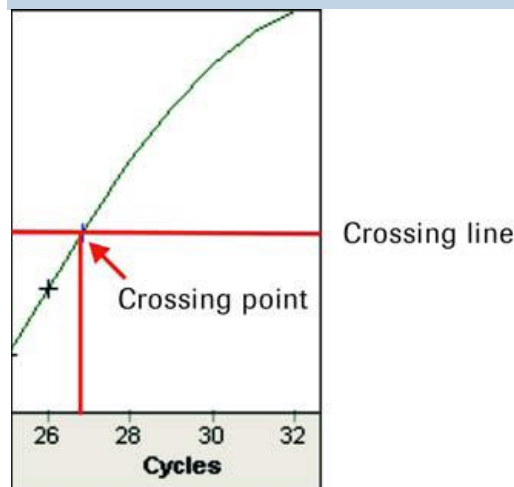
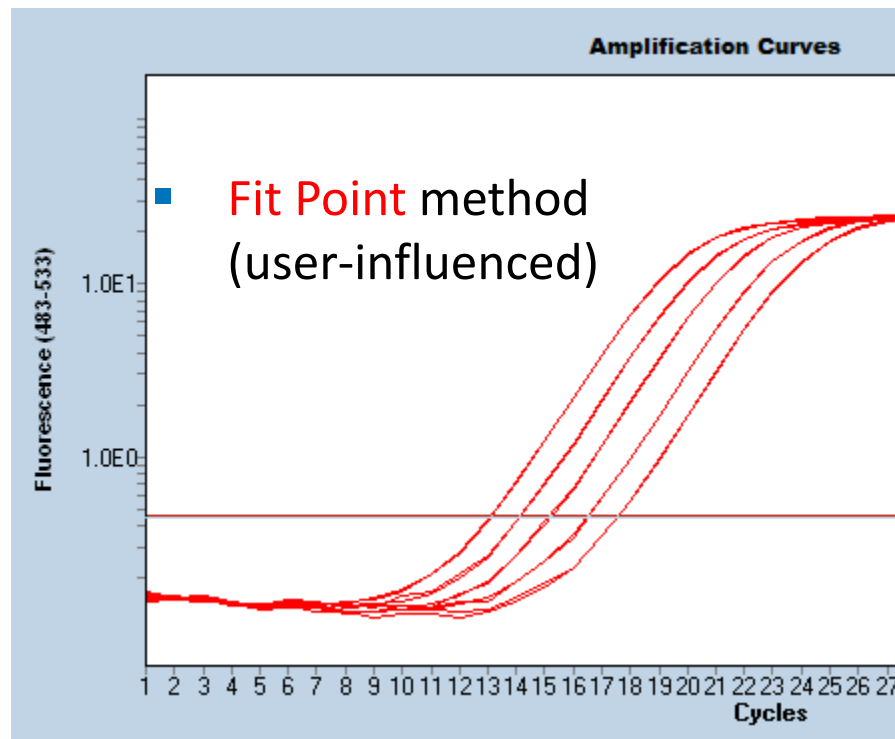
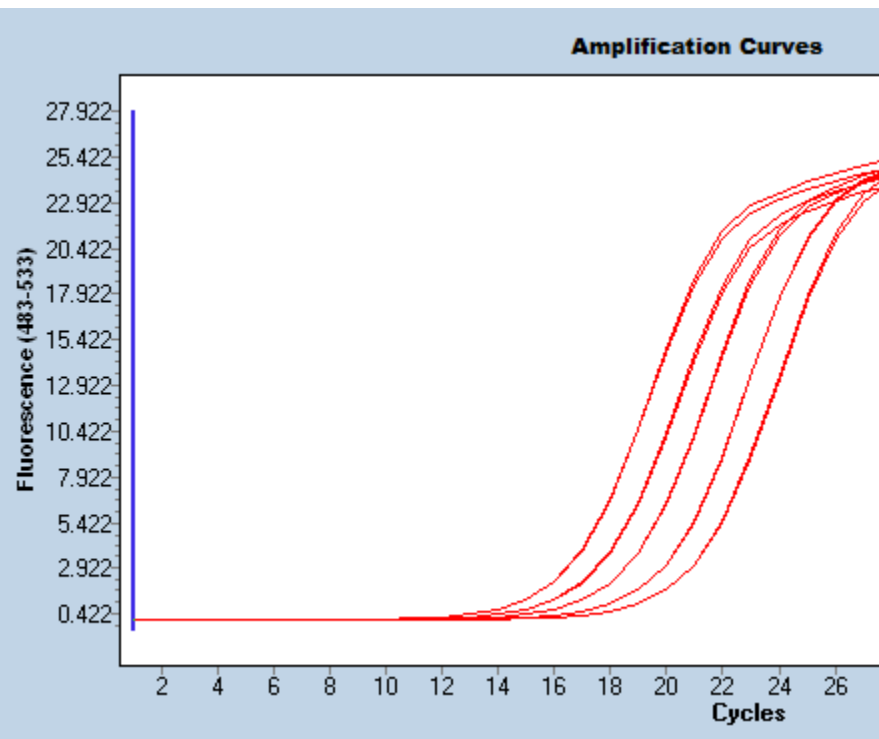
Concentrations and Crossing Points



Calculation of crossing points (Cp)

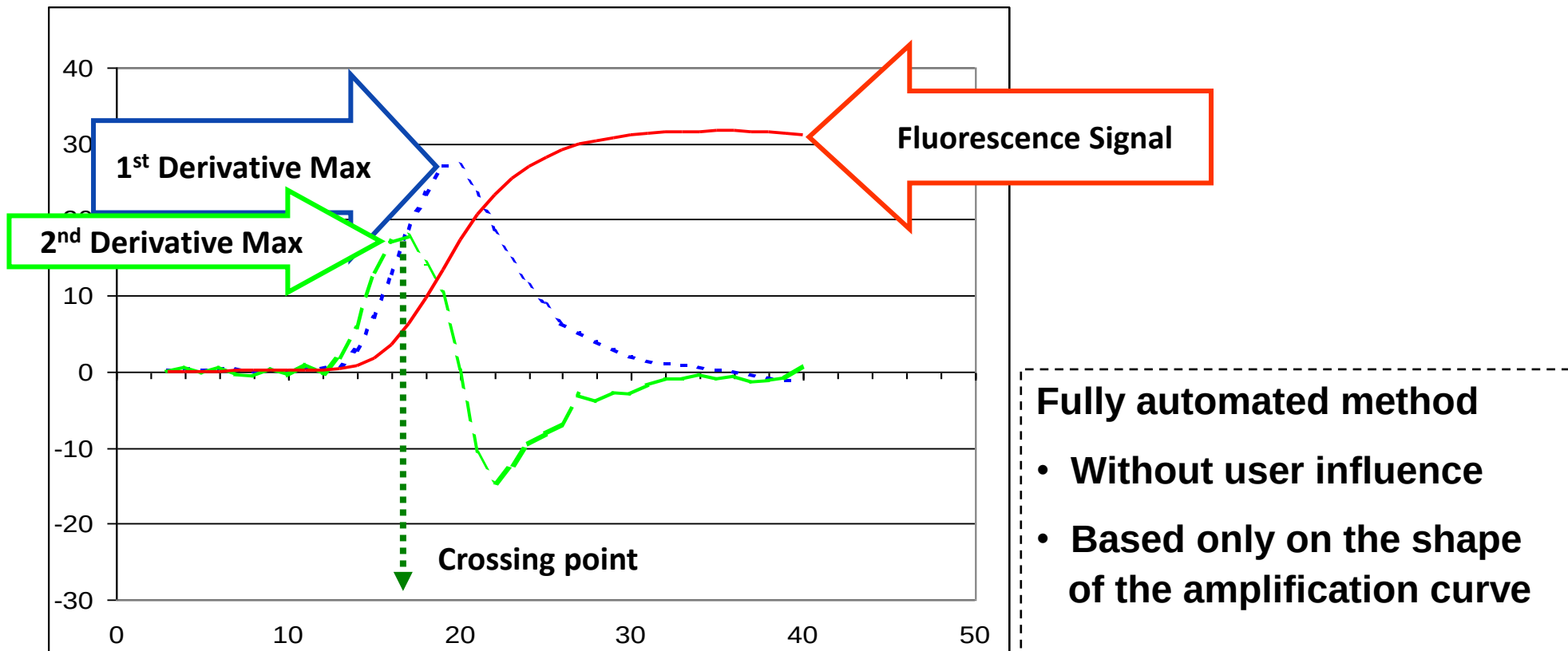
- Optional with **Fit Point** method (user-influenced)
- Standard method (automatic)
2nd Derivative Maximum Method
二次微分最大値

Fit Point method (user-influenced)



Crossing Point Calculation

2nd Derivative Maximum Method



Analysis

Experiment

Subset Editor

Sample Editor

Analysis

Report

Create New Analysis

- 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

Create new analysis

Analysis Type * Abs Quant/2nd Derivative Max

Subset

Program * Amplification

Name

* Abs Quant/2nd Derivative Max for All

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B																								
C																								
D																								
E																								
F																								
G																								
H																								
I																								
J																								
K																								
L																								
M																								
N																								
O																								
P																								

☒

☐

AQ Analysis

Experiment

Subset Editor

Sample Editor

Analysis

Report

Subset: Quantification

Abs Quant results

☒ Positive
 ☒ Negative
 ☒ Standard

Samples			Results			
Include	Color	Pos Name	Cp	Concentration	Stand...	Statu
☒		C8 no template contrc				
☒		C9 negative control				
☒		C10 Standard 1E1			1.00E1	
☒		C11 Standard 1E2			1.00E2	
☒		C12 Standard 1E3			1.00E3	
☒		C13 Standard 1E4			1.00E4	
☒		C14 Standard 1E5			1.00E5	

Apply Template

Notes

Calculate

Experiment

Subset Editor

Sample Editor

Analysis

Report

Subset: Quantification

Abs Quant results

☒ Positive
 ☒ Negative
 ☒ Standard

Samples			Results			
Include	Color	Pos Name	Cp	Concentration	Stand...	Statu
☒		C8 no template contrc				
☒		C9 negative control	35.39	2.82E1		
☒		C10 Standard 1E1	37.45	5.49E0	1.00E1	

Replicate Statistics

Samples	MeanCp	STD Cp	Mean conc	STD conc
C8, D8, E8				
C9, D9, E9	36.31	0.93	1.61E1	1.11E1

Apply Template

Notes

Calculate

Analysis

Experiment

Subset Editor

Sample Editor

Analysis

Report

Create New Analysis

- 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

Create new analysis

Analysis Type * Abs Quant/Fit Points

Subset * All Samples

Program * amplification

Name * Abs Quant/Fit Points for All Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Navigation controls: Previous, Next, First, Last, and a scrollbar.

Confirm (checkmark icon) **Cancel** (X icon)

AQ Analysis

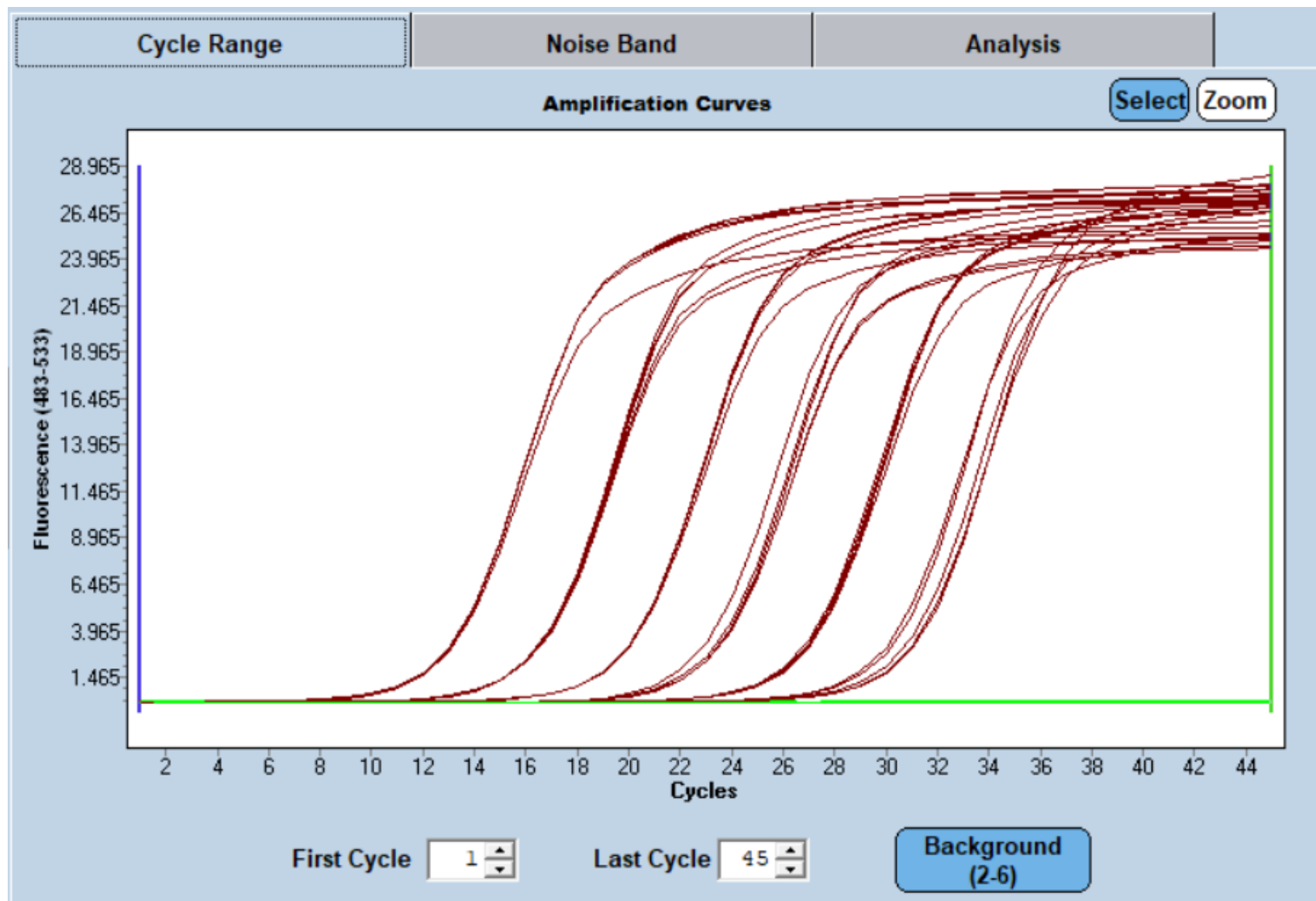
Experiment

Subset Editor

Sample Editor

Analysis

Report



AQ Analysis



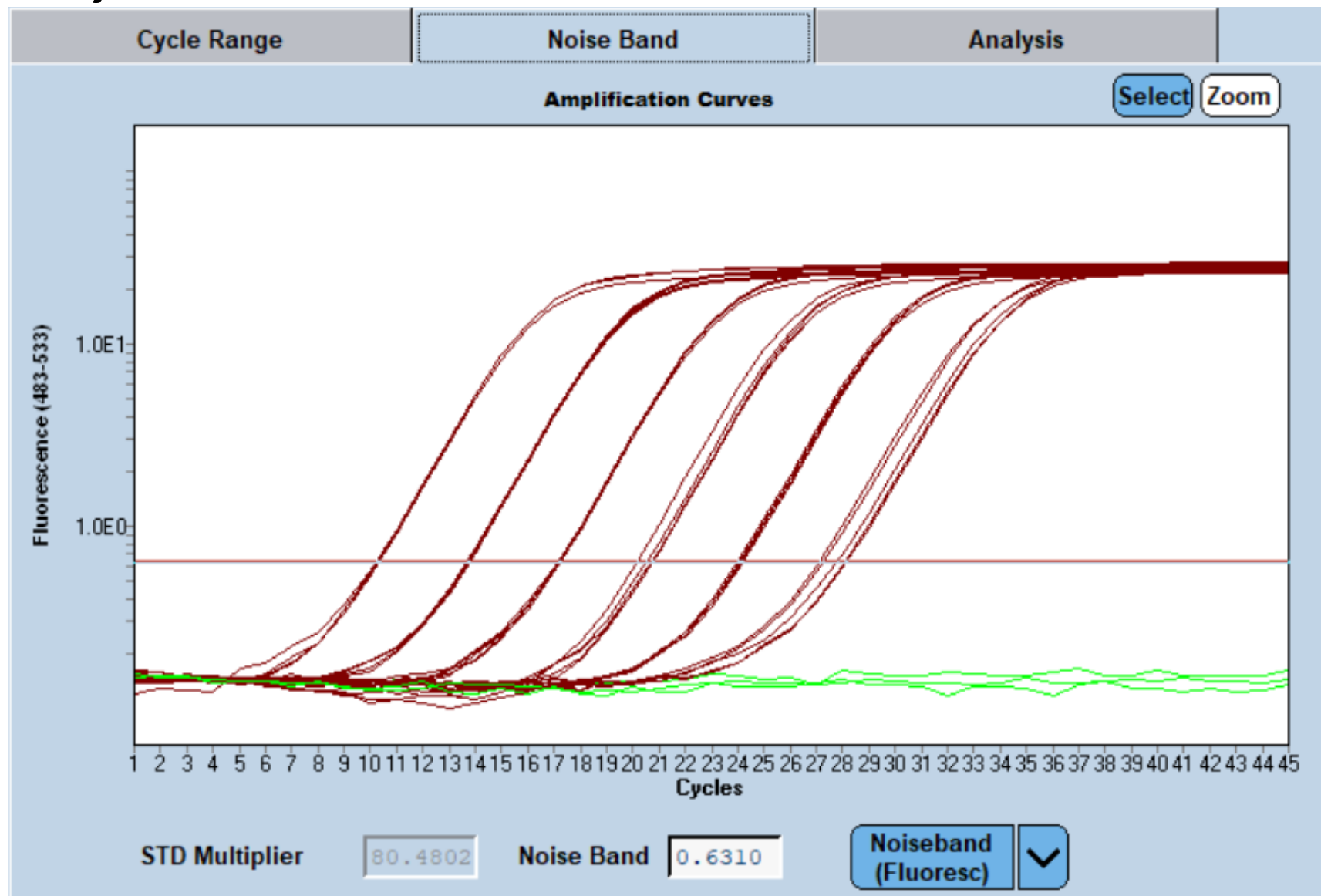
Experiment

Subset Editor

Sample Editor

Analysis

Report



AQ Analysis

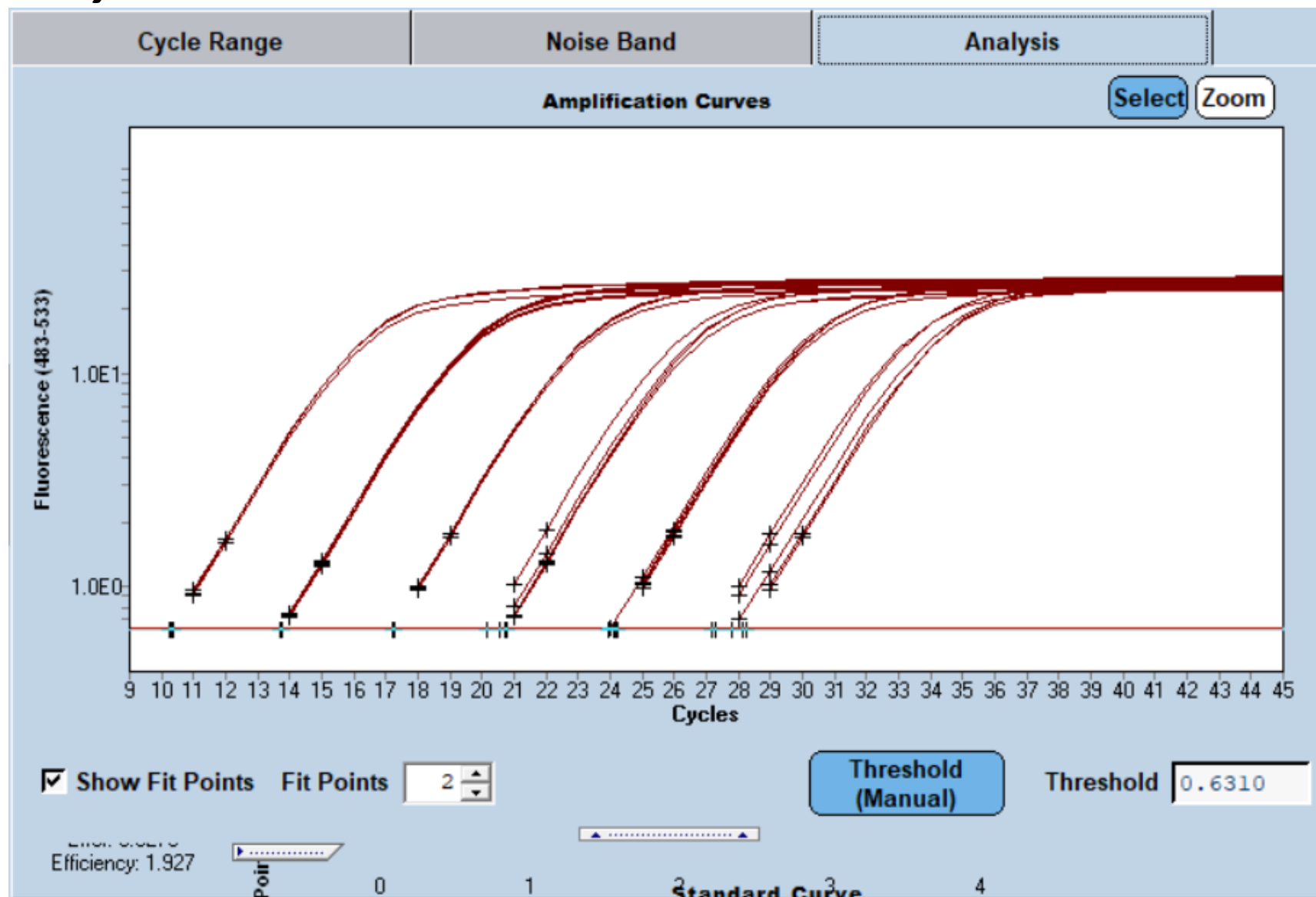
Experiment

Subset Editor

Sample Editor

Analysis

Report



AQ Analysis

Experiment

Subset Editor

Sample Editor

Analysis

Report

Calculate

Subset: Standards and Unknowns

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	●												●	●	●	●	●	●	●	●
B	●																			
C	●																			

Abs Quant results

☒ Positive
 ☒ Negative
 ☒ Uncertain
 ☒ Standard

Samples				Results			
Include	Color	Pos	Name	Cp	Concentration	Stand...	Status
☒	●		A1 Sample 1	16.13	1.01E5		
☒	●		A13 Sample 1	16.10	1.04E5		
☒	●		A14 Sample 1	16.13	1.02E5		
☒	●		A15 Sample 2				
☒	●		A16 Sample 2	17.12	5.26E4		

Export Table

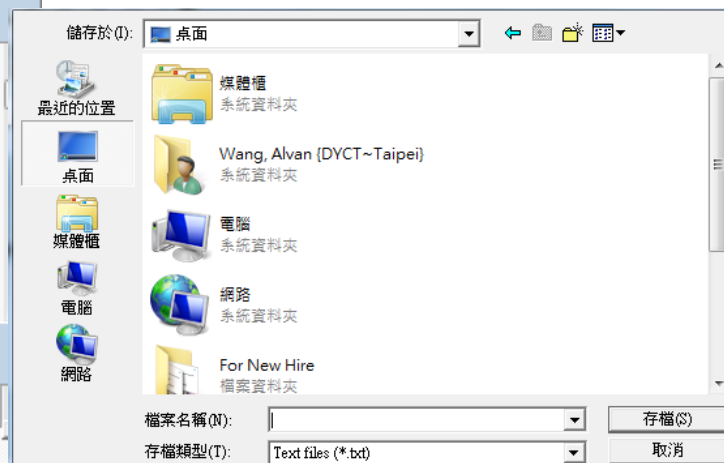
Replicate Statistics

Samples	MeanCp	STD Cp	Mean conc	STD conc
A1, A13, A14,	16.13	0.02	1.02E5	1.44E3
A15, A16, C1,	17.13	0.03	5.26E4	1.17E3
A17, A18, E1,	18.27	0.02	2.47E4	2.85E2

Apply Template

Notes

Calculate



AQ Analysis

Experiment

Subset Editor

Sample Editor

Analysis

Report

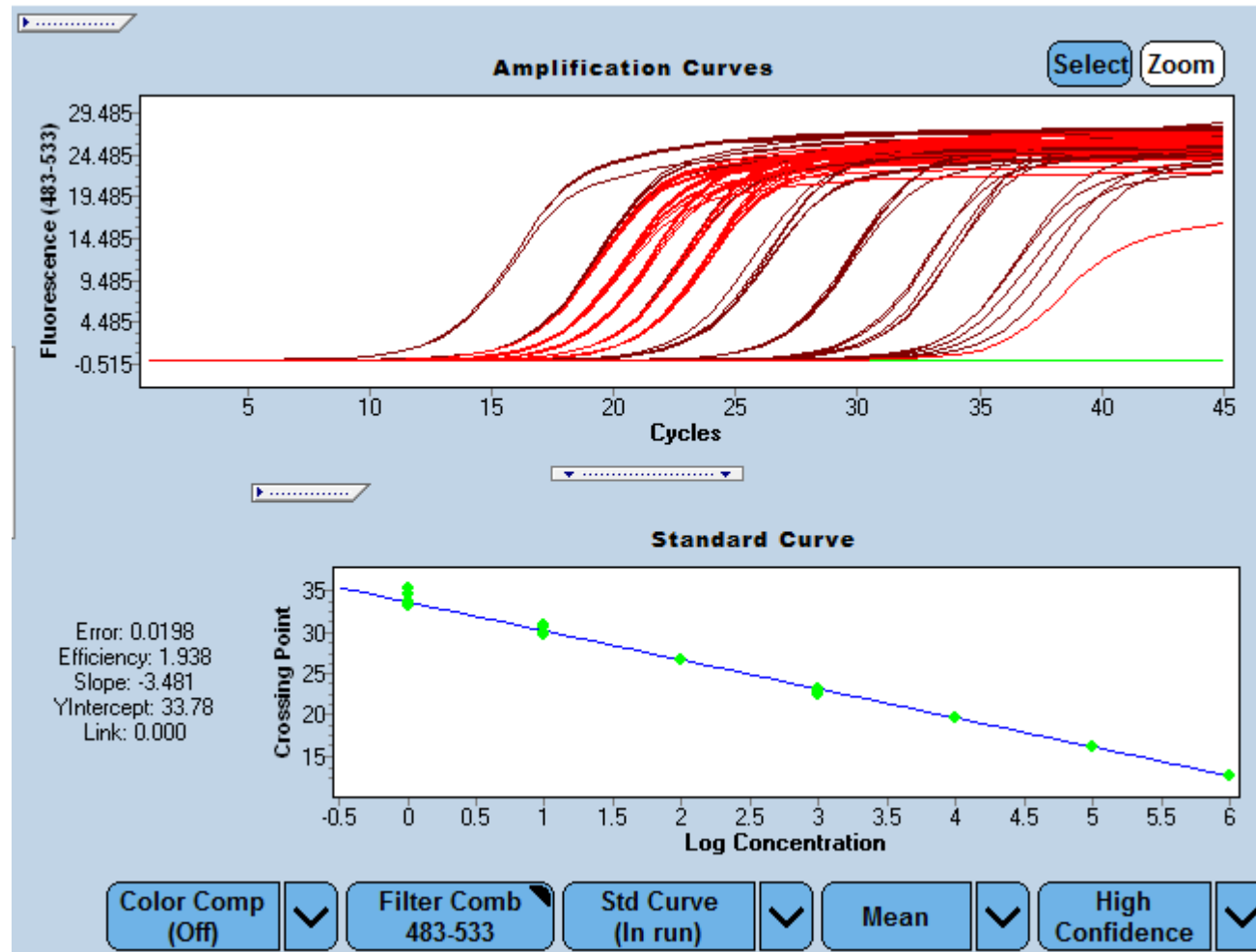


Chart Preferences

Print

Export Chart

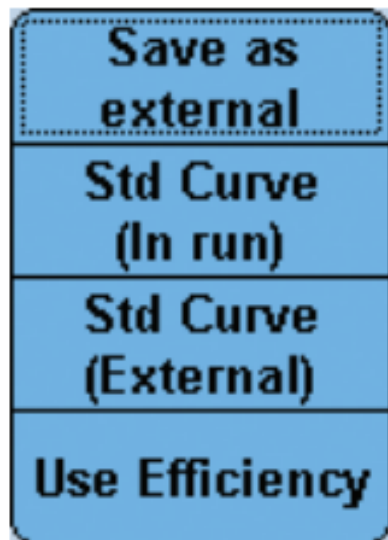
Copy to clipboard

Providing the Standard Curve

- ▶ Use a previously saved standard curve (called an external standard curve). An external standard curve can be loaded into experiments that do not have a standard curve, thus allowing quantitative analysis of those runs. This is especially suitable for applications where the same parameter is analyzed in multiple runs.
- ! *At least one sample (or replicates of this sample) of known concentration must be included in every experiment. This sample should be designated as a standard and should fall within the range of the imported standard curve. The detection format, the analysis mode, and the Color Compensation data (if any) used for the run must be the same as those used for the imported standard curve.*
- ! *For the valid use of the external standard curve, PCR amplification must be highly reproducible and reaction conditions must be constant for all experiments. We recommend running tests to ensure stable PCR efficiency and using replicate samples (especially for low concentrations) to create the standard curve. Also, include a previously quantified sample in each analyzed run, to verify that the calculated values are reproducible.*

To save a standard curve

On the *Standards* multi-select button, select *Save as external*.



The LightCycler[®] 480 Software automatically navigates to the location *User folder – Special Data – Std Curve* subfolder. To save the standard curve, enter a file name, and click .

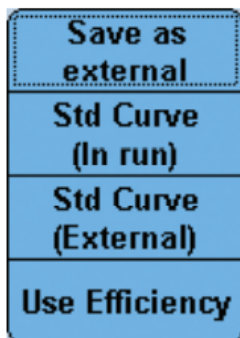
You can use the saved external standard curve in other quantification analyses for experiments that have the same experiment parameters as those used to create the standard curve.

To use an external standard curve

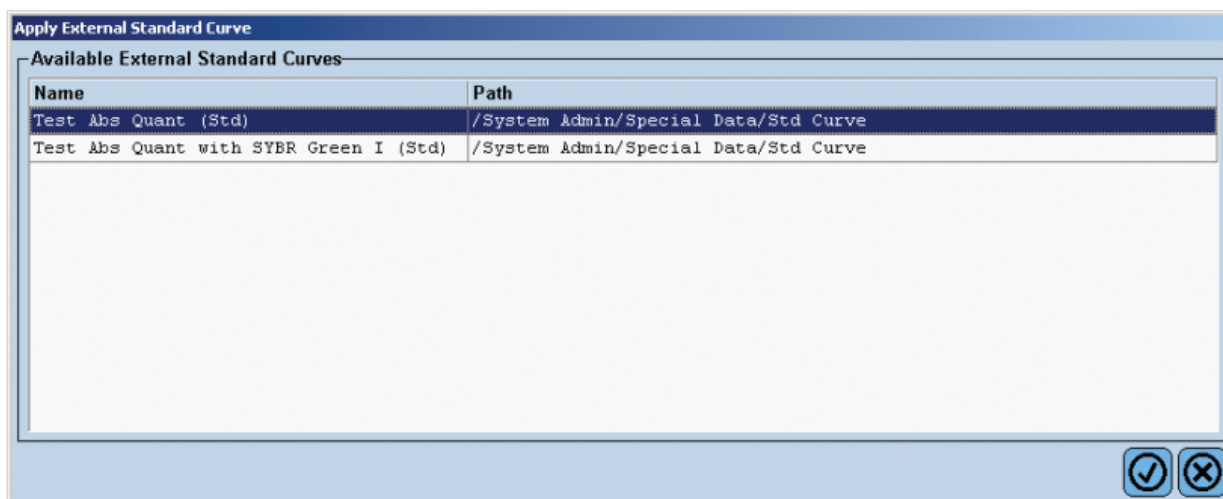
Select *Standard* as the sample type for the standard sample and specify the standard concentration.

For detailed information on the *Sample Editor* see section [Entering Sample Information](#).

On the *Standards* multi-select button, select *Std Curve (External)*.



The *Apply External Standard Curve* dialog opens. Select an appropriate external standard curve object from the list:



RQ Analysis

76

Experiment

Subset
EditorSample
Editor

Analysis

Report

Create New Analysis

Abs Quant/2nd Derivative Max

Abs Quant/Fit Points

Advanced Relative Quantification

Basic Relative Quantification

Create new analysis

Analysis Type * Advanced Relative Quantification

Subset * All Samples

Program * amplification

Name * Advanced Relative Quantification for

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												



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

RQ Analysis

Experiment

Subset Editor

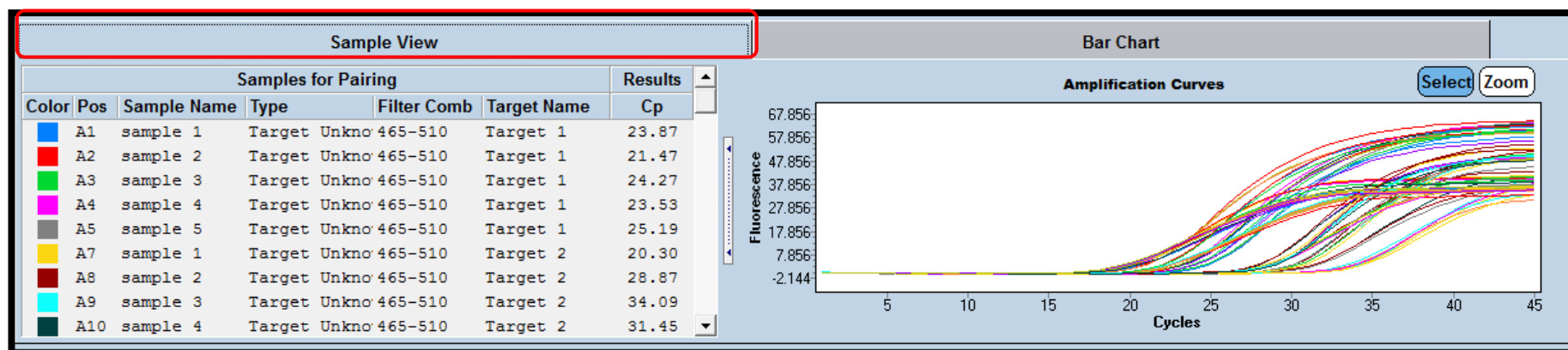
Sample Editor

Analysis

Report

Calculate

Results			Manual Pairing		Target Name				
Bar			Target Name		Target	Reference	Ratios		
Chart	Pairing	Sample Name	Targets	References	Mean Cp	Mean Cp	Target/Ref	Normalized	Status
☒		calibrator	Target 1	Reference 1;Refere	22.43	24.14	3.266	1.000	
☒	A1/D1	sample 1	Target 1	Reference 1;Refere	23.76	26.31	5.859	1.794	
☒	A2/D2	sample 2	Target 1	Reference 1;Refere	21.34	24.58	9.490	2.906	
☒	A3/D3	sample 3	Target 1	Reference 1;Refere	24.30	26.18	3.675	1.125	
☒	A4/D4	sample 4	Target 1	Reference 1;Refere	23.56	25.27	3.267	1.000	
☒	A5/D5	sample 5	Target 1	Reference 1;Refere	25.11	23.90	0.4320	0.1323	
☒		calibrator	Target 2	Reference 1;Refere	28.49	24.14	4.91E-2	1.000	
☒	A7/D1	sample 1	Target 2	Reference 1;Refere	20.21	26.31	68.81	1402	
☒	A8/D2	sample 2	Target 2	Reference 1;Refere	28.84	24.58	5.23E-2	1.066	
☒	A9/D3	sample 3	Target 2	Reference 1;Refere	33.98	26.18	4.48E-3	9.14E-2	
☒	A10/D4	sample 4	Target 2	Reference 1;Refere	31.32	25.27	1.51E-2	0.3069	
☒	A11/D5	sample 5	Target 2	Reference 1;Refere	33.66	23.90	1.16E-3	2.36E-2	



RQ Analysis

Experiment

Subset Editor

Sample Editor

Analysis

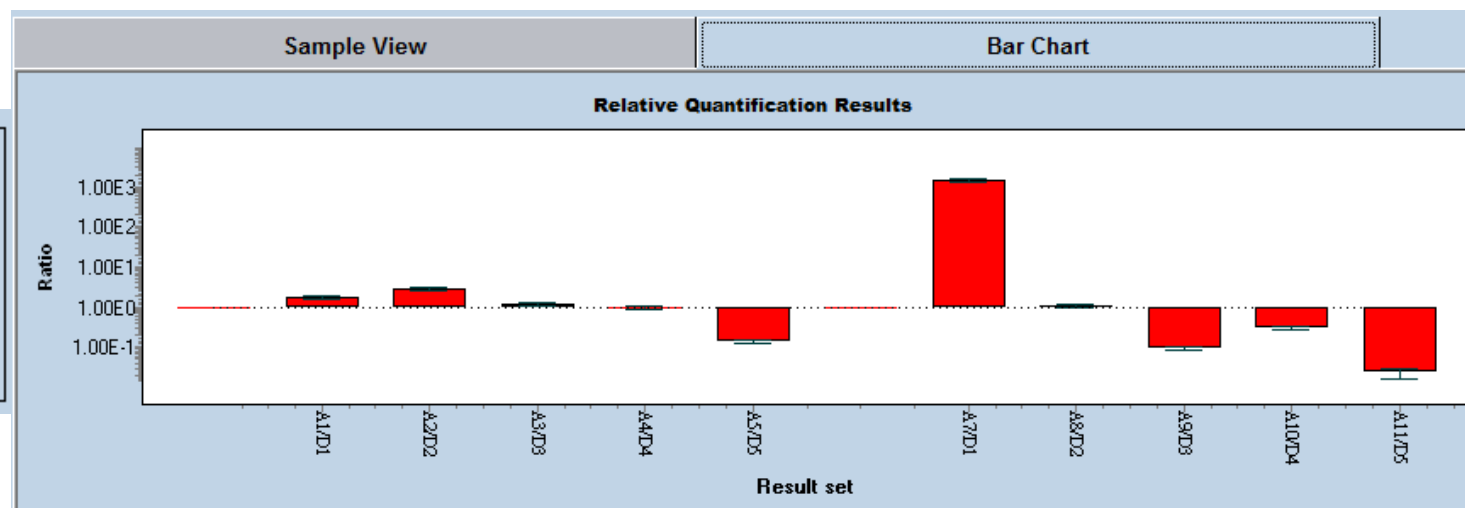
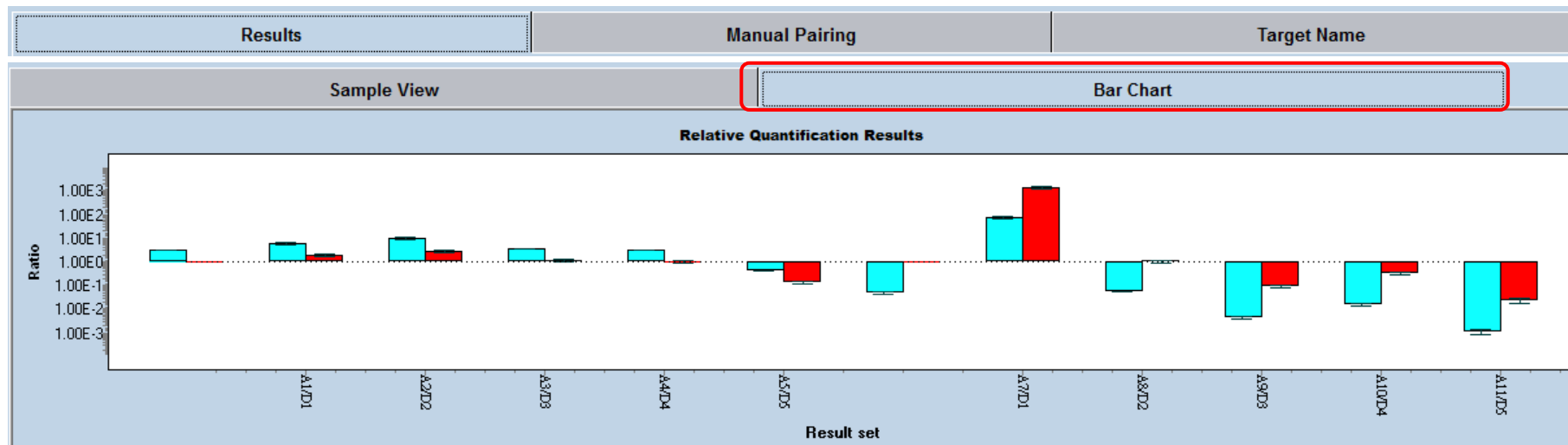
Report

Settings

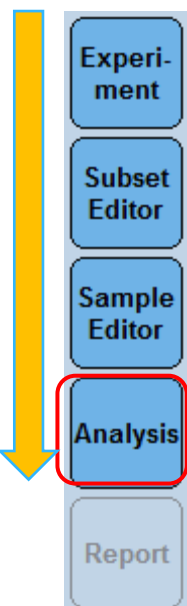
Ratios

- ☐ Display Target/Reference Ratio
- ☒ Display Normalized Ratio
- ☐ Show Ratio Errors

If checked, the ratio shows in the result table and the bar chart.



Analysis



Experiment

Subset Editor

Sample Editor

Analysis

Report

Create New Analysis

- 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

Create new analysis

Analysis Type * Tm Calling

Subset * All Samples

Program * Melting Curve

Name * Tm Calling for All Samples

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B																								
C																								
D																								
E																								
F																								
G																								
H																								
I																								
J																								
K																								
L																								
M																								
N																								
O																								
P																								

☒ ☐

T_m calling/Melting curve

Experiment

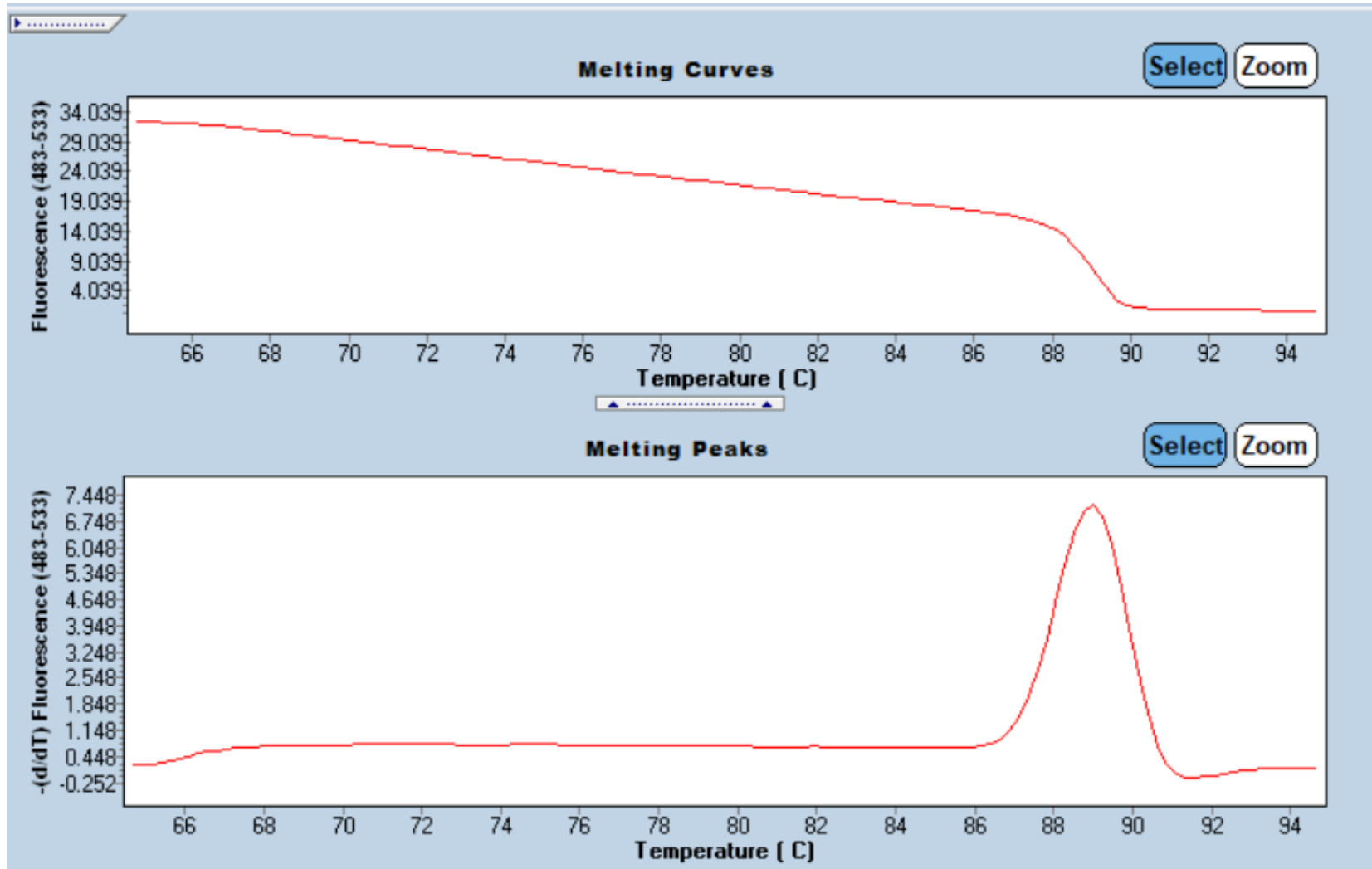
Subset Editor

Sample Editor

Analysis

Report

Calculate



Report

Experiment

Subset Editor

Sample Editor

Analysis

Report



Report

Report Settings

Subset: All Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

General **Detailed**

- ☒ Demo Rel Quant Mono Color
 - ☒ Experiment
 - ☒ Protocol
 - ☐ Samples
 - ☐ Instrument
 - ☐ Revision History
 - ☒ Relative Quantification
 - ☐ Settings
 - ☐ Calibrators
 - ☐ Target Names
 - ☐ Pairings
 - ☐ Results
 - ☐ Results Bar Chart

Default Settings

Apply Template **Generate**



LightCycler® 480 Software

Report

Demo Rel Quant Mono Color

Experiment

Creation Date	2013/7/24 上午 09:12:10	Last Modified Date	2014/3/19 下午 04:44:35
Operator	Demo	Owner	System Admin
Start Time	2007/10/15 下午 04:47:45	End Time	2007/10/15 下午 05:58:07
Run State	Completed	Software Version	LCS480 1.4.9.115
Macro	Macro Owner		
Macro Status			
Templates	Mono Color Hydrolysis Probe - UPL Probe 96-0	Plate ID	00153282
Test ID		Lot ID	
Color Comp ID			
Run Notes	Mono color Relative Quantification experiment. Two target genes and two reference genes are detected with Universal ProbeLibrary probes, all labeled with fluorescein (FAM).		

Programs

Program Name: pre-incubation							
Cycles	1	Analysis Mode: None					
Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (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 (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (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 (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step size (°C)	Step Delay (cycles)
40	None	00:00:30	2.20		0	0	0

Report

Experiment

Subset Editor

Sample Editor

Analysis

Report



Report

Report Settings

Subset: All Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

General | Detailed

- ☒ Demo Rel Quant Mono Color
 - ☒ Experiment
 - ☒ Protocol
 - ☐ Samples
 - ☐ Instrument
 - ☐ Revision History
 - ☒ Relative Quantification
 - ☐ Settings
 - ☐ Calibrators
 - ☐ Target Names
 - ☐ Pairings
 - ☐ Results
 - ☐ Results Bar Chart

Default Settings

Apply Template **Generate**



LightCycler® 480 Software

Report

Demo Rel Quant Mono Color

Experiment

Creation Date	2013/7/24 上午 09:12:10	Last Modified Date	2014/3/19 下午 04:44:35
Operator	Demo	Owner	System Admin
Start Time	2007/10/15 下午 04:47:45	End Time	2007/10/15 下午 05:58:07
Run State	Completed	Software Version	LCS480 1.4.9.115
Macro	Macro Owner		
Macro Status			
Templates	Mono Color Hydrolysis Probe - UPL Probe 96-0	Plate ID	00153282
Test ID		Lot ID	
Color Comp ID			
Run Notes	Mono color Relative Quantification experiment. Two target genes and two reference genes are detected with Universal ProbeLibrary probes, all labeled with fluorescein (FAM).		

Programs

Program Name: pre-incubation							
Cycles	1	Analysis Mode: None					
Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (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 (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (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 (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step size (°C)	Step Delay (cycles)
40	None	00:00:30	2.20		0	0	0

Tool Bar



Exit the application



Save changes to selected object



Log into a database



Export the selected object to a file



Show the overview



Close the selected object



Show the navigator



Print the screen



Open Tools



Change the password

Roche

Tools

- [-] User Access
 - Current Password**
 - Users and Groups
 - System Settings
- Report Settings
- Error Log

Old Password: *

New Password: *

Confirm Password: *

Close



Create a new User

Roche

Tools

- [-] User Access
 - Current Password
 - Users and Groups**
 - System Settings
- Report Settings
- Error Log
- [-] Database Information
 - View Logged In Users
 - Update Query Engine
 - Clean-up Database
- Instruments
- Detection Formats

Users

Groups

Roles

Users

 System Admin

☒ Show users with disabled access

New

Delete

User System Admin

Enter the user's full name:

System Admin

Enter the name the user wants to log in as:

admin

Enter the user's password:

Confirm the password:

Select the user's role:

Local Administrator

Select the groups the user belongs to:

☐ Access Disabled

This is the currently logged in user!

Close



Create a new User

Roche

Tools

- [-] User Access
 - Current Password
 - Users and Groups
 - System Settings**
 - Report Settings
 - Error Log
- [-] Database Information
 - View Logged In Users
 - Update Query Engine
 - Clean-up Database
- Instruments
- Detection Formats

Logout on Inactivity

☐ Enabled

Minutes of Inactivity before Auto-Logout 15

Disable Access on Invalid Password

☐ Enabled

Maximum Login Attempts 99

Password

Password expiration days 100000

Standard User can

☒ Include/exclude samples from analyses

Roche can

- ☒ Access objects owned by Standard Users
- ☒ Access objects owned by Local Administrators
- ☒ Access objects owned by Expert Users

Expert User can

- ☒ Delete items they own
- ☒ Edit detection formats

Close



Create a new User

Roche

Tools

- [-] User Access
 - Current Password
 - Users and Groups
 - System Settings
 - Report Settings
 - Error Log
- [-] Database Information
 - View Logged In Users
 - Update Query Engine
 - Clean-up Database
 - Instruments**
 - Detection Formats

Instruments Virtual LightCycler 480 96 System II



Connection

Operation Log

Self Test

Connection Settings

Name Virtual LightCycler 480 96 System II

IP Address 0.0.0.0

Test Connection

Lamp

Absolute Intensity 1

Operation Time (h) 1

Reset values after Lamp Change

Instrument Settings

☐ Barcode Enabled

Plate Type

☒ White Plates

☐ Clear Plates

☐ Mixed Plates (user configurable)

Instrument Information

Instrument ID Virtual-96-SysII

Block Size 96

Block Type Aluminum

Technical Information

Not Connected

Excitations Filters

Pos	Wavelength
1	440
2	465
3	498
4	533

Emission Filters

Pos	Wavelength
0	488
1	510
2	580
3	610

Make Default

Close



Link to Instrument

Roche

Tools

- [-] User Access
 - Current Password
 - Users and Groups
 - System Settings
- Report Settings
- Error Log
- [-] Database Information
 - View Logged In Users
 - Update Query Engine
 - Clean-up Database
 - Instruments**
 - Detection Formats

Instruments Virtual LightCycler 480 96 System II



LightCycler?480 Instrument

Edit Name:

New Instrument



Connection

Operation Log

Self Test

Connection Settings

Name New Instrument

IP Address 192.168.95.41

Test Connection

Lamp

Absolute Intensity 0

Operation Time (h) 0

Reset values after Lamp Change

Make Default

or Please ask user call 0800258458



Create a new detection Format

Tools

User Access

- Current Password
- Users and Groups
- System Settings

Report Settings

Error Log

Database Information

- View Logged In Users
- Update Query Engine
- Clean-up Database

Instruments

Detection Formats

Detection Formats

Active	Name
☒	SYBR Green I / HRM Dye
☒	SimpleProbe
☒	Mono Color Hydrolysis Probe
☒	Dual Color Hydrolysis Probe
☒	3 Color Hydrolysis Probe
☒	4 Color Hydrolysis Probe
☒	Mono Color HybProbe
☒	Multi Color HybProbe

New

Copy

Rename

Delete

Filter Combination Selection

Emission						
E	488	510	580	610	640	660
x						
c						
i						
t						
a						
t						
i						
o						
n						

Clear

Selected Filter Combination List

Excitation Filter	Emission Filter	Name	Melt Factor	Quant Factor	Max Integration Time (Sec)
465	510	Fluos	2	1.5	2
498	610	Red 610	1.2	5	2
498	640	Red 640	1.2	5	2
498	660	Cy 5 / Cy 5.5	1.2	5	2

Close



Assay Formats and Cp Calculation

Absolute/Relative Quantification

Hardware Overview for LightCycler® Systems

System Operation Procedures

LC 96/LC 480 QC Report

Q&A

LC96 QC Report Template



Qualification Service



Instrument Verification Run Report

SYSTEM INFORMATION

System Type LightCycler® 96
Instrument Serial No. 00000000014401
Instrument SW Version 1.02.00.0086
Verification Run Started 14-Feb-2022 14:56:17
Experiment - File ExperimentTemplate_QC-Test_20220214.lc96p
Reagent Lot Number 59016500
Comment

REPORT INFORMATION

Report Generated With CHECK REPORT TOOL
Software Version 8.0.6
Parameter Version 1.0

VERIFICATION RUN RESULT

Report Generated By wanga51
Report Created 17-Feb-2022
Check Result **Passed**

Document Version 1.0

Page 1/2

Qualification Service



Instrument Verification Run Report

VERIFICATION RUN REPORT DATA

Parameter	Limit	Value
-----------	-------	-------

Quantification Cycle [Cq]		Passed
Lowest Cq	≥ 21.61	21.95
Highest Cq	≤ 22.61	22.2
Calculated SD	≤ 0.2	0.04

Initial Fluorescence [Avg. Cycle 1 to 17]		Passed
Lowest Initial Fluorescence	≥ 0.016	0.028
Highest Initial Fluorescence	≤ 0.047	0.036
Calculated CV [%]	≤ 20	3.991

Melting Temperature [Tm]		Passed
Median [°C]	84 ± 2	83.11
Lowest Tm [°C]	≥ 82.71	82.995
Highest Tm [°C]	≤ 83.51	83.191
Calculated SD	≤ 0.15	0.04

Number of Excluded Samples		Passed
Max. Excluded	≤ 3	0

Document Version 1.0

Page 2/2

LC480 QC Report Template

Q⁺ Qualification Services
Performance Qualification (PQ) Check Report

LightCycler® 480 Instrument

Serial No: 30705
 Date: 17-Jan, 2022



Roche Diagnostics Ltd.
 Applied Science Business Area

Qualification Service
 Instrument Verification Run Report

SYSTEM INFORMATION

System: LightCycler® 480II
 Instrument Serial No: 30705
 Instrument SW Version: 1.5.1.62
 Verification Run Started: 17-Jan-2022 12:28:44
 Experiment - File: QC-20220117-30705.bxo
 Reagent Lot Number: 57252820
 Detection - Unit: Type II (Filter-Set II)
 Block - Size: 96
 Comment:

REPORT INFORMATION

Report Generated With: CHECK REPORT TOOL
 Software Version: 8.0.6
 Parameter Version: 1.0

VERIFICATION RUN RESULT

Reported Generated By: wanga51
 Report Created: 18-Jan-2022
 Check Result: Passed

Qualification Service
 Instrument Verification Run Report

VERIFICATION RUN REPORT DATA

Parameter	Lower Limit	Upper Limit	Value
Fluorescence 510 offset (Blank Solution)			Passed
Median	0.05	5.94	1.686
CV [%]		30	22.567
Fluorescence 510 background cycle 2-6			Passed
Mean	29.309	134.932	51.423
CV [%]		10	8.61
Fluorescence 640 signal dynamics			Passed
Mean	0.573	1.219	0.848
CV [%]		10	8.361
Crossing Point			Passed
Mean [Cycles]	20.9	22.9	21.882
SD [Cycles]		0.5	0.036
Tm PCR Mix (640)			Passed
Mean [°C]	61.1	63.5	62.409
SD [°C]		0.3	0.053
Tm Melting Mix (510) TmA1			Passed
Median [°C]	76.9	79.3	78.149
Range [°C]		0.9	0.121
Tm Melting Mix (510) TmB1			Passed
Median [°C]	76.9	79.3	78.043
Range [°C]		0.9	0.333
Tm Melting Mix (510) dTm1			Passed
Median [°C]	-0.3	0.3	-0.097
Range [°C]		0.5	0.255
Tm Melting Mix (510) TmA2			Passed
Median [°C]	90.5	92.9	91.482
Range [°C]		1.1	0.168

Qualification Service
 Instrument Verification Run Report

VERIFICATION RUN REPORT DATA

Parameter	Lower Limit	Upper Limit	Value
Tm Melting Mix (510) TmB2			Passed
Median [°C]	90.3	92.7	91.42
Range [°C]		1.1	0.216
Tm Melting Mix (510) dTm2			Passed
Median [°C]	-0.35	0.25	-0.074
Range [°C]		0.5	0.067

Blank Samples	Excluded	Passed
PCR Mix Samples	1	0
Melting Mix Samples	2	0
Total Samples	3	0

Document Version 1.0 Page 3/3

Document Version 1.0

Page 2/3

CERTIFICATE

FOR INSTRUMENT VERIFICATION RUN

LightCycler® 480II

Verification Run Started: 17-Jan-2022
 Instrument Serial No.: 30705
 Certificate Generated By: wanga51
 Check Report SW Version: 8.0.6
 Instrument SW Version: 1.5.1.62
 Reagent Lot: 57252820
 Experiment - File: QC-20220117-30705.bxo

An instrument Verification Run was carried out with the aforementioned LightCycler® 480II. This certificate confirms that at the time of the verification run this instrument was in accordance with the Roche specifications for the LightCycler® 480II.

Instrument Location:

Authorized Roche Representative:

Date:

Signature:



Document Version 1.0

1/1



Assay Formats and Cp Calculation

Absolute/Relative Quantification

Hardware Overview for LightCycler® Systems

System Operation Procedures

LC 96/LC 480 QC Report

Q&A

Automated Workflows From Sample to Result

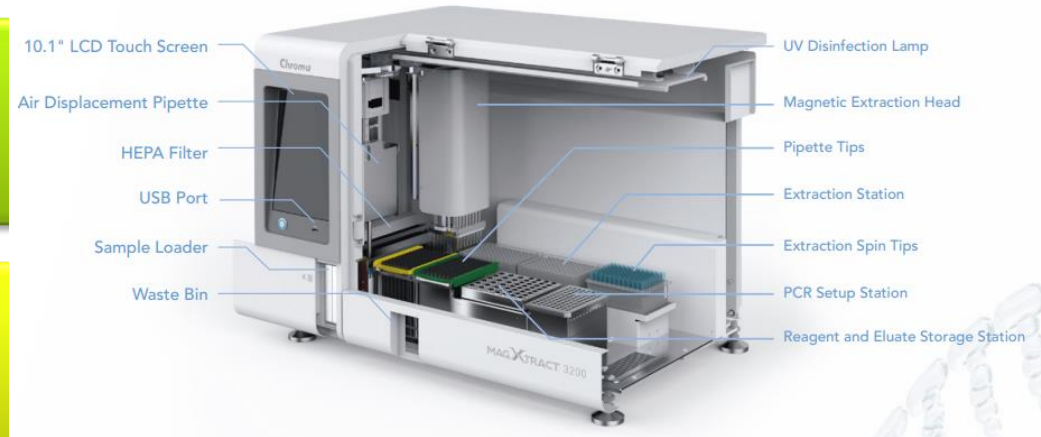
Roche

Sample disruption

Purification

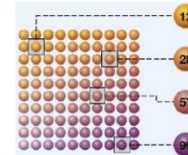
Assay setup

Detection and analysis

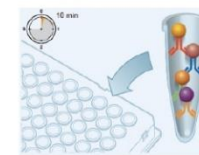


操作時間只需要3小時・最多可同時檢測100種標的物

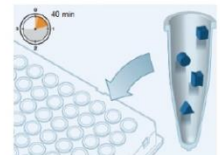
1. 選擇特定含抗體之磁珠



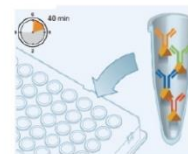
2. 將磁珠混合放入盤中



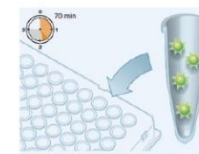
3. 加入樣品



4. 加入Biotin label之抗體



5. 加入呈色劑



6. 上機分析資料

