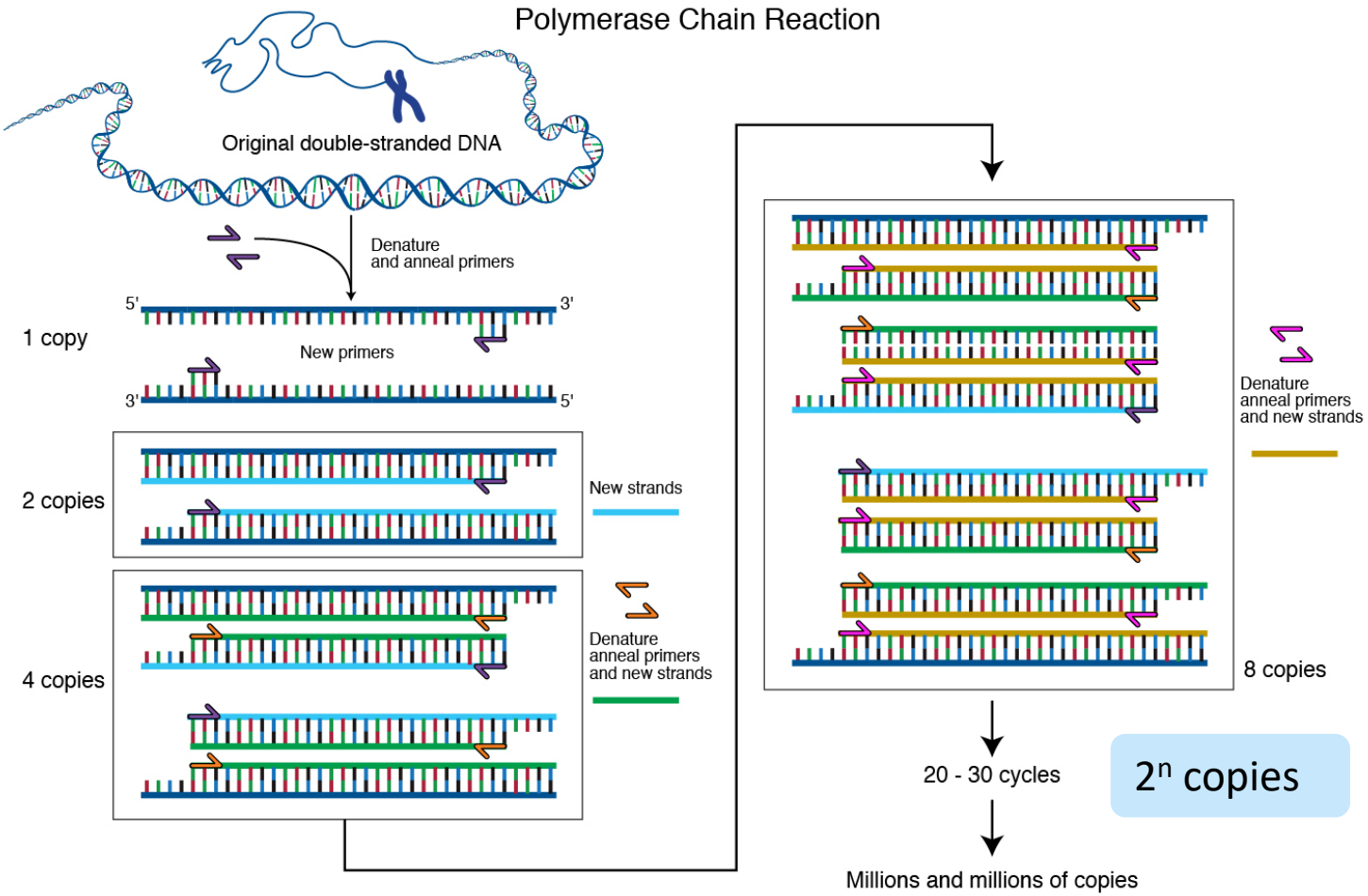
LIGHTCYCLER® 480 REAL-TIME PCR SYSTEM

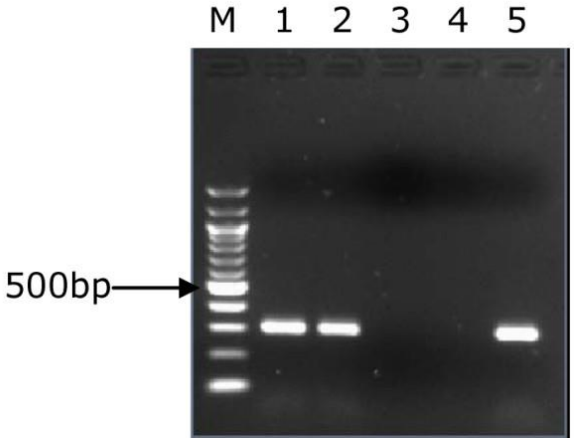
Sophia Lin

- Real Time PCR Basic Training
- Troubleshooting cases sharing
- System Operation Procedures
- LC 96/LC 480 QC Report
- Q&A

Polymerase Chain Reaction (PCR)

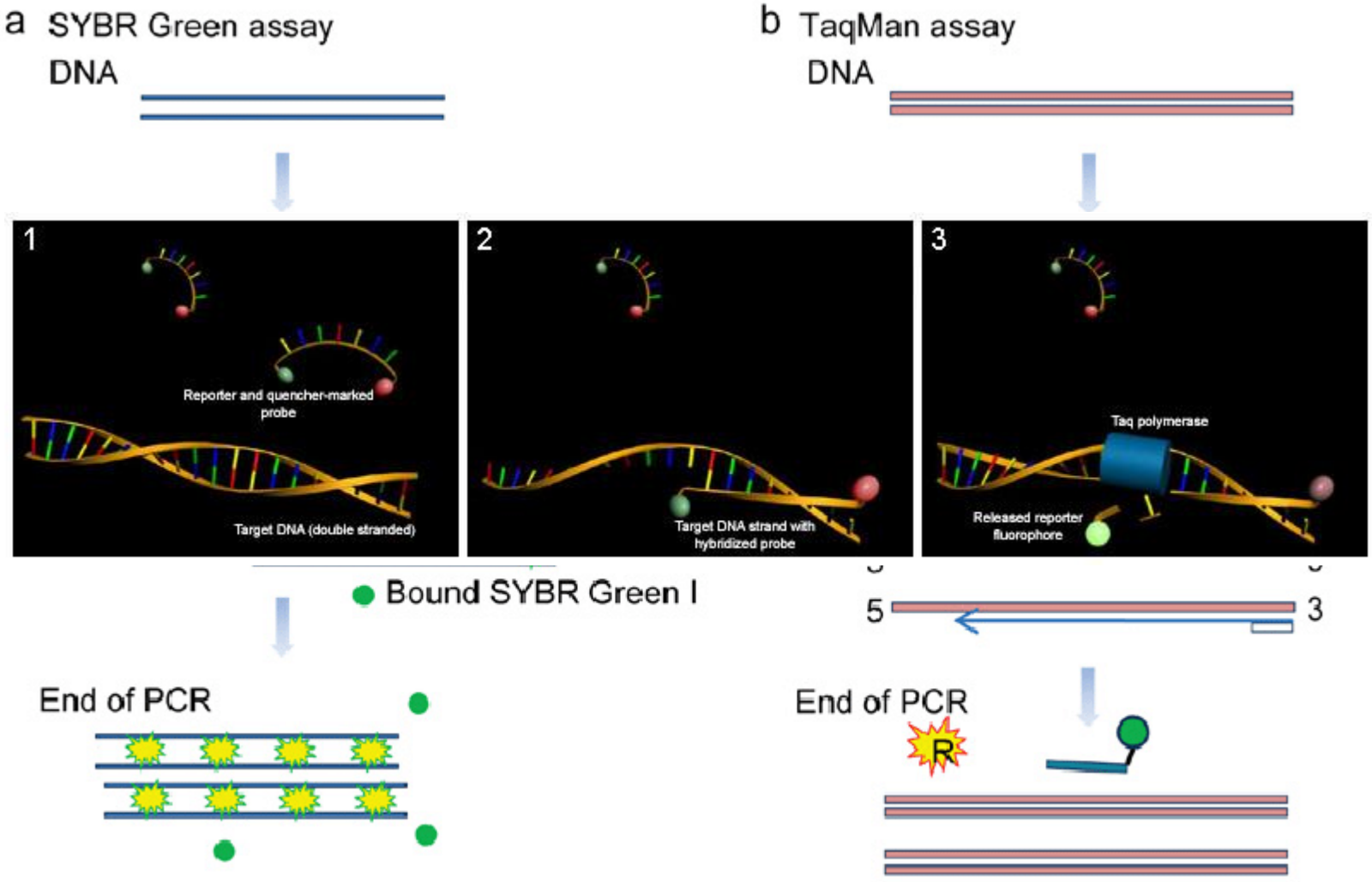


2^n copies



Denature → **Annealing** → **Extension**

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



LightCycler® Detection Formats

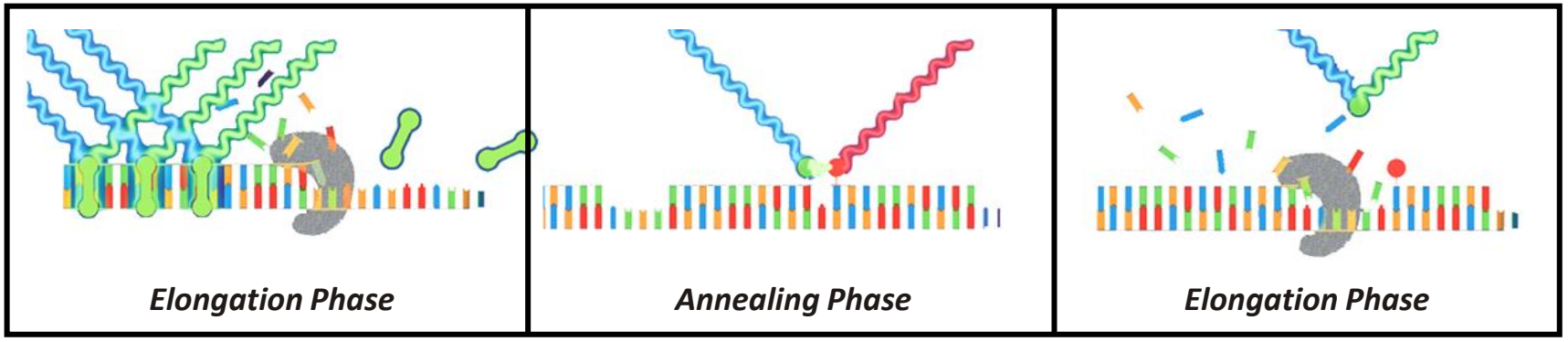
Non-specific binding dye

Specific labeled probes

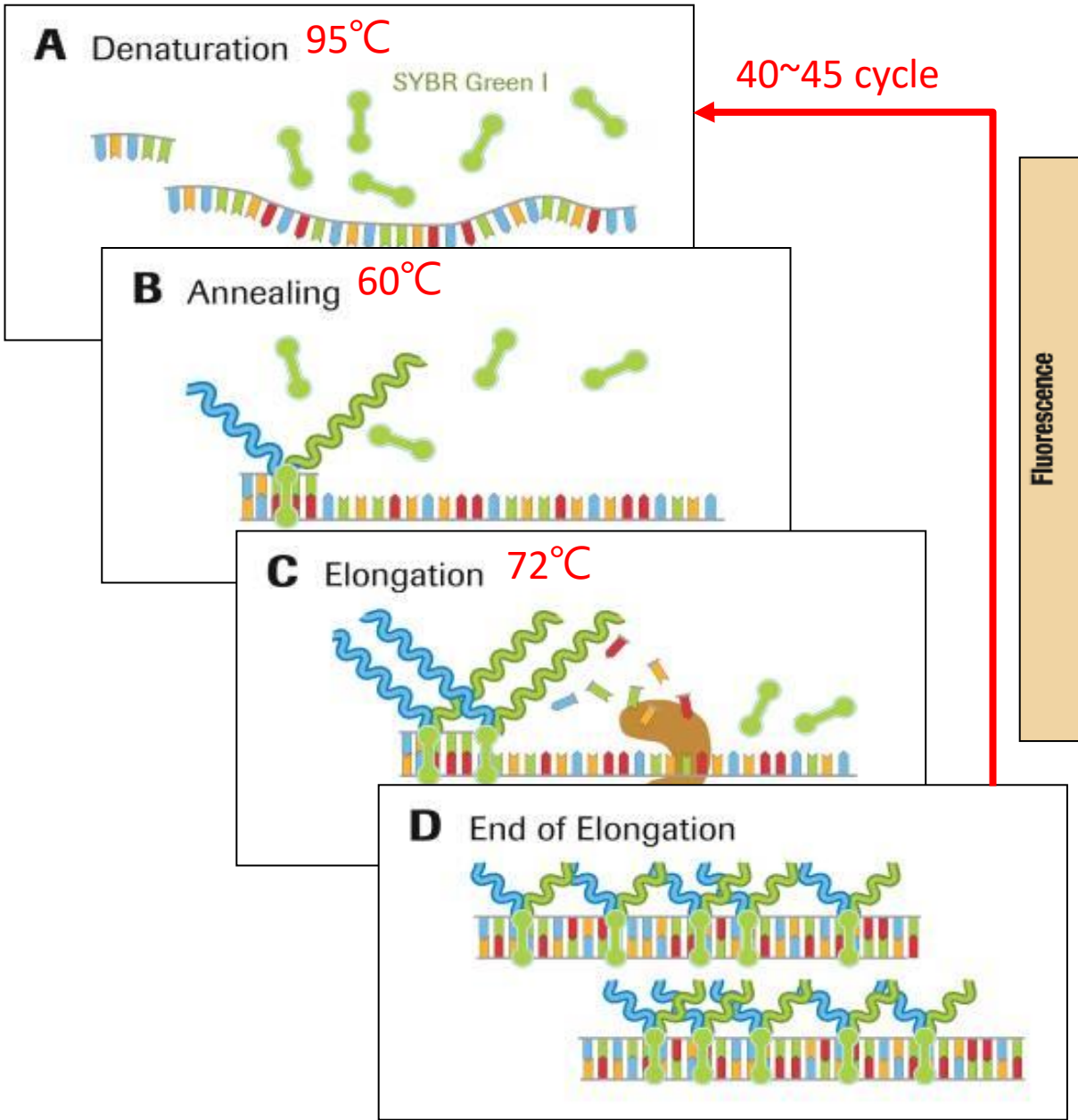
SYBR Green I

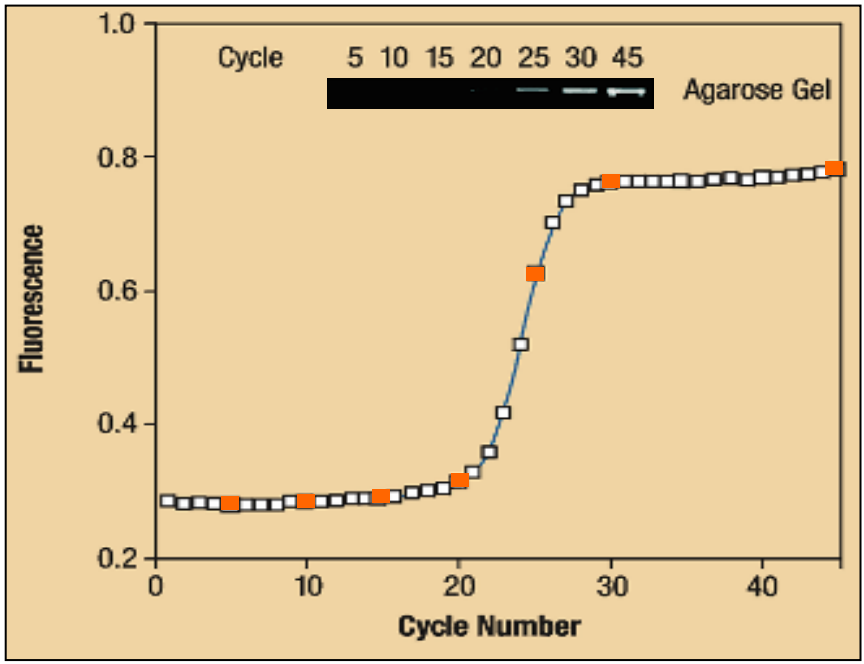
Hybridization Probe

Hydrolysis Probe (TaqMan)

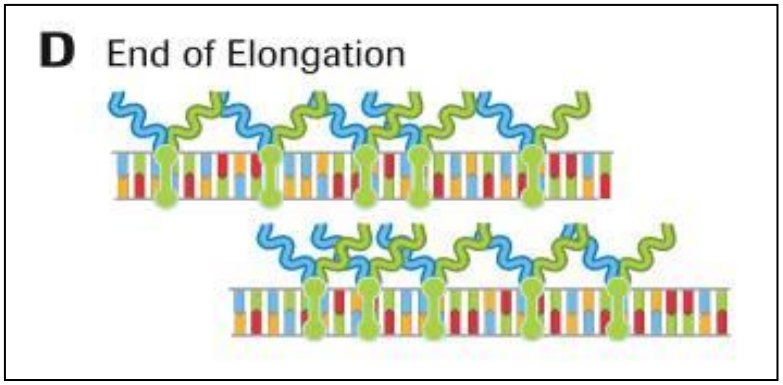


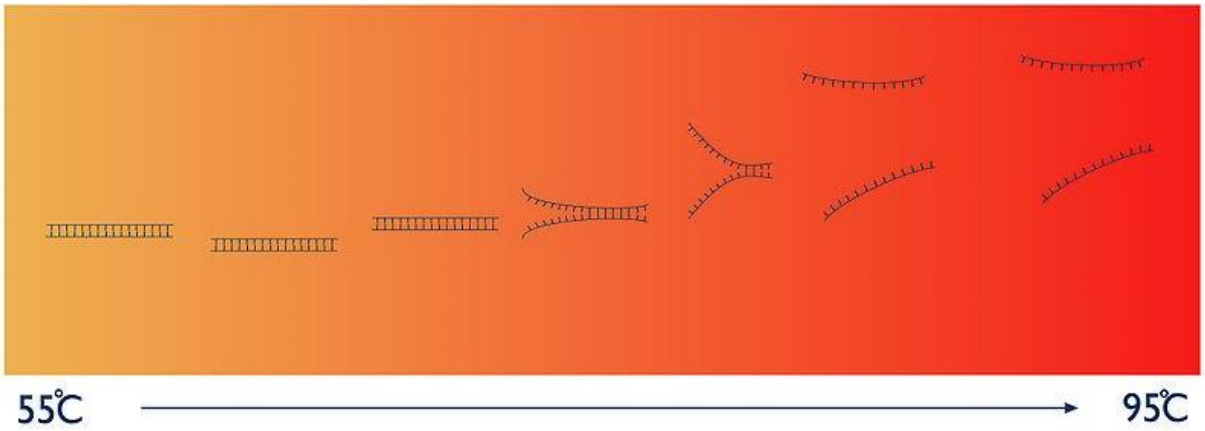
LightCycler® Assay Formats *SYBR Green I Format*

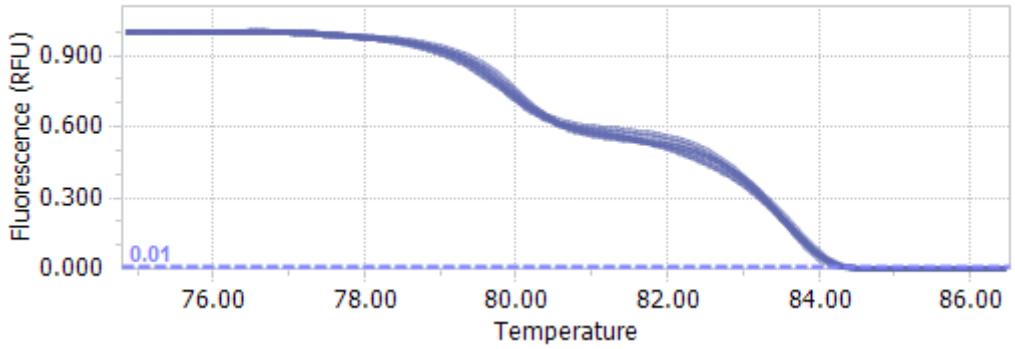


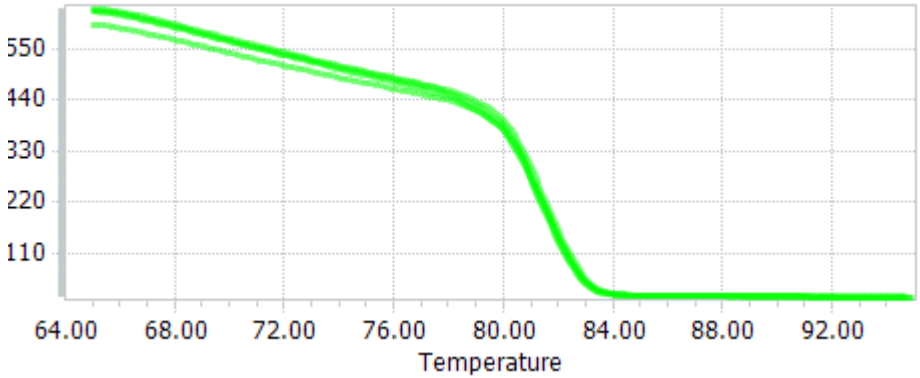


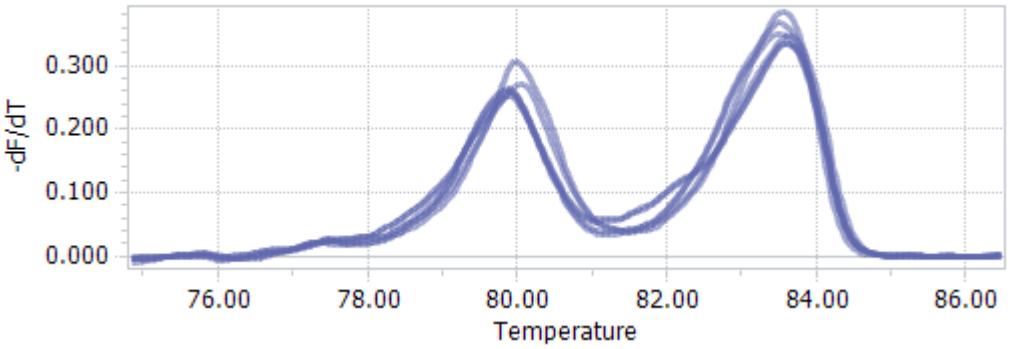
Analysis Module Tm Calling/Melting Curve

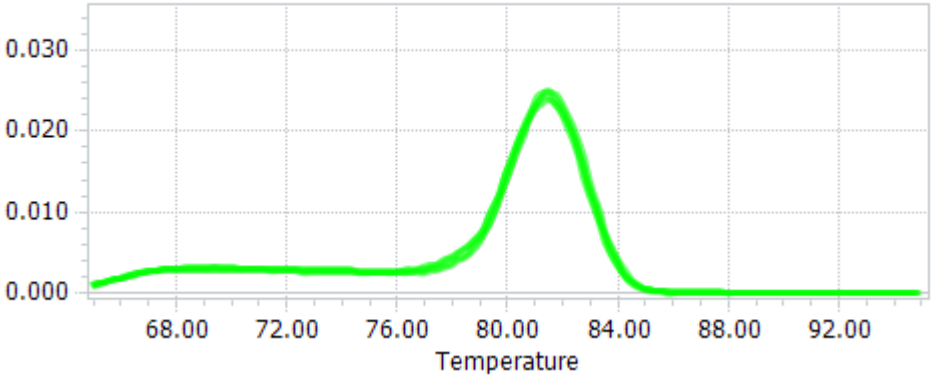






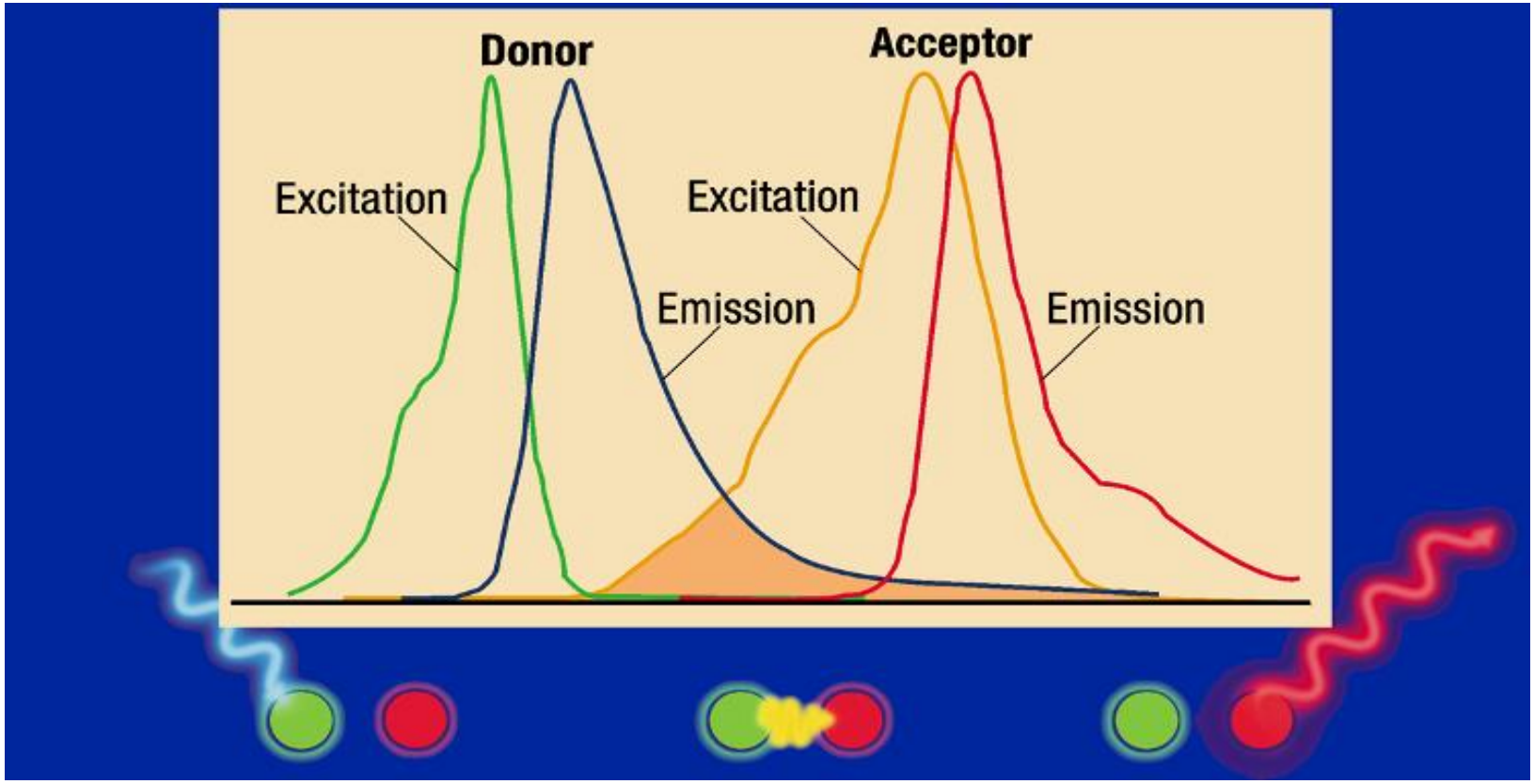






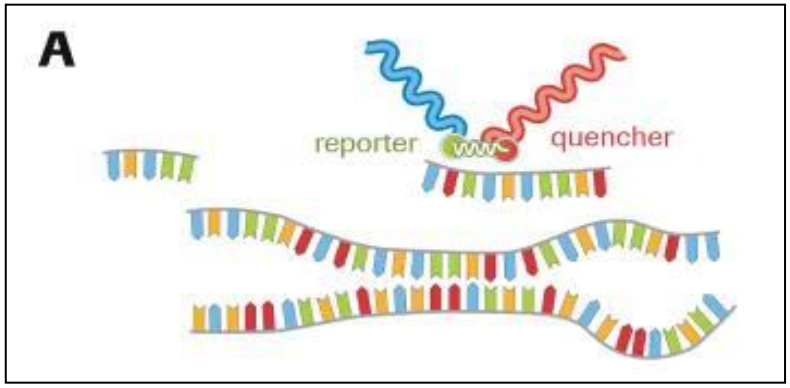
LightCycler® Assay Formats

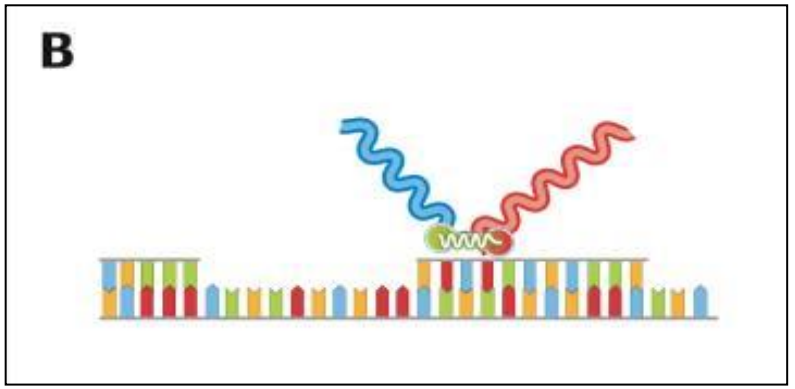
Fluorescence Resonance Energy Transfer (FRET)

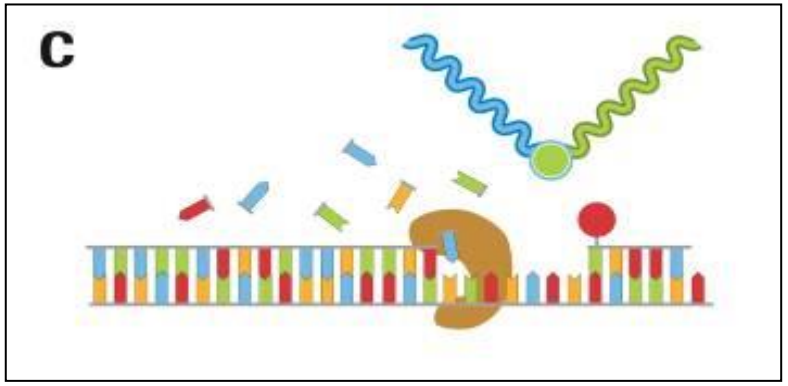


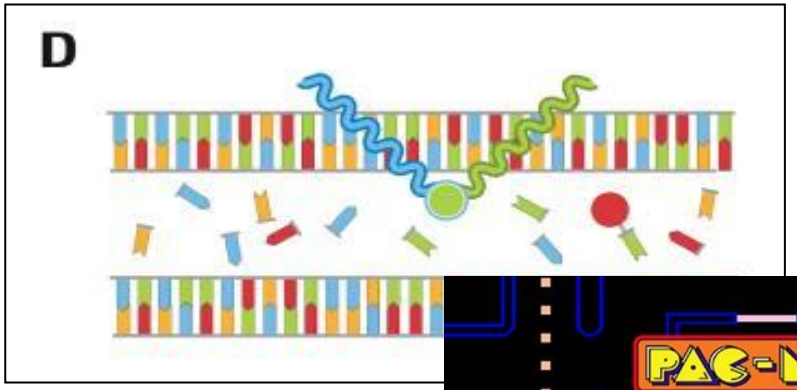
LightCycler® Assay Formats

TaqMan Probe





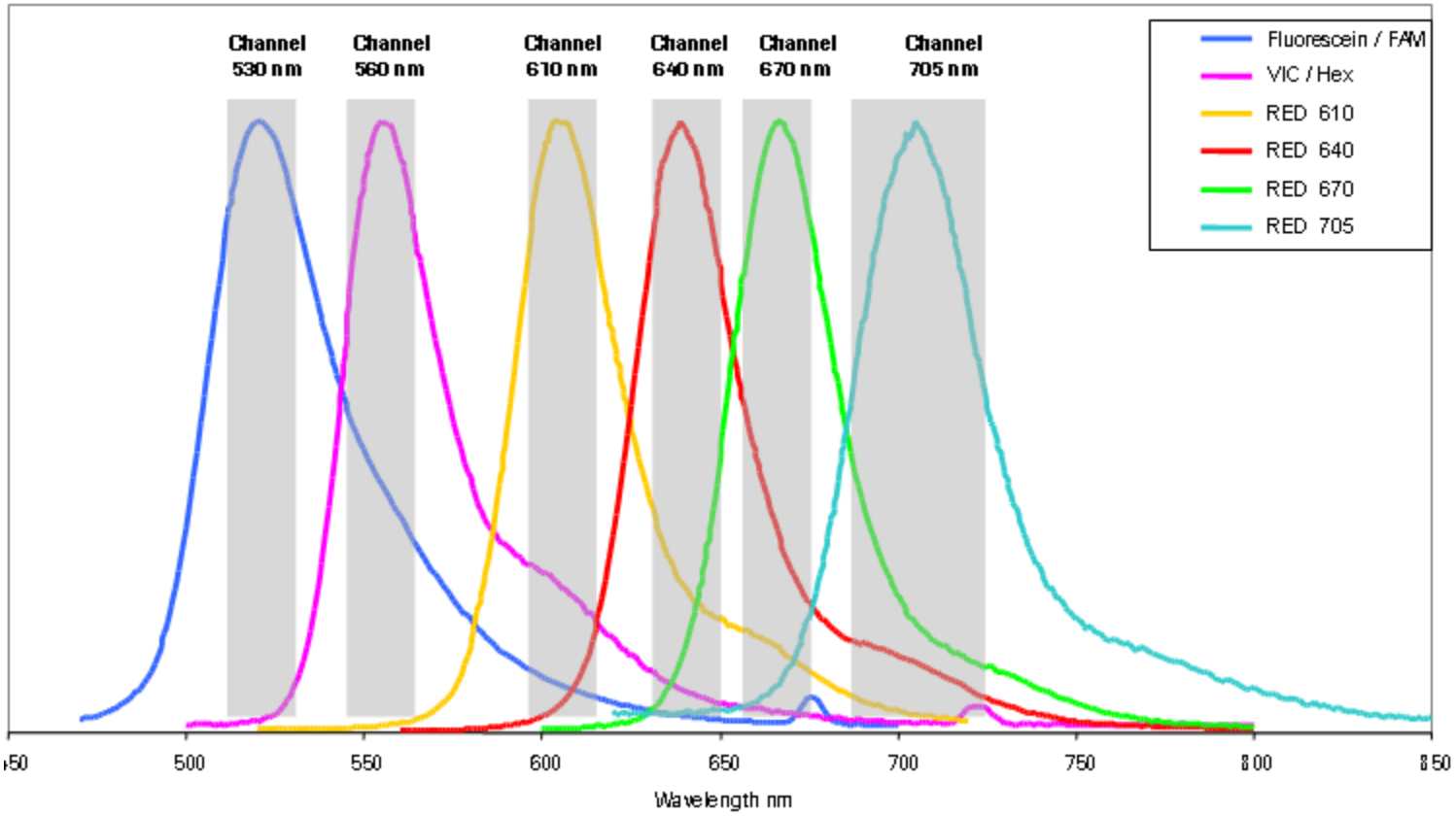




1. *多色Multiplex*
2. *不需melting curve*

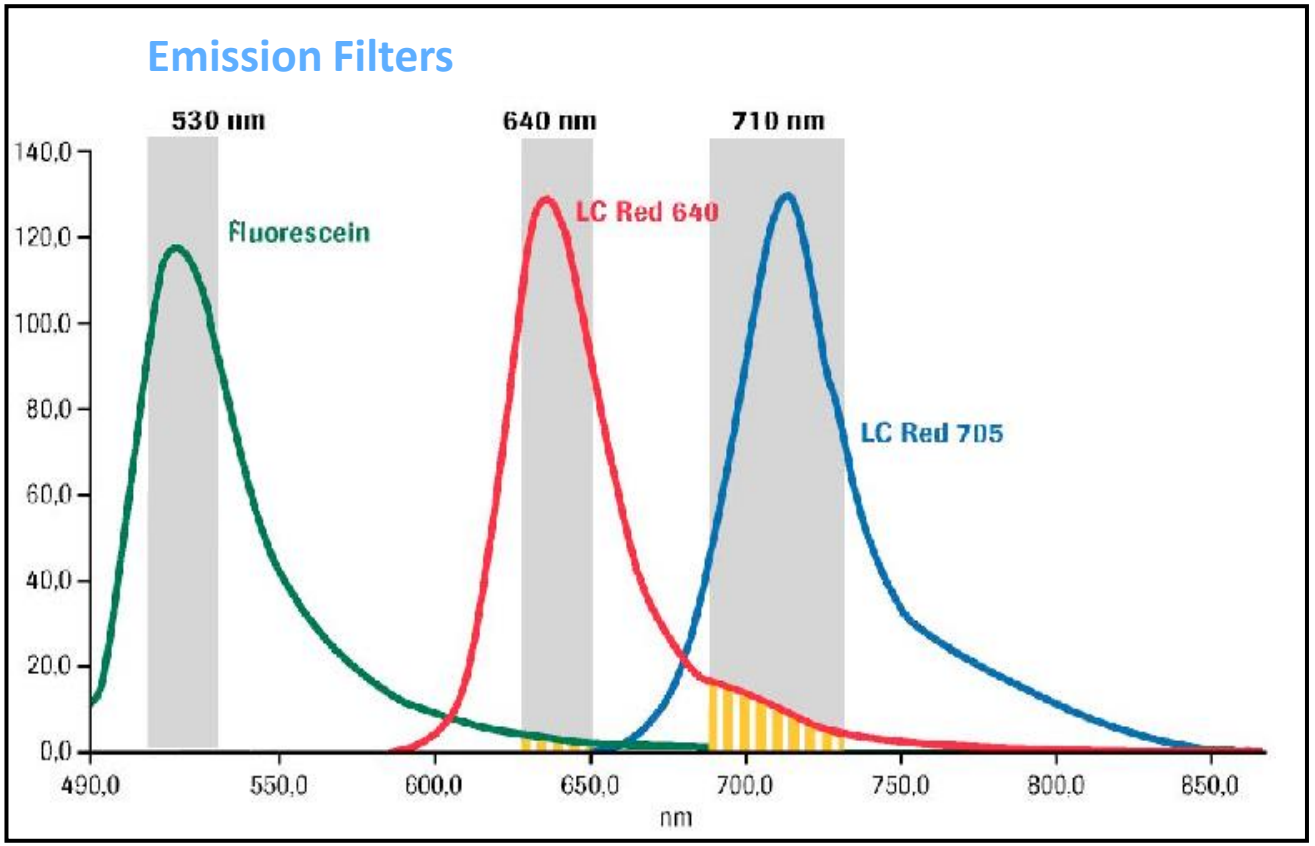
LightCycler® Assay Formats

Dye Emission Spectra



LightCycler® Assay Formats

Dye Emission Spectra and Crosstalk

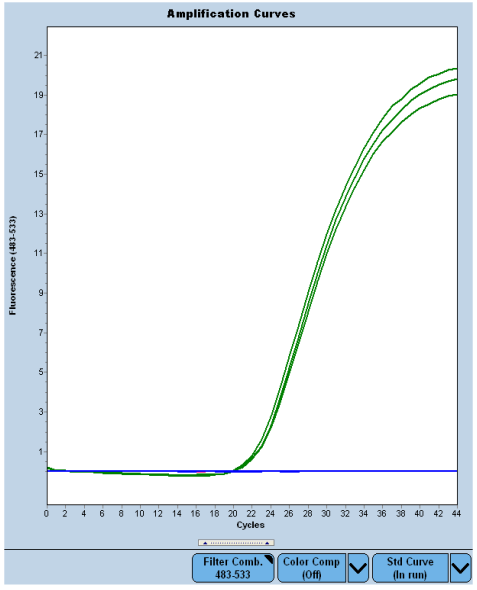


Tricolor Hydrolysis Probe - Example

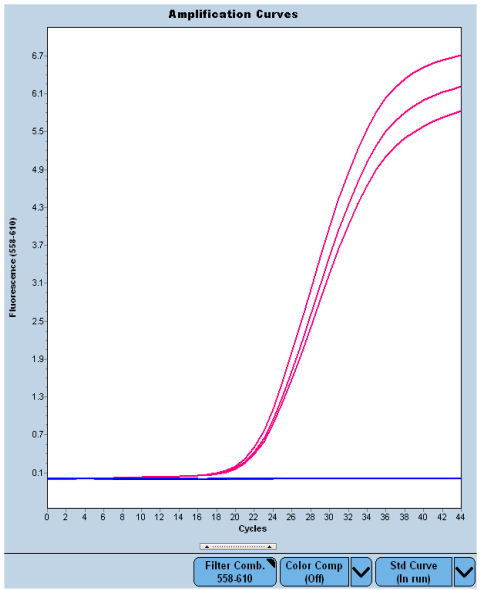
Application with Spectral Crosstalk

FAM – Red 610 – Cy 5 **(PBGD + G6PDH + CyP2 C9)**

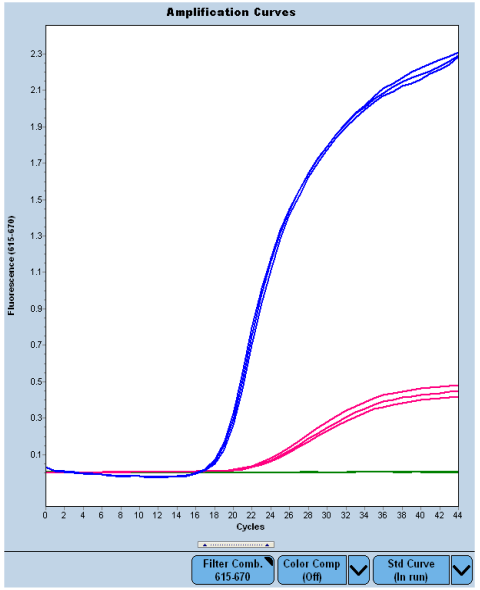
Crosstalk of Cy 5 into channel 610 is reliably compensated



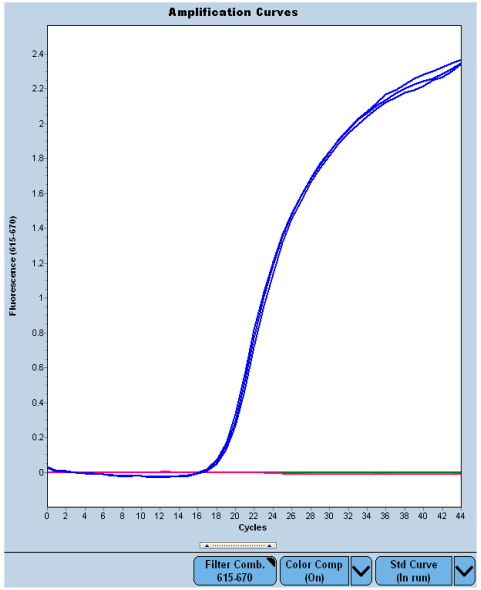
533



610



670 no CC

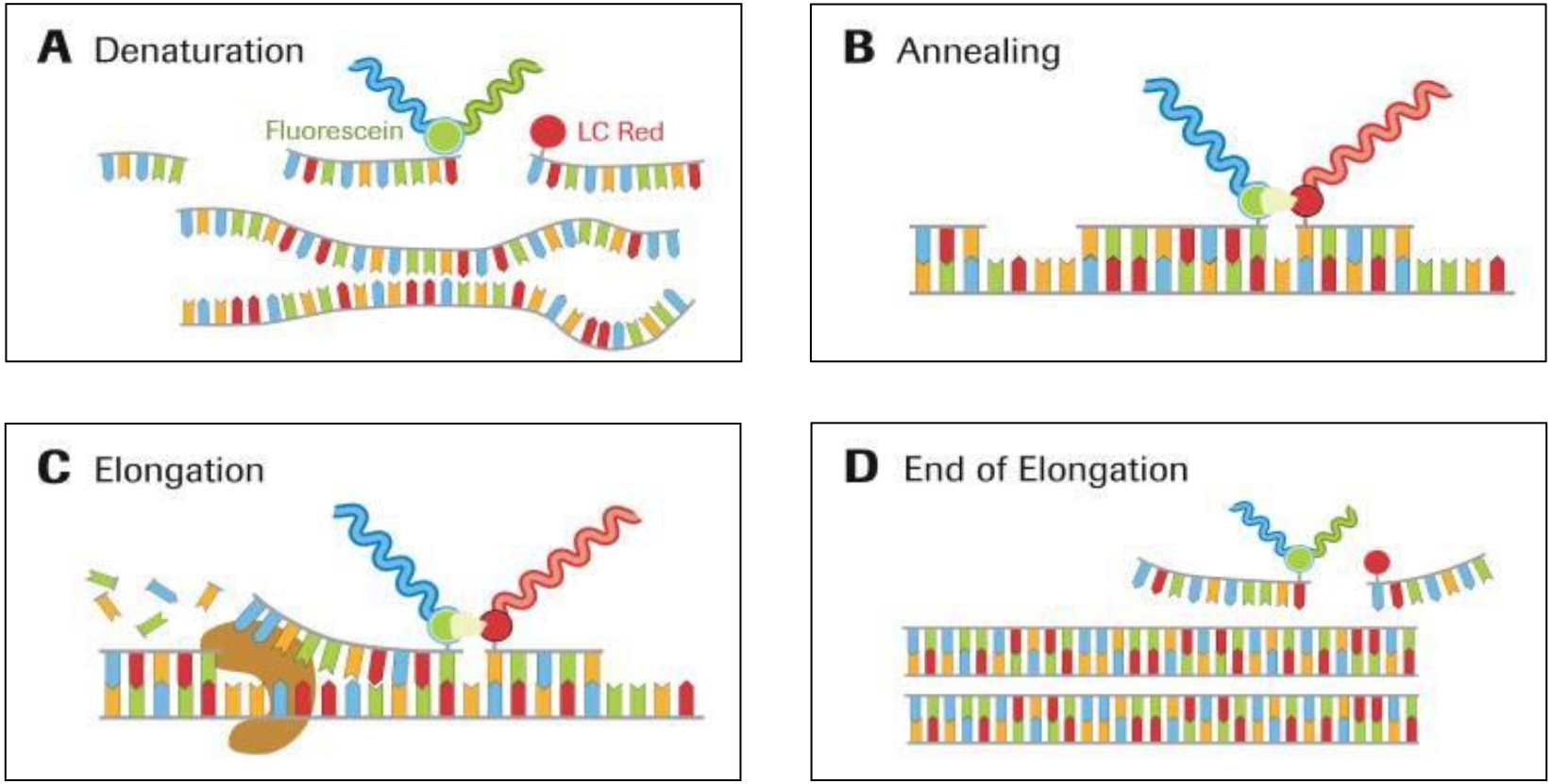


670 with CC

LightCycler® Assay Formats

Hybridization Probes

HybProbe

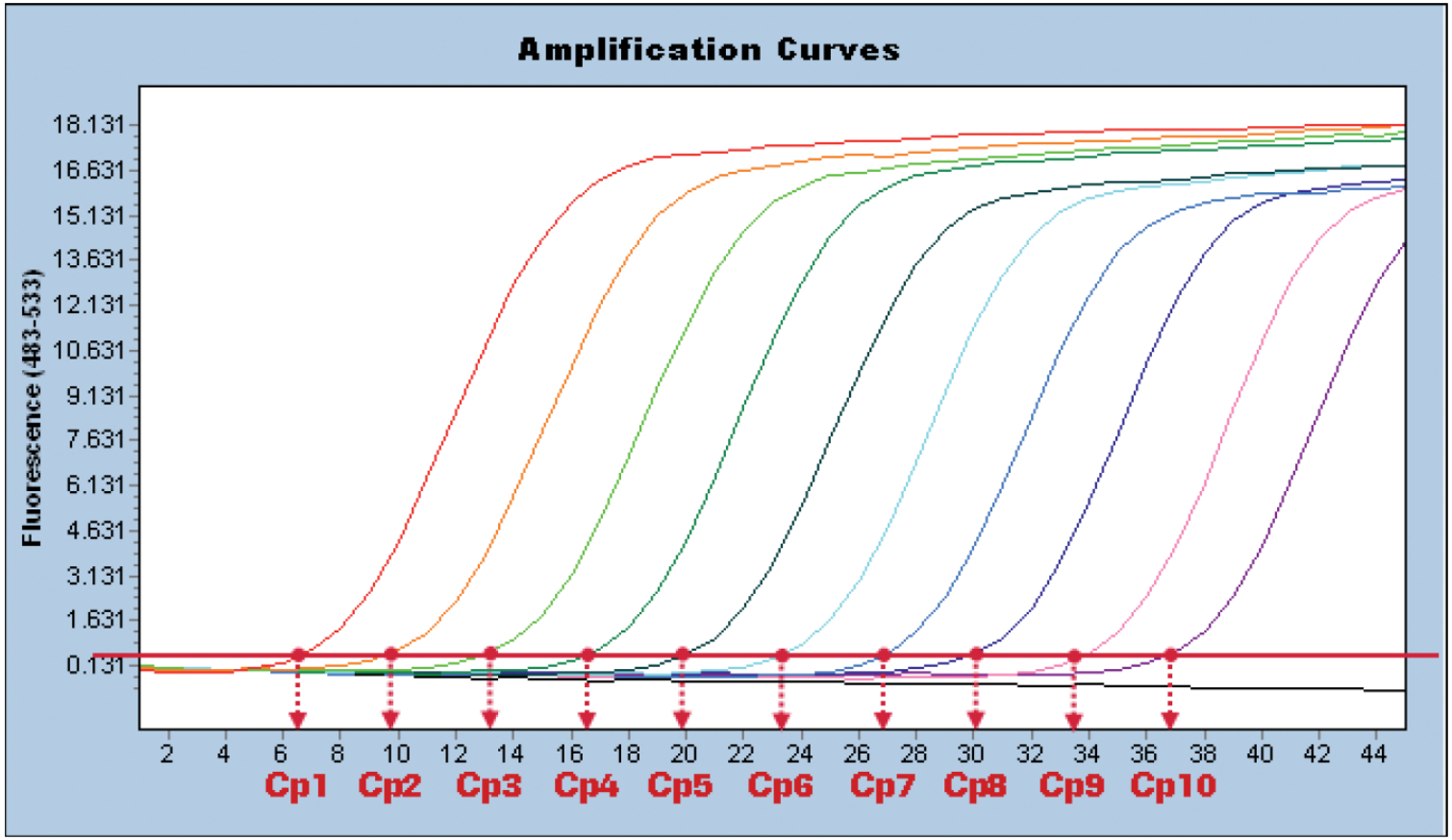


No primer-dimer or side-products' disturbance ! **– High specificity**

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

Clinical Chemistry 55:4
611–622 (2009)

Special Report

The MIQE Guidelines: *M*inimum *I*nformation for Publication of Quantitative Real-Time PCR *E*xperiments

Stephen A. Bustin,[1*] Vladimir Benes,[2] Jeremy A. Garson,[3,4] Jan Hellemans,[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 confu-

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*.

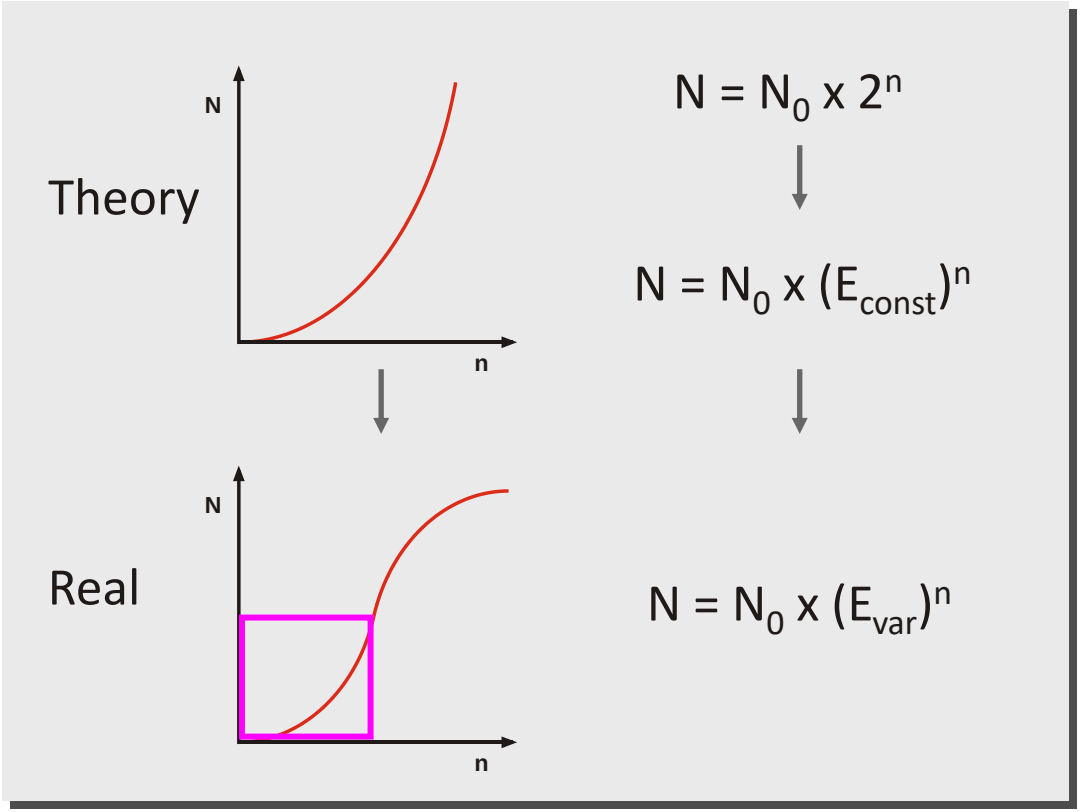
1.4 The term *FRET probe* (fluorescence resonance energy transfer probe) refers to a generic mechanism in which emission/quenching relies on the interaction between the electron-excitation states of 2 fluorescent dye molecules. LightCycler-type probes should be referred to as *dual hybridization probes*.

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

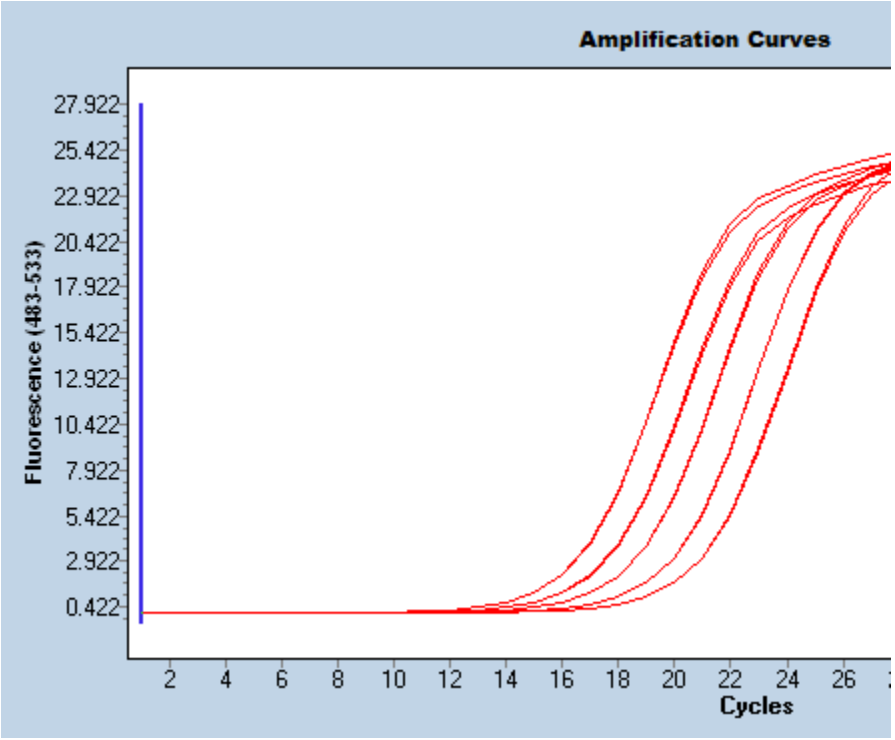
N: number of amplified molecules

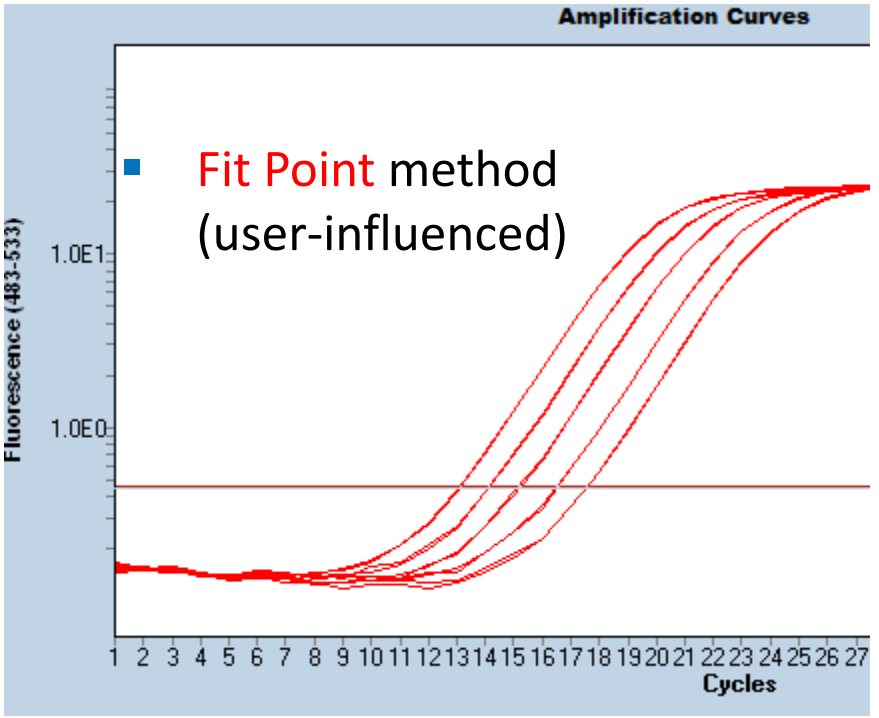
$N_{0:}$ initial number of molecules

n: number of amplification cycles

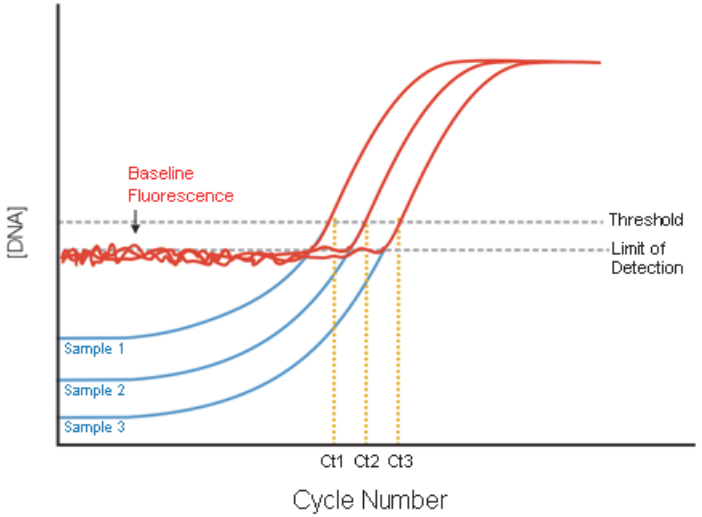
E: amplification efficiency

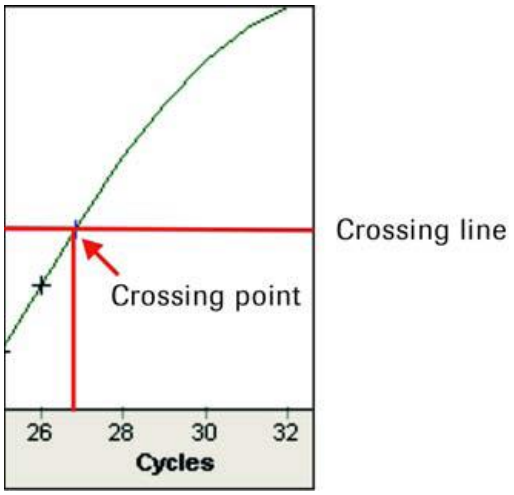
How to calculate the Ct/Cp/Cq value



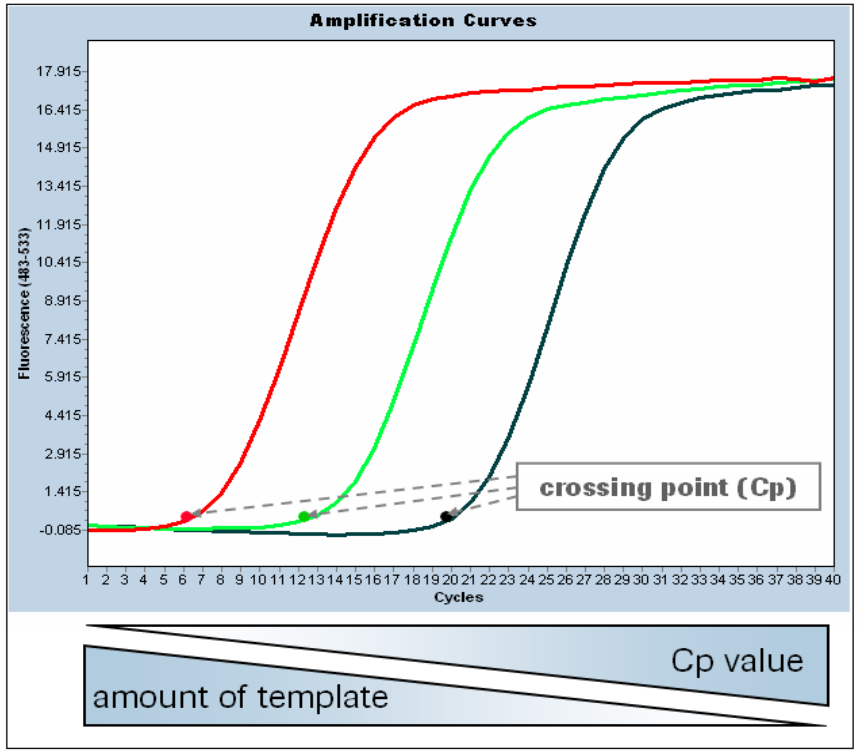


- Fit Point method (user-influenced)





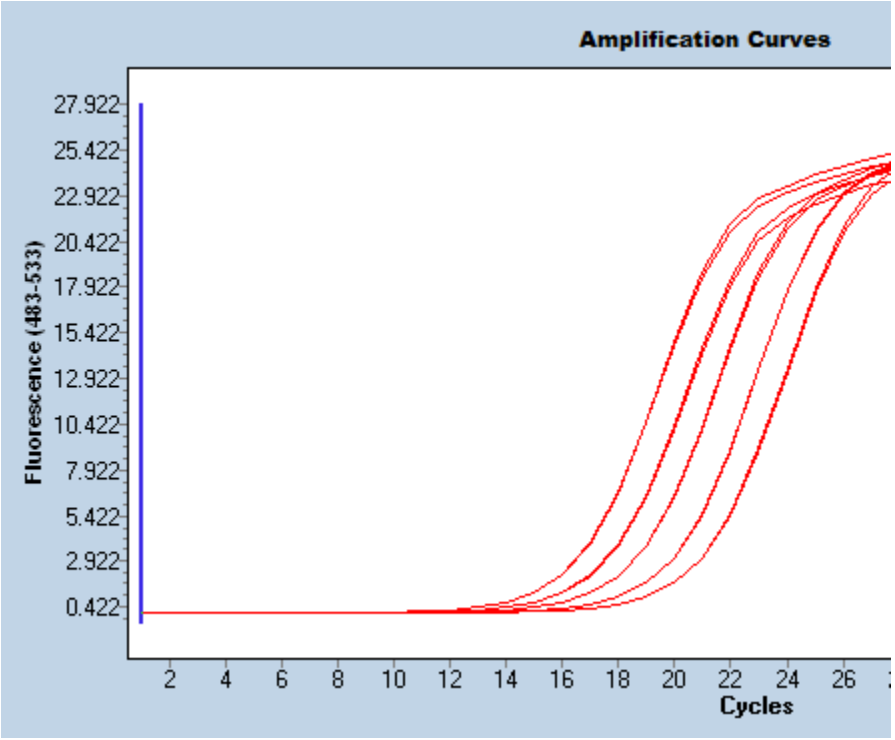
Concentrations and Crossing Points

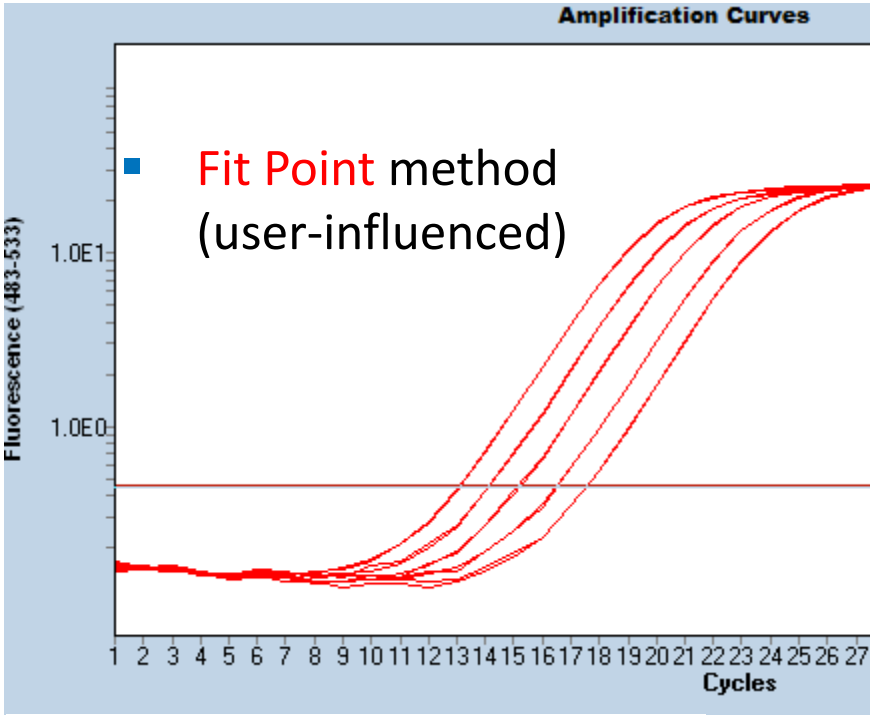


Calculation of crossing points (Cp)

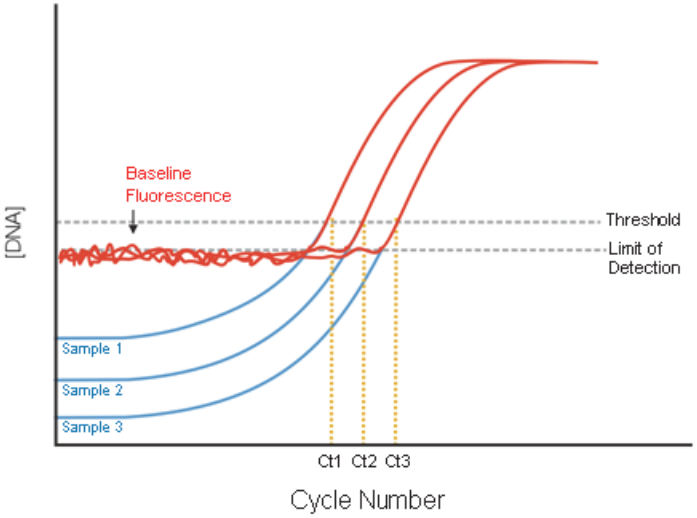
- Optional with Fit Point method (user-influenced)
- Standard method (automatic)
  2nd Derivative Maximum Method
  二次微分最大値

Fit Point method (user-influenced)

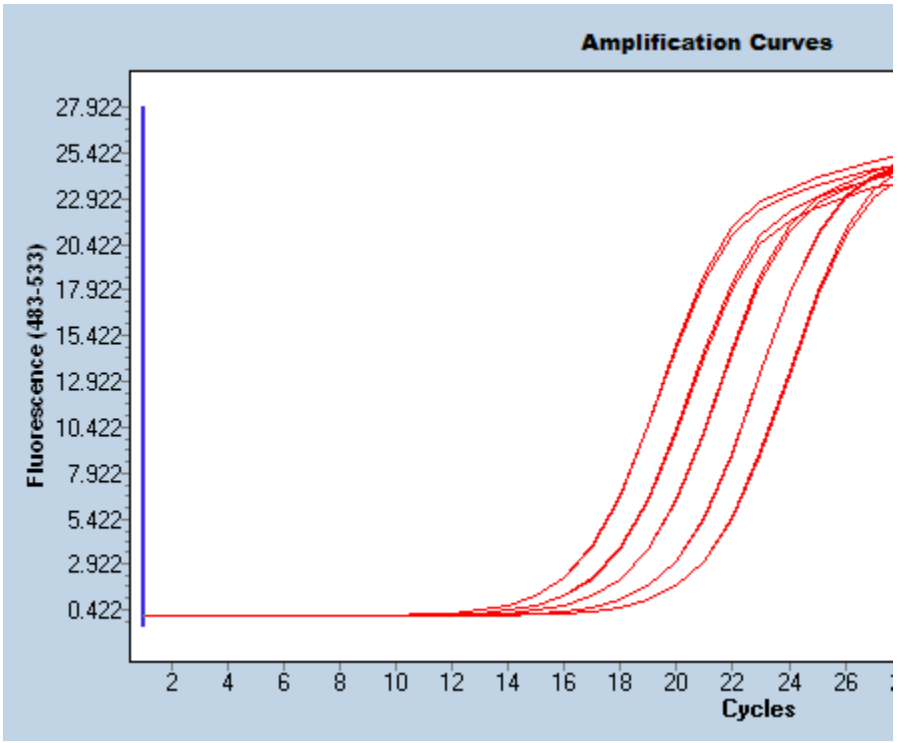


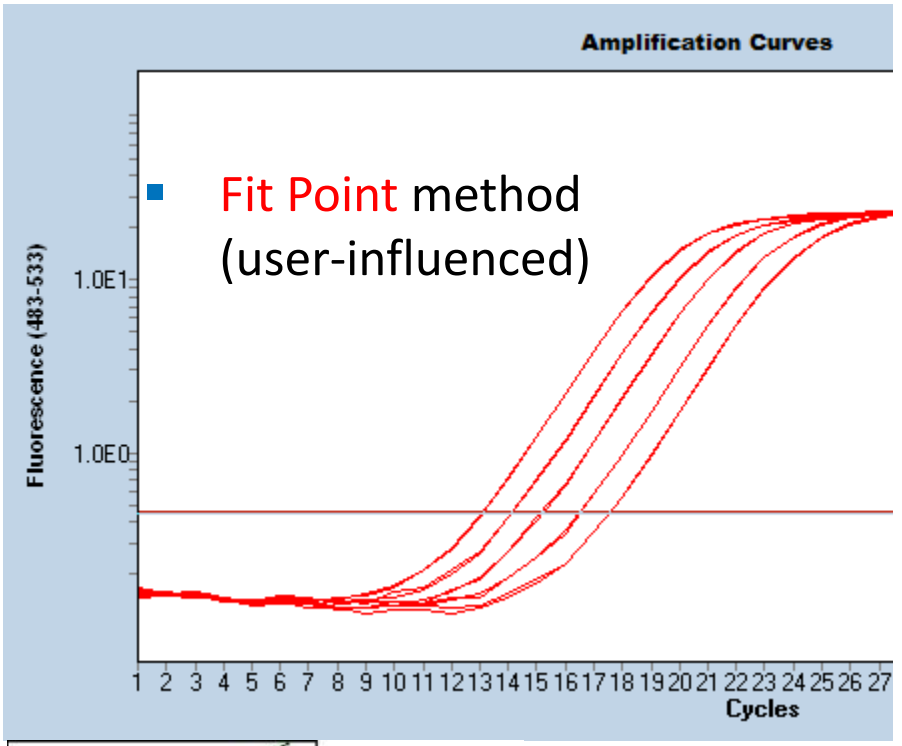


- Fit Point method (user-influenced)

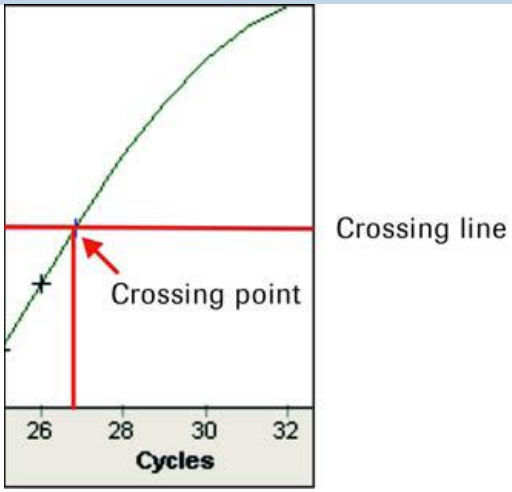


Fit Point method (user-influenced)



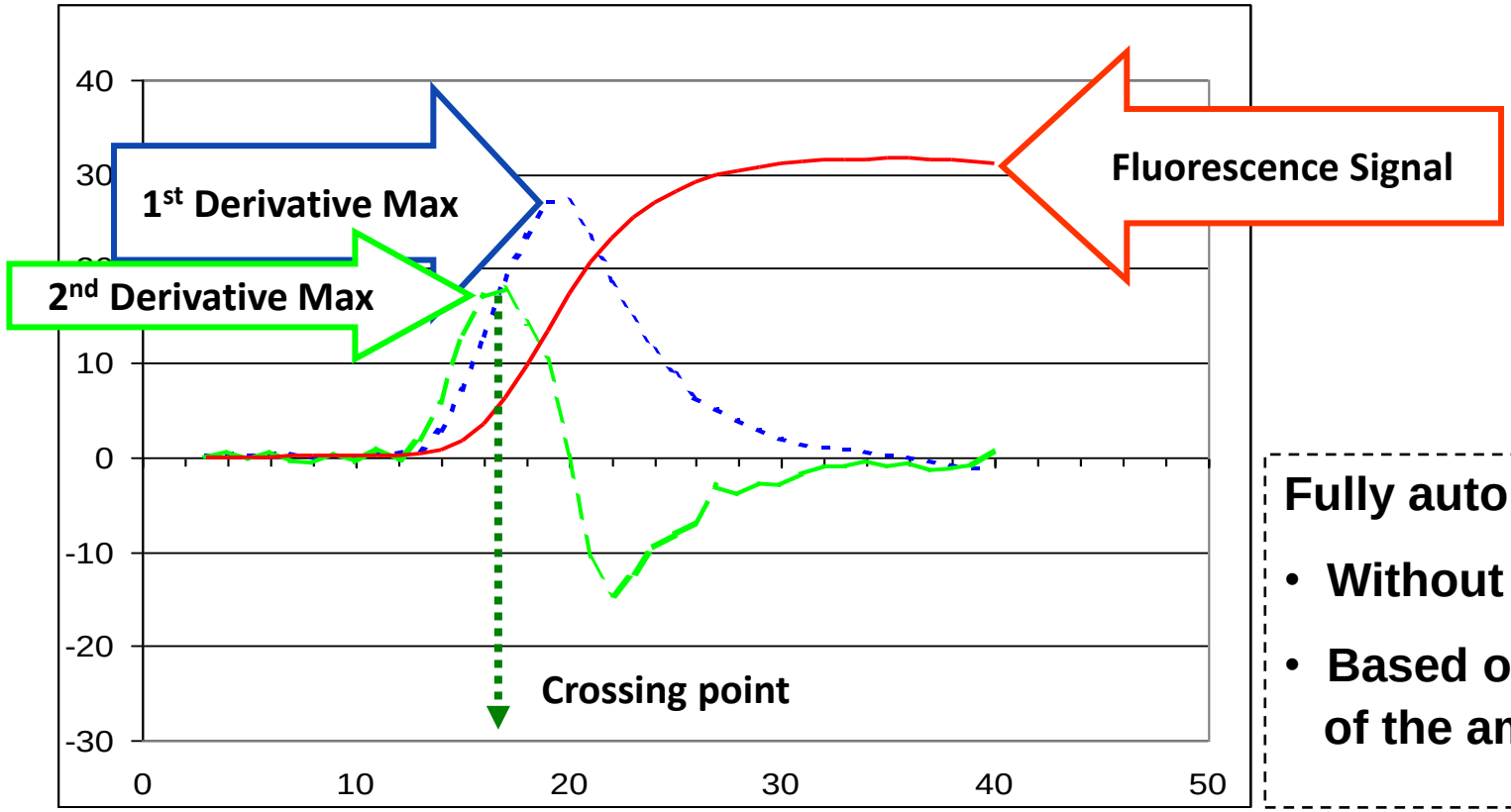


- Fit Point method (user-influenced)



Crossing Point Calculation

2nd Derivative Maximum Method

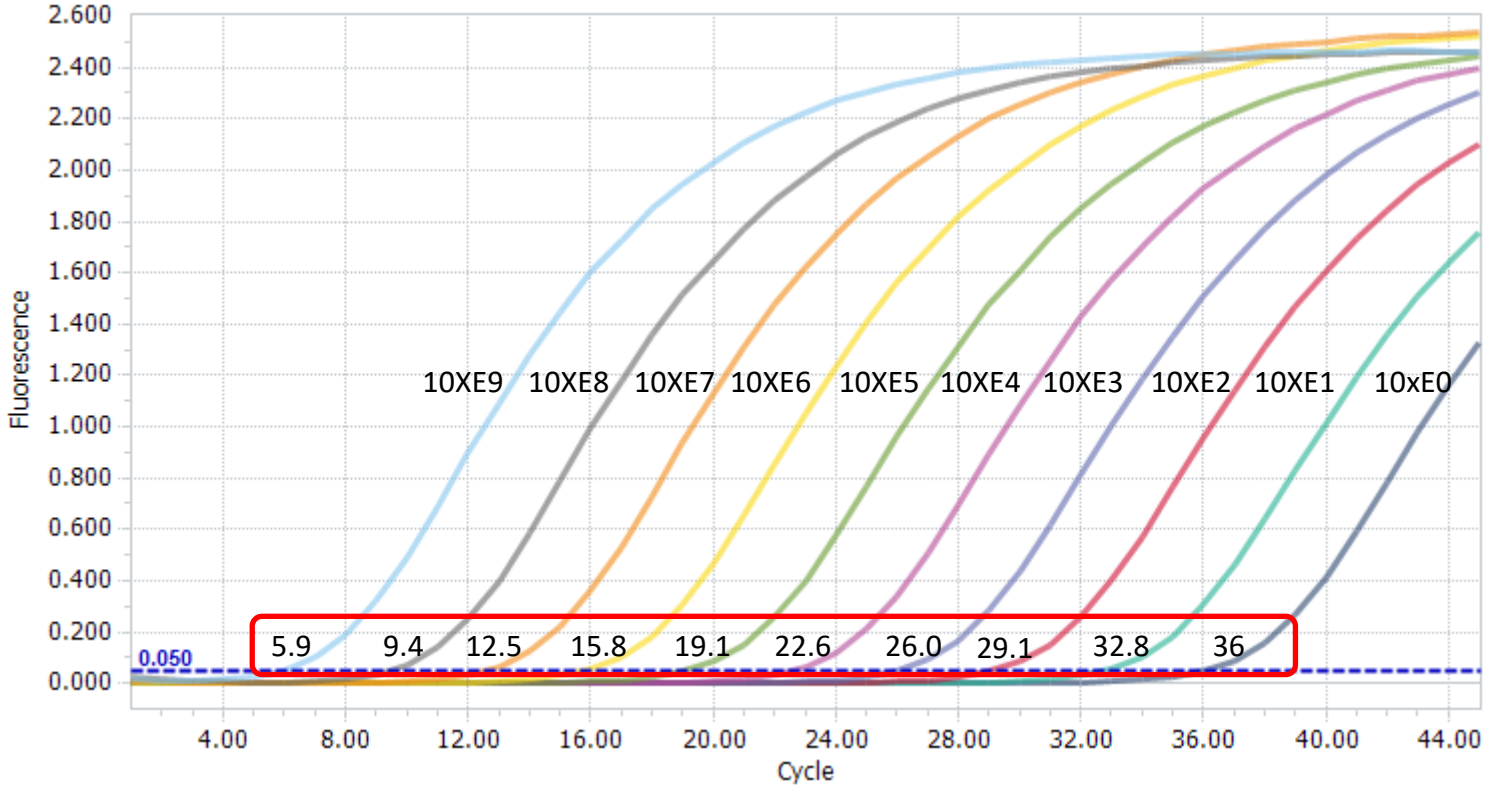


Fully automated method

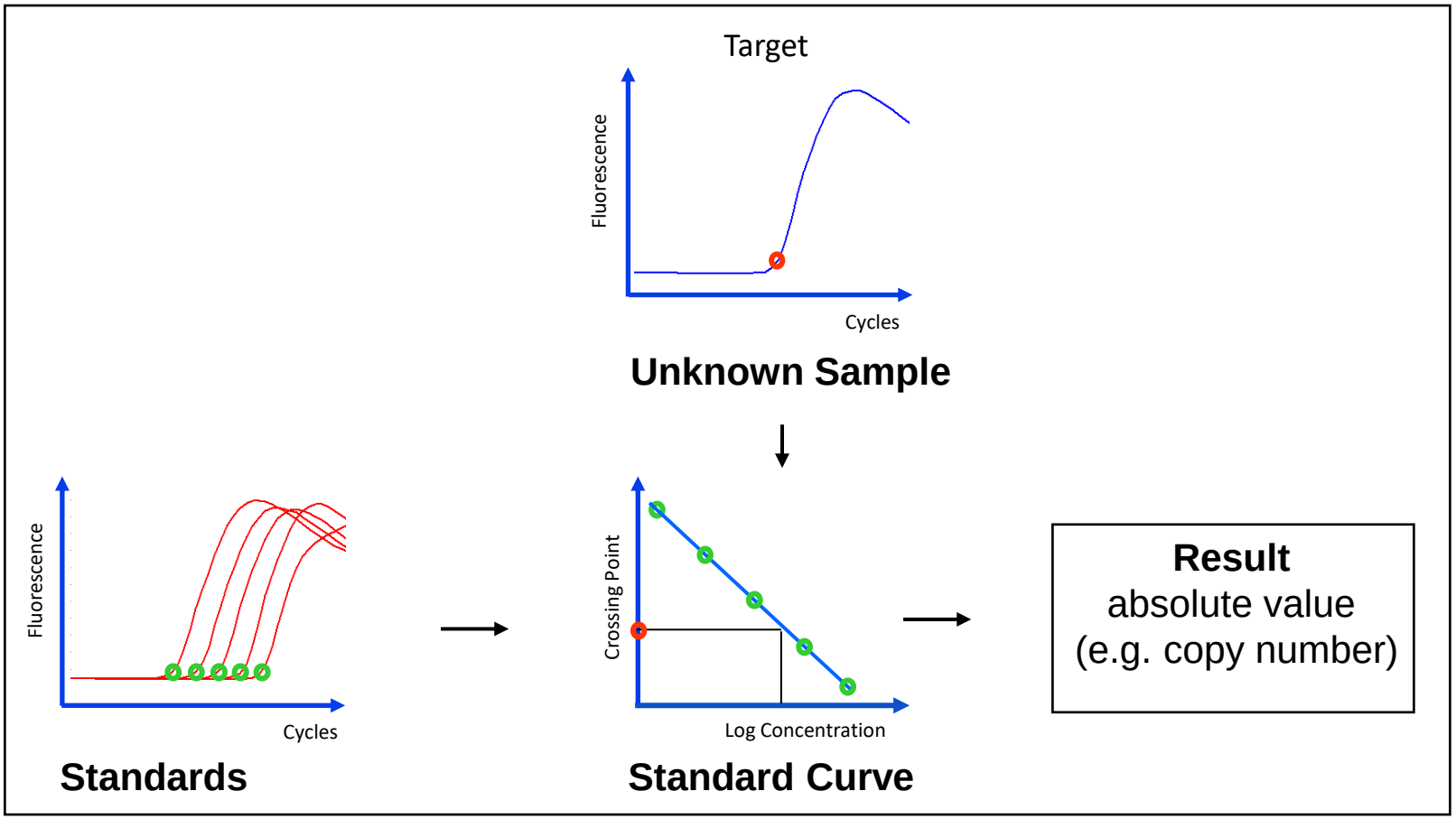
- **Without user influence**
- **Based only on the shape of the amplification curve**

There is a correlation between Cp and concentration

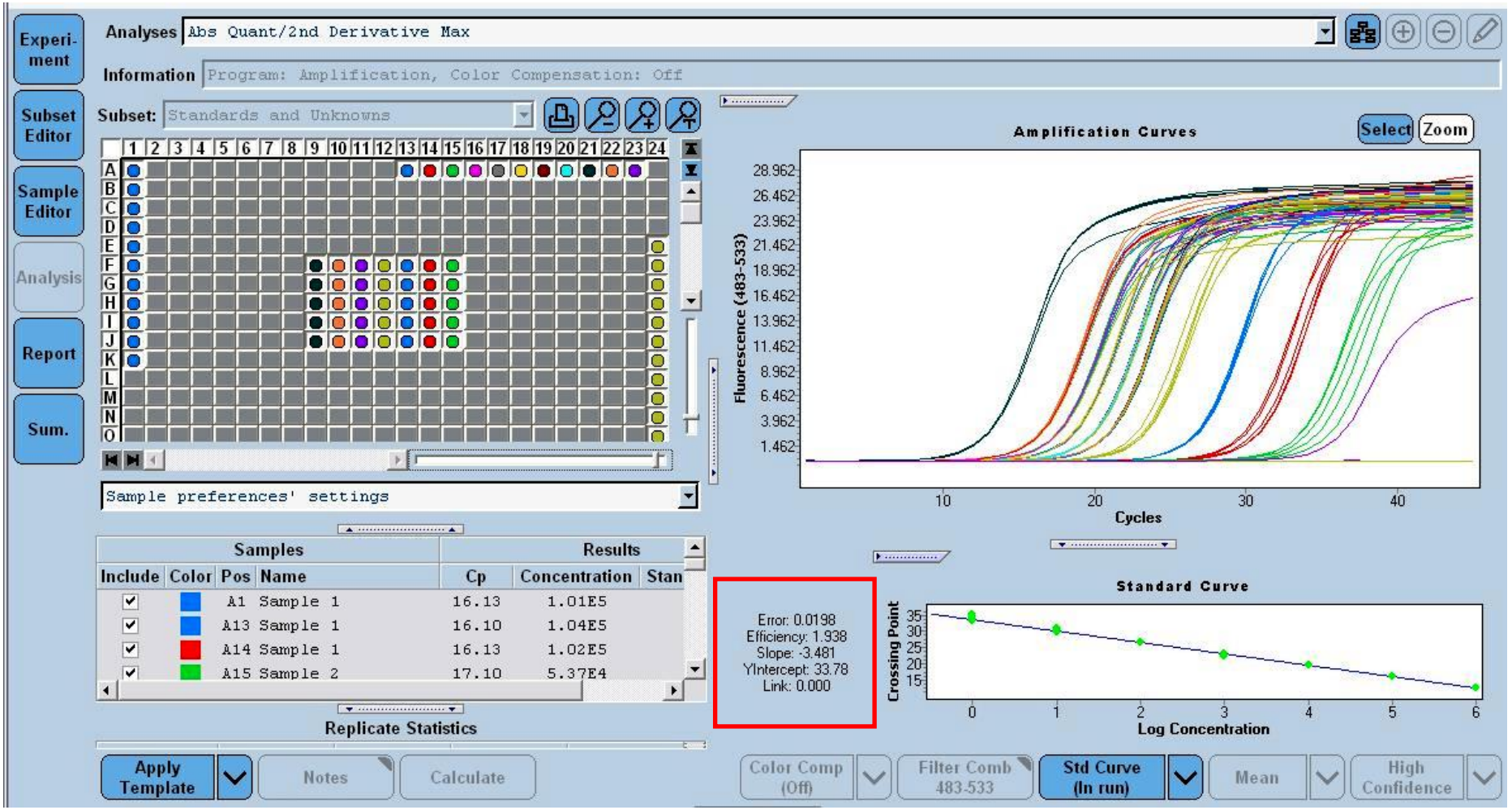
The higher concentration of target nucleic acid in the starting material, the sooner a significant increase in fluorescent signal will be observed, yielding a lower Cycle no.



Absolute Quantification with External Standards: Principle



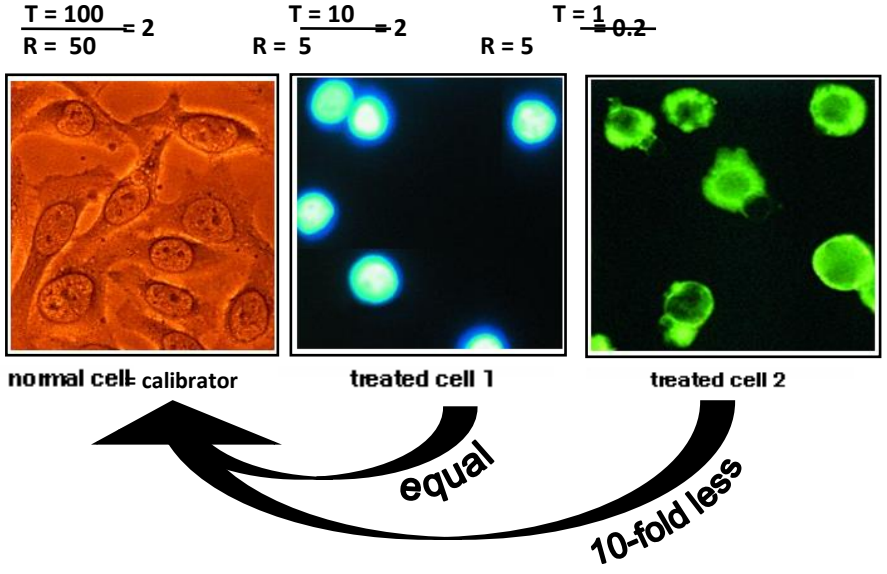
Absolute Quantification with External Standards: Example



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

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

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

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

Type	gene	Cp
Calibrator (control)	Target	25
	Reference	20

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

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

Relative Quantification - Without Efficiency correction

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

Type	gene	Cp
Sample	Target	22
	Reference	21

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

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

Type	gene	Cp
Calibrator (control)	Target	25
	Reference	20

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

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

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

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

Example for Relative Quantification

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

I. Without efficiency correction

$$\text{Relative amount} = 2^{-\Delta\Delta CT}$$

$$= 2^{-[(22-21)-(25-20)]} = 2^{-[1-5]} = 2^{4} = 16$$

II. With efficiency correction

$E_T = 1.6$

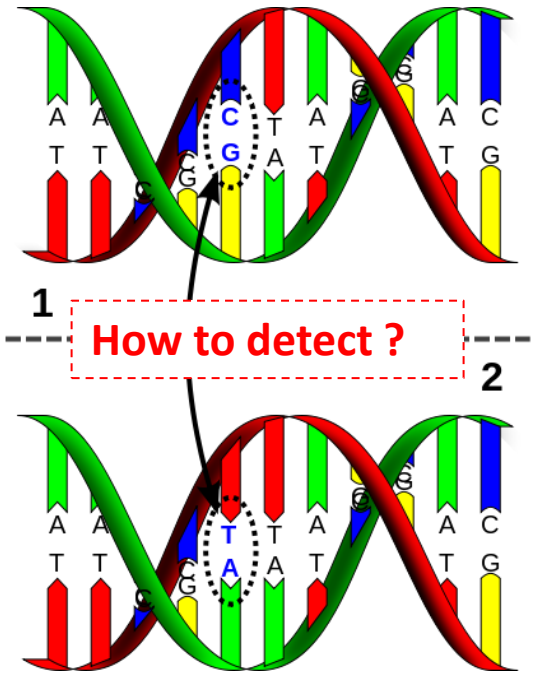
$E_R = 1.8$

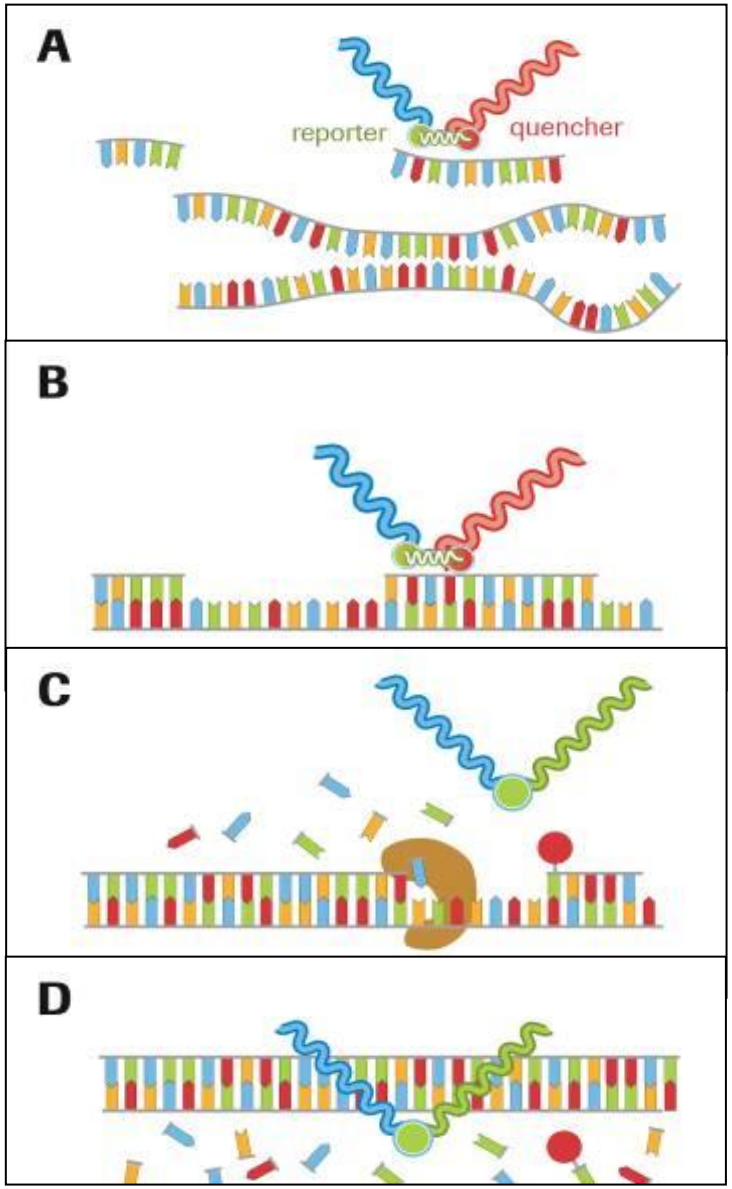
$$\text{Relative amount} = E_T^{CpT(C) - CpT(S)} \times E_R^{CpR(S) - CpR(C)}$$

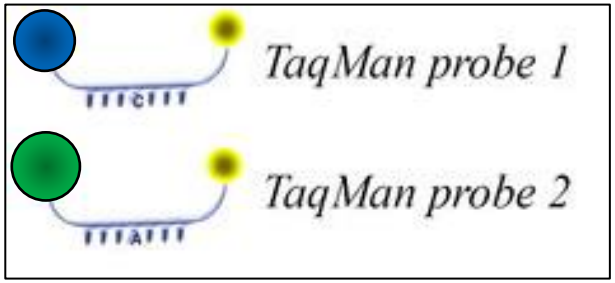
$$= 1.6^{(25-22)} \times 1.8^{(21-20)} = 1.6^{3} \times 1.8^{1} = 7.37$$

Endpoint Genotyping (allelic discrimination)

Principle

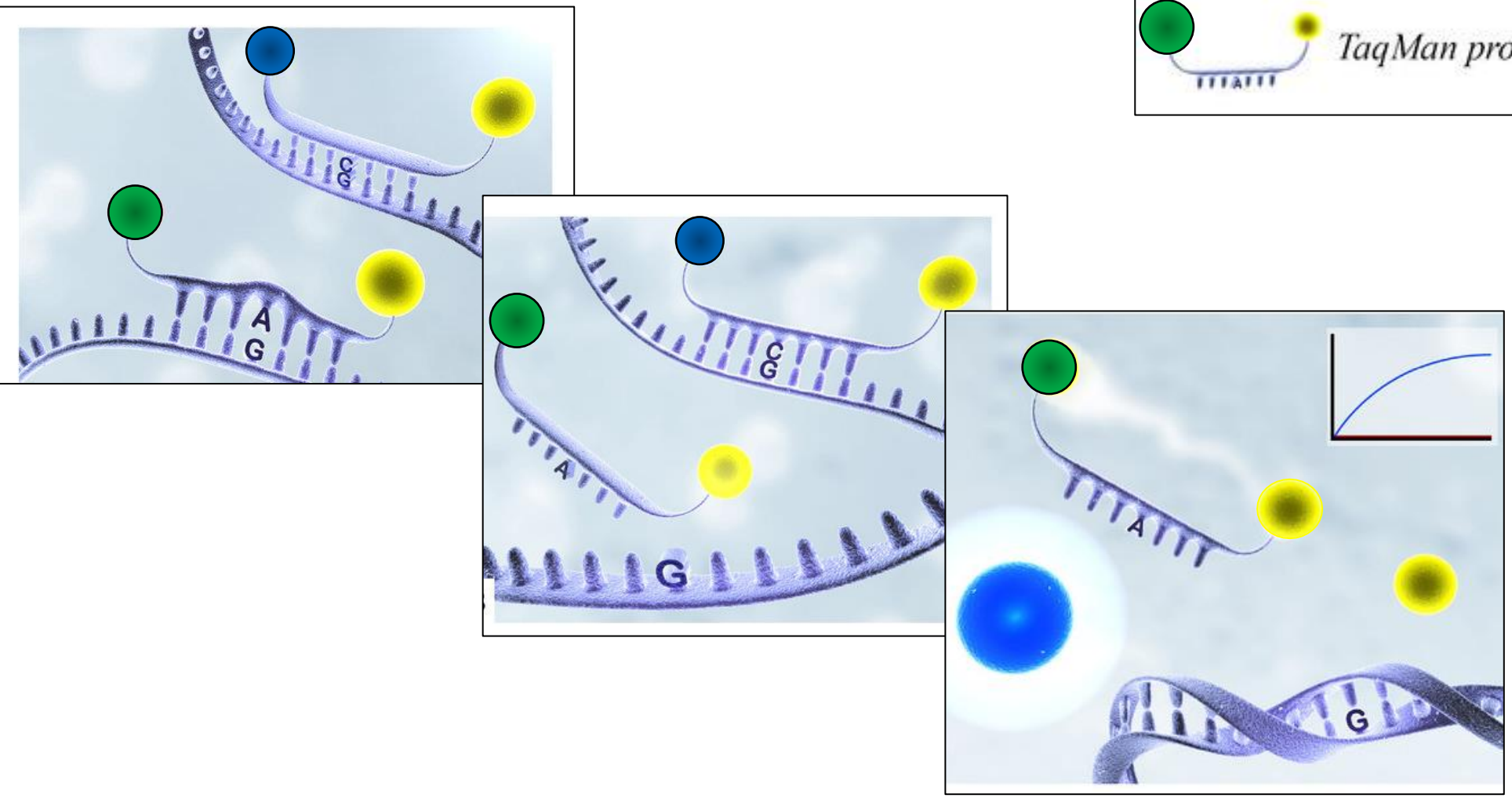






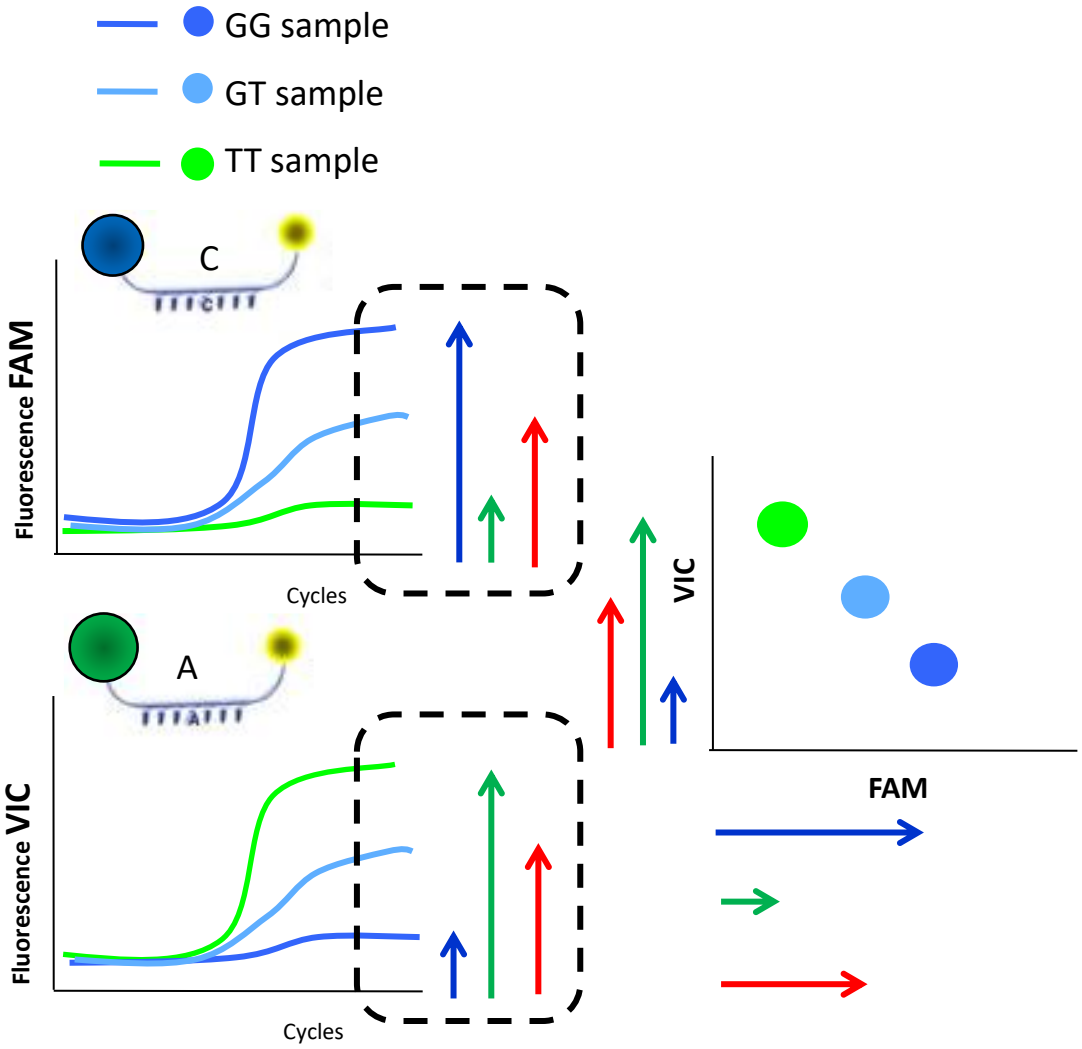
Endpoint Genotyping (allelic discrimination)

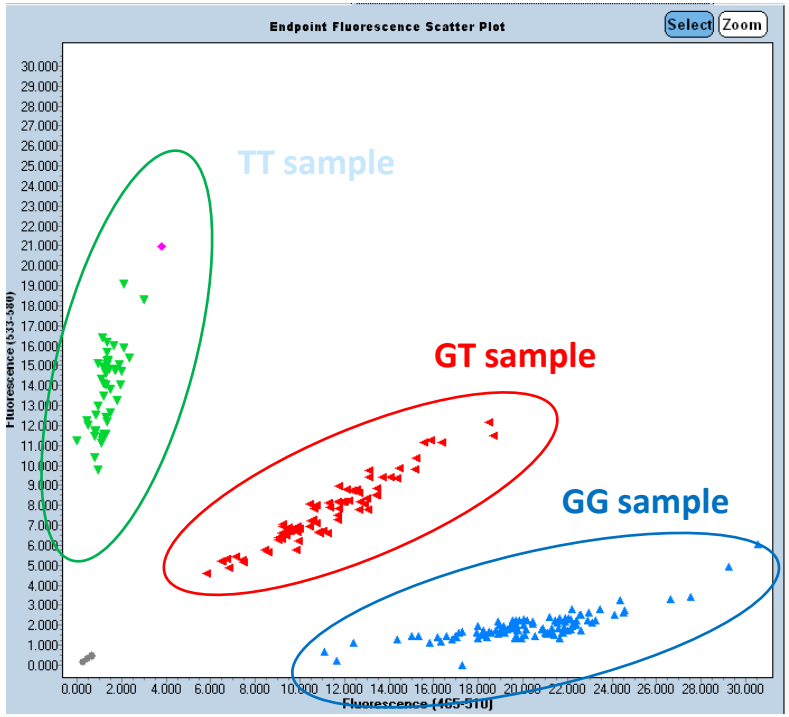
Principle



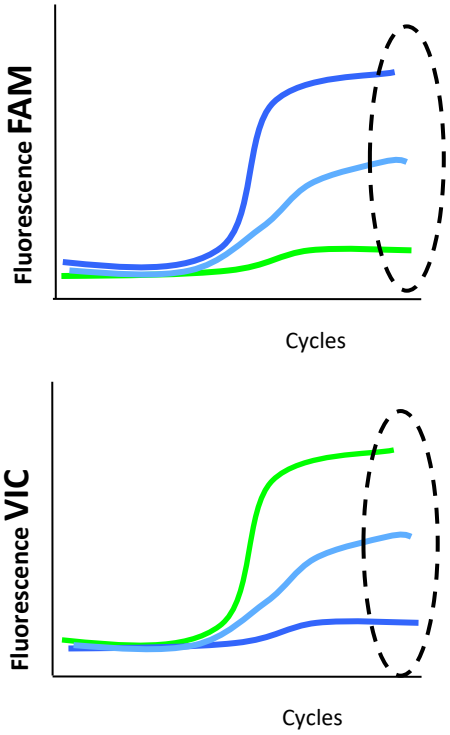
Endpoint Genotyping (allelic discrimination)

Principle

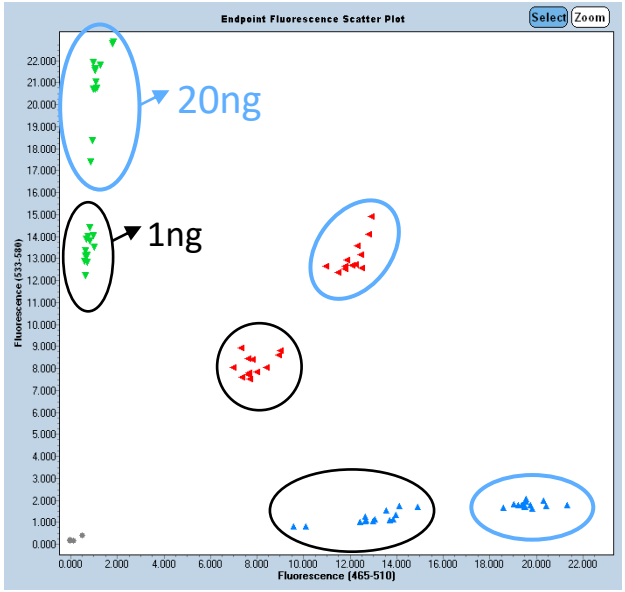


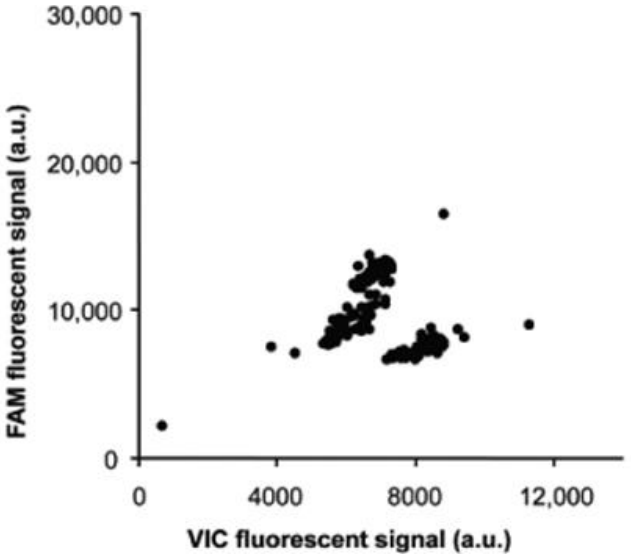


Endpoint Genotyping Data
Influence of DNA Concentration



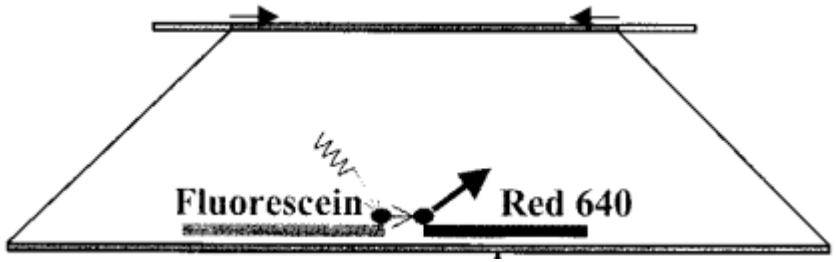
1 and 20 ng DNA

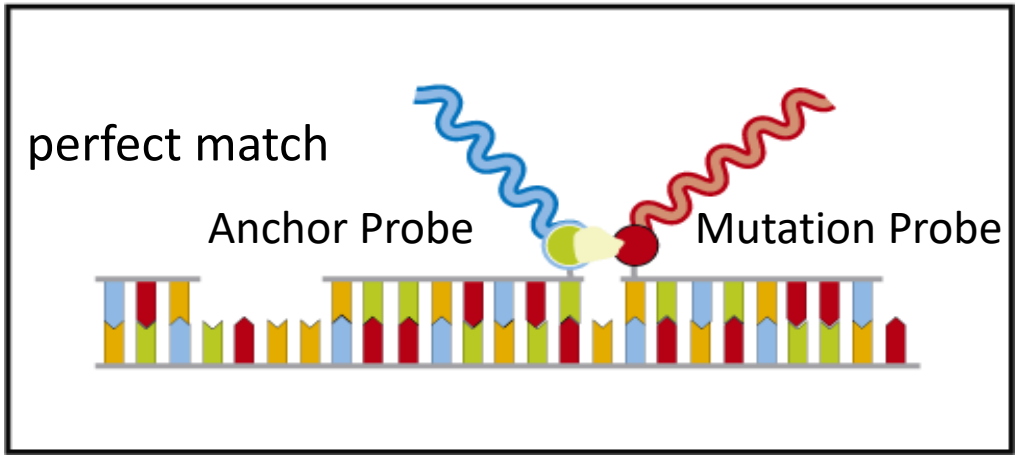


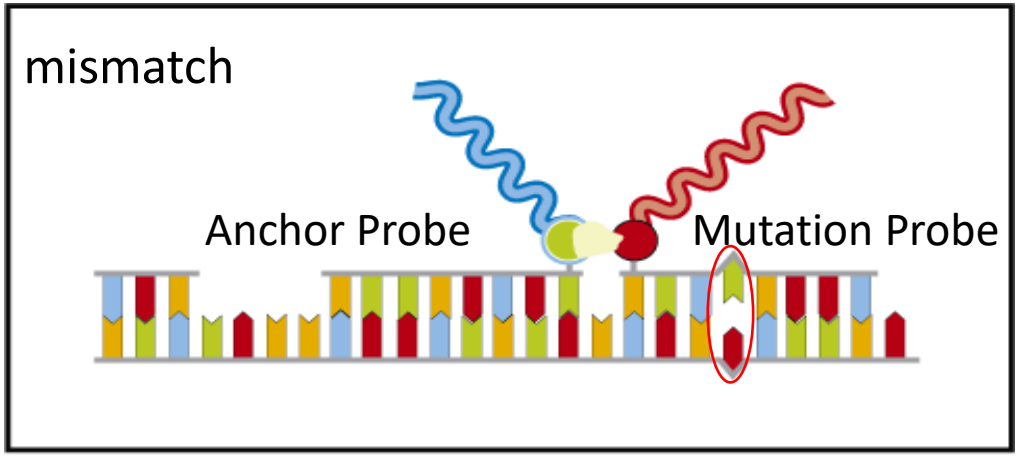


Melting Curve Genotyping

Principle

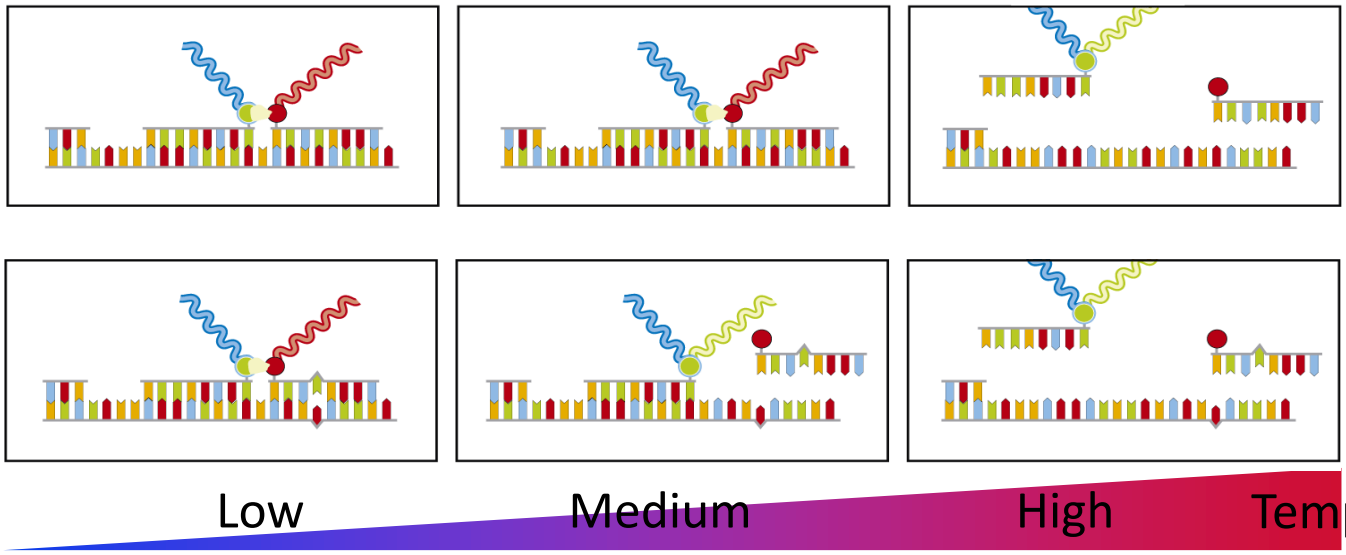


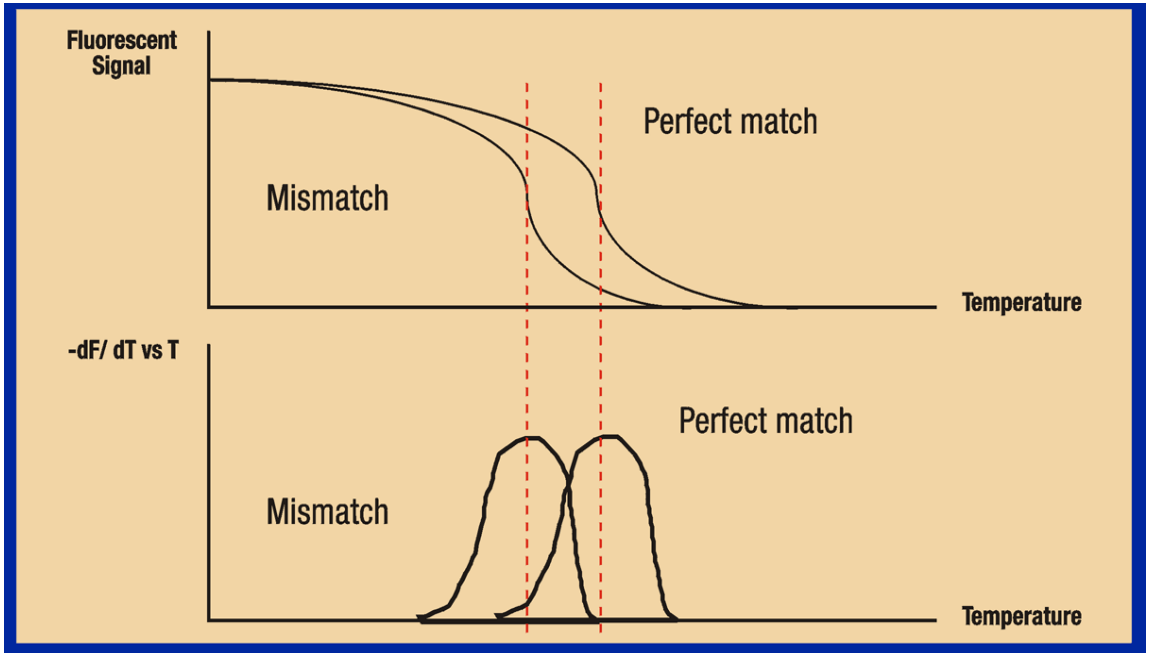




Melting Curve Genotyping

Principle





Melting Curve Based Genotyping

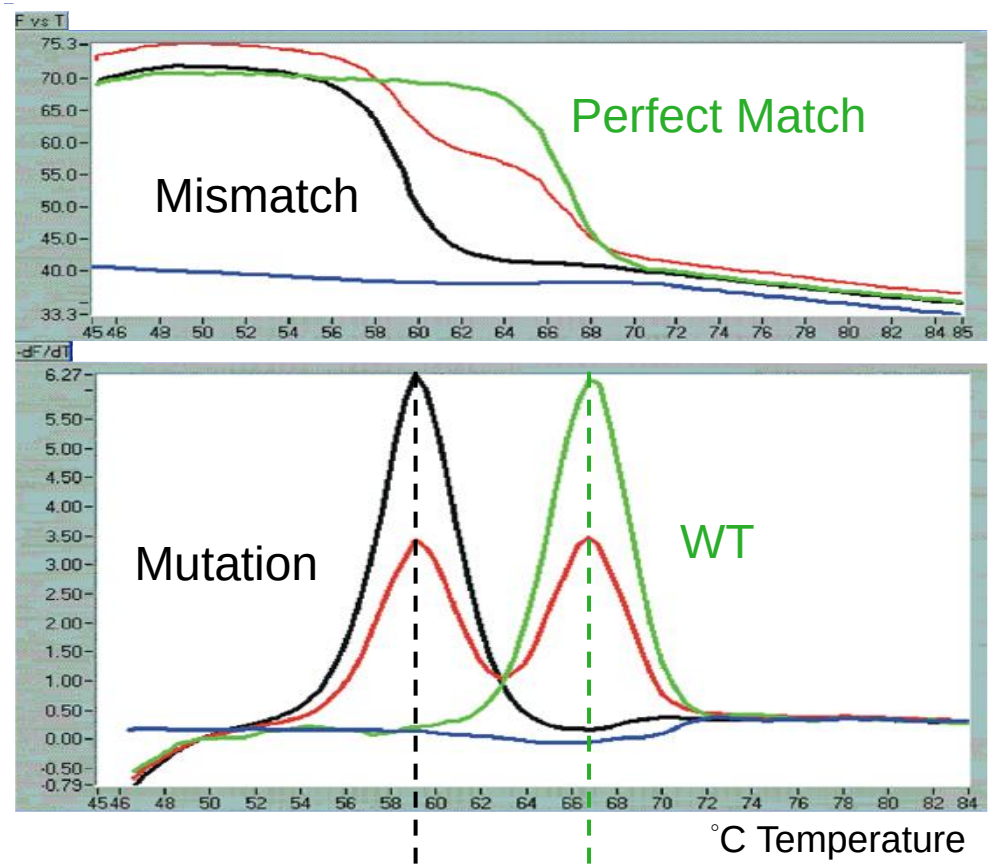
Genotyping of Single Point Mutation

Melting Curves

Fluorescence

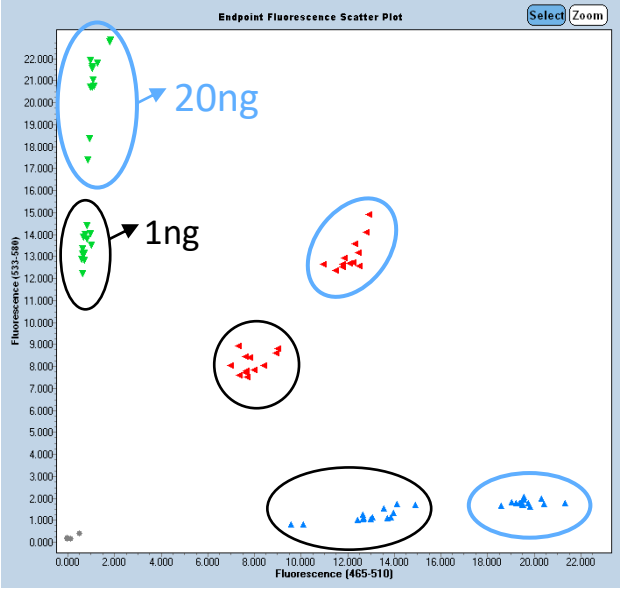
Melting Peaks

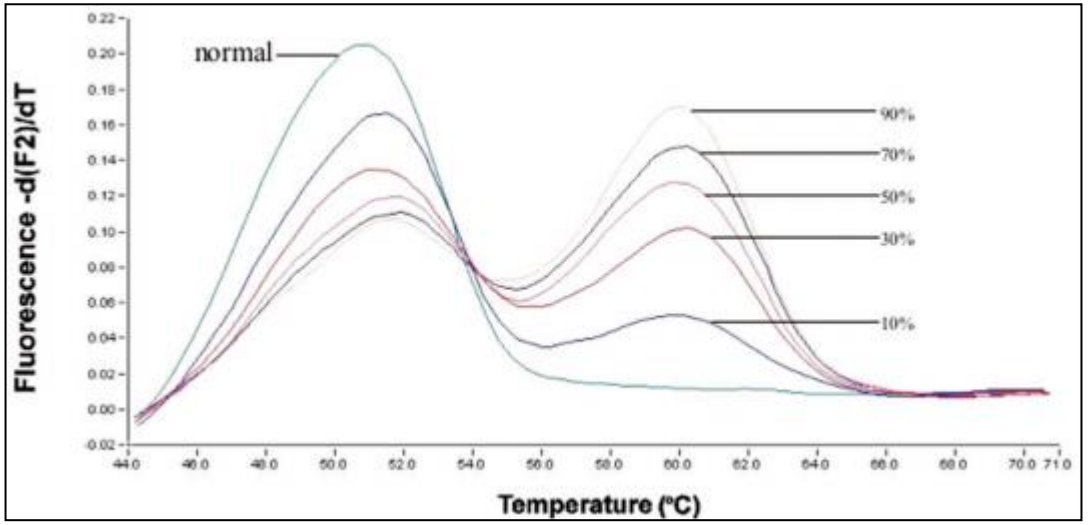
-dF/dT



Endpoint & Melting Genotyping Data
Influence of DNA Concentration

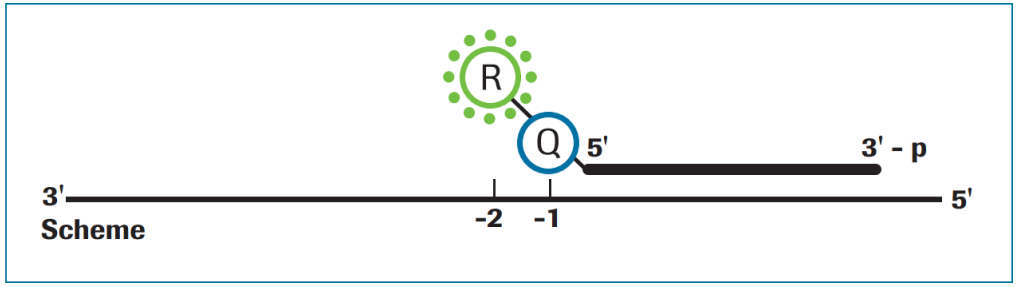
1 and 20 ng DNA

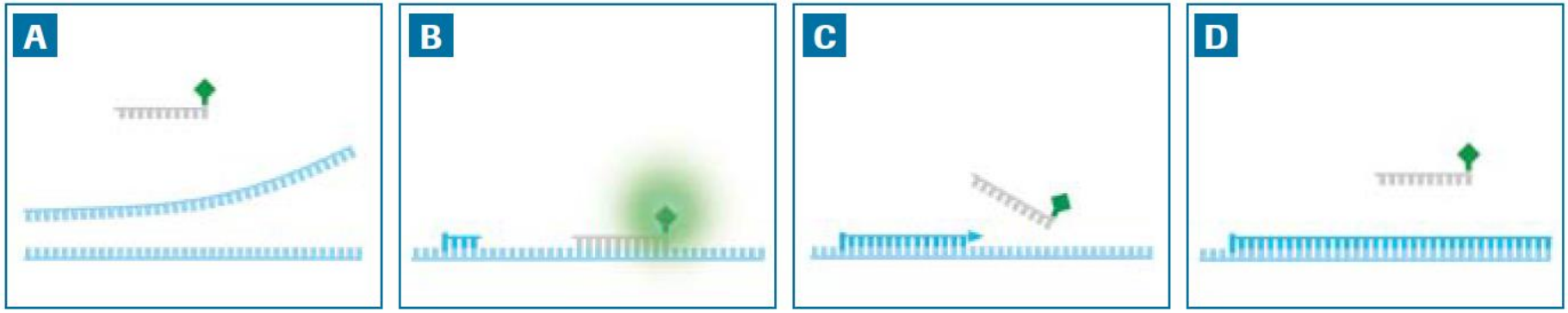




Melting Genotyping Data

SimpleProbe



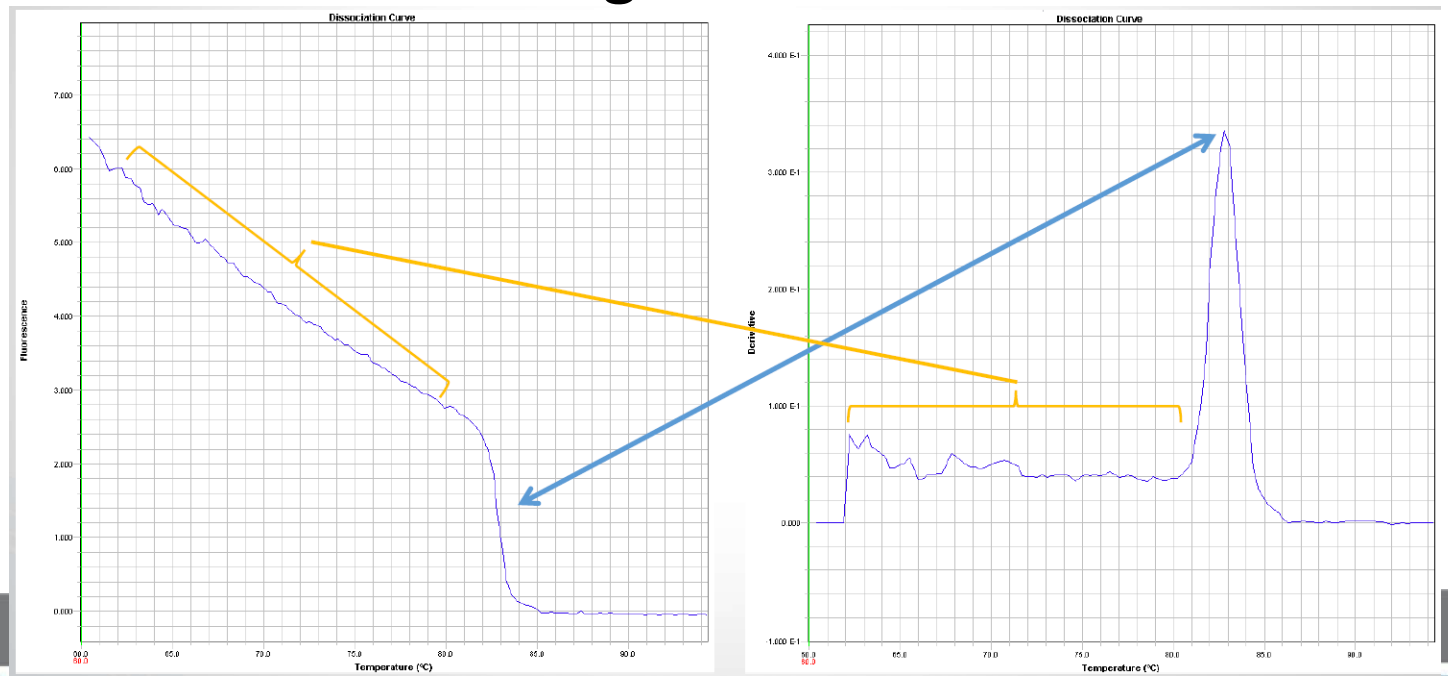


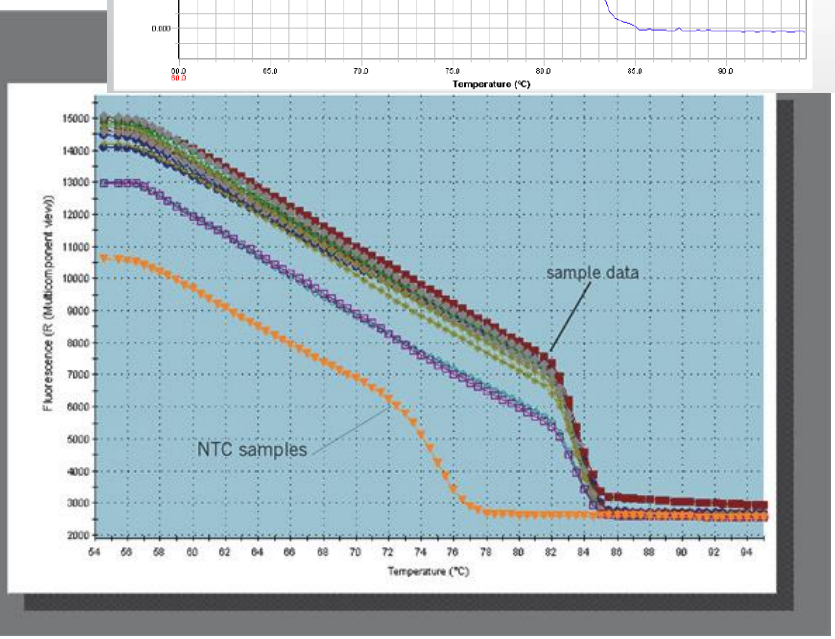
- Real Time PCR Basic Training
- Troubleshooting cases sharing
- System Operation Procedures
- LC 96/LC 480 QC Report
- Q&A

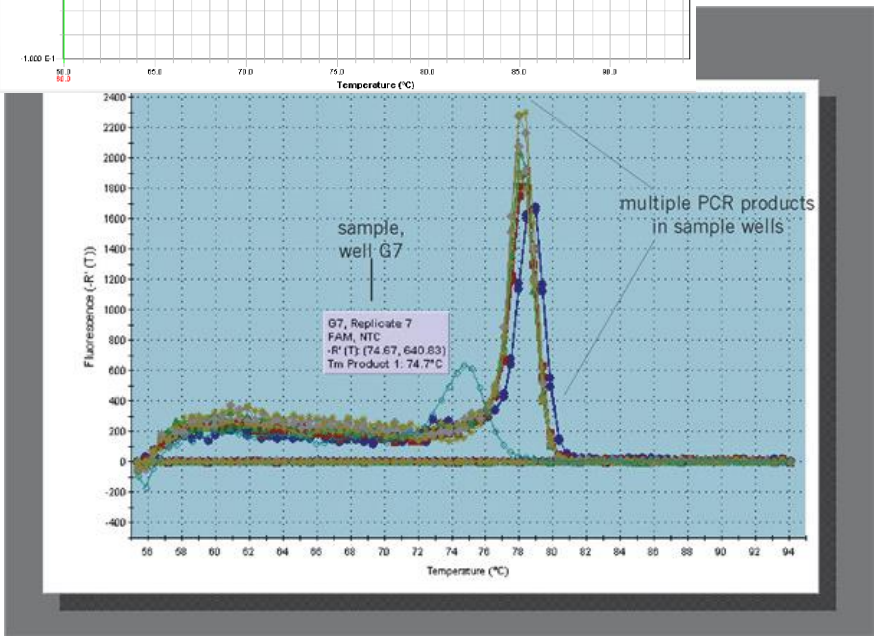
Troubleshooting cases sharing:

- Shoulder of Melting Curve
- Primer Dimmer 1
- Primer Dimmer 2
- gDNA contamination
- Effect of master mix

Case 1: Shoulder of Melting Curve

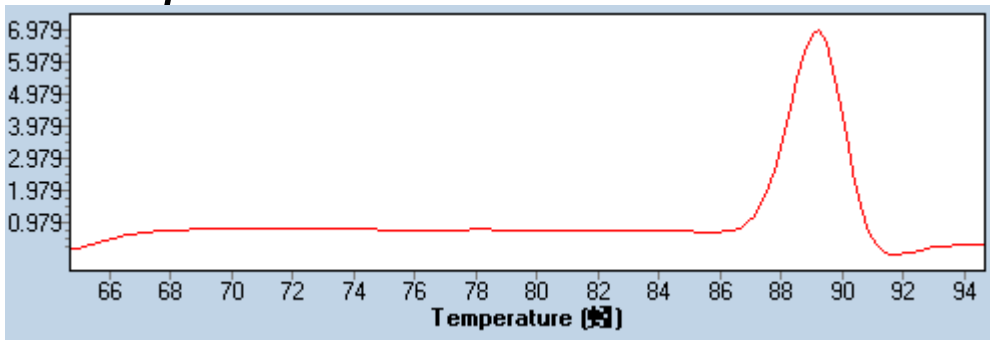




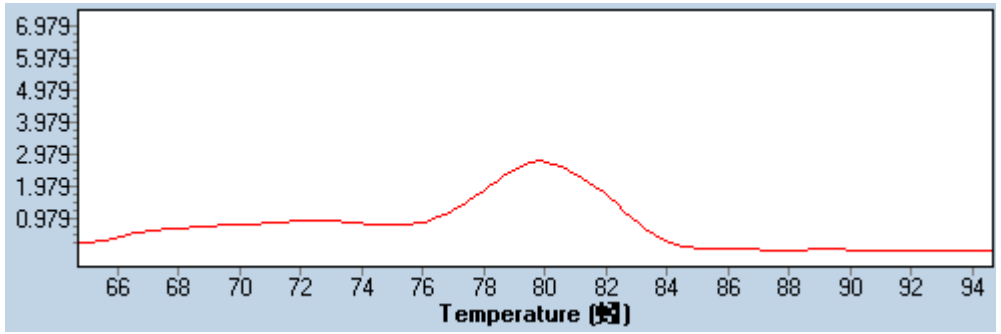


Case 2: Primer Dimmer

No Template Control



Positive Sample Melting curve

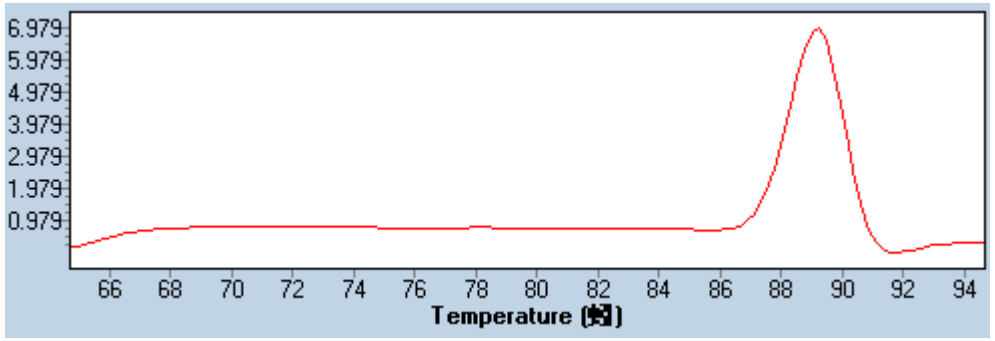


1.Primer dimer：

Primer conc. ↓

Primer design

Amplicon length：100bps↑

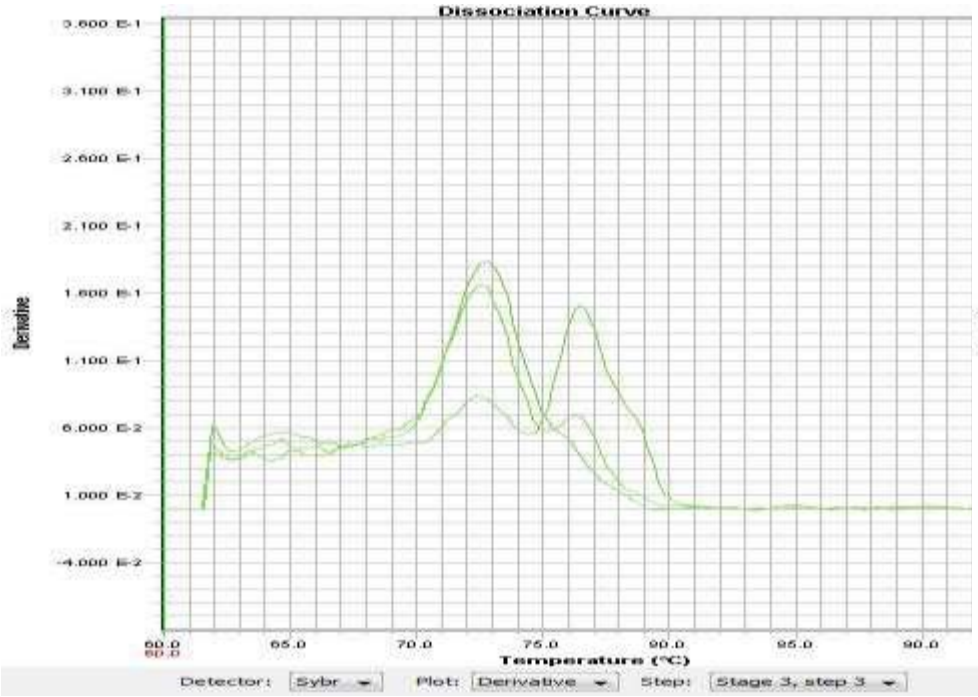


2.Contamination

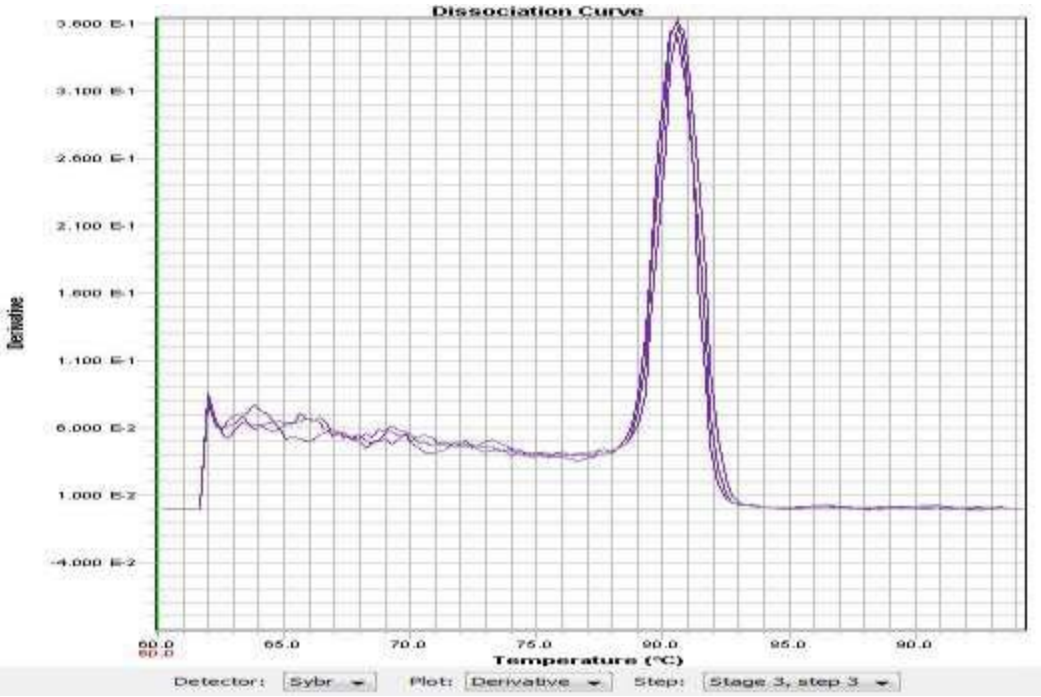
Case 3: Primer Dimmer

Not all primer dimmers are a problem for an Assay

NTC



Sample Results



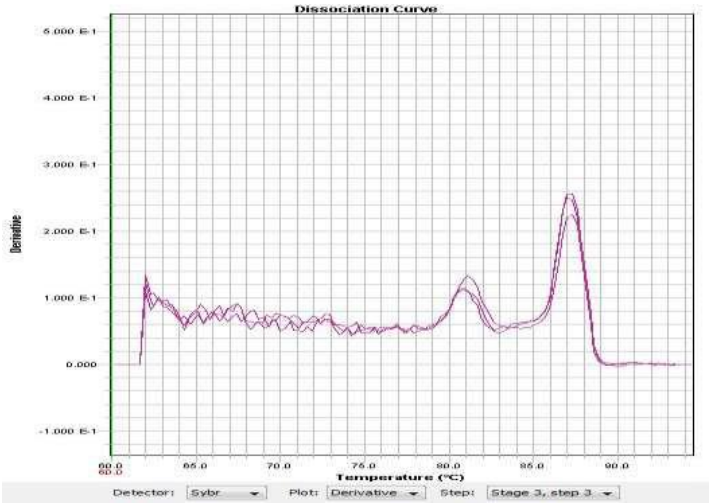
How to Design Primers for QPCR

- PCR product/Amplicon size : 50-210 base pairs.
- Primer length : 19-23 nucleotides.
- GC content : 35-65%.
- Melting temperature (Tm) : 60-68 °C. The annealing temperature for the assay is 5 °C lesser than the Tm of the primers.
- Exon-exon junction : When amplifying cDNA by QPCR, the primers should span exon-exon junction to avoid the amplification of contaminating DNA.
- Repeats and runs : Dinucleotide repeats (TCTCTCTCTC) and repeated nucleotides (eg. TAAAAAAAGC) should be avoided.
- 3’ Complementarity : The complementary regions of the 3’ ends of forward and reverse primers should be avoided to prevent the formation of primer-dimers.
- 3’ Stability : G or C residues should be included at the 3’ end of the primer to increase the stability of the annealing.
- GC clamp : One or two GC clamps at the 5’ end of the primer increases the specificity of the annealing.
- Specificity : The specificity of the primers should be checked by BLAST
- SNPs : Primers should not contain any known SNP (single nucleotide polymorphism) variations

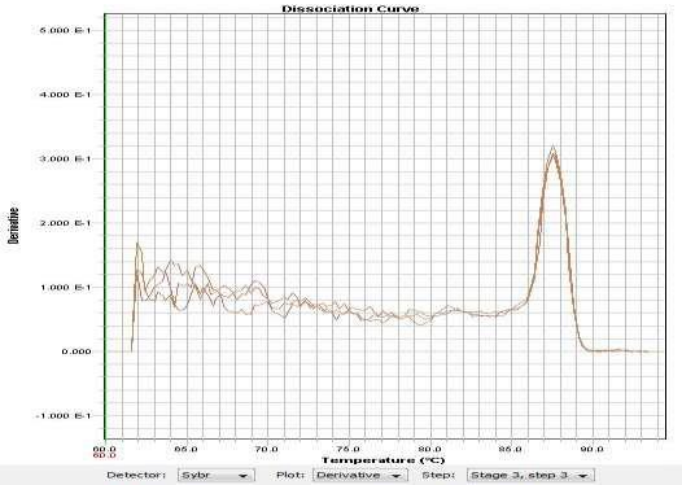
Case 4: gDNA contamination

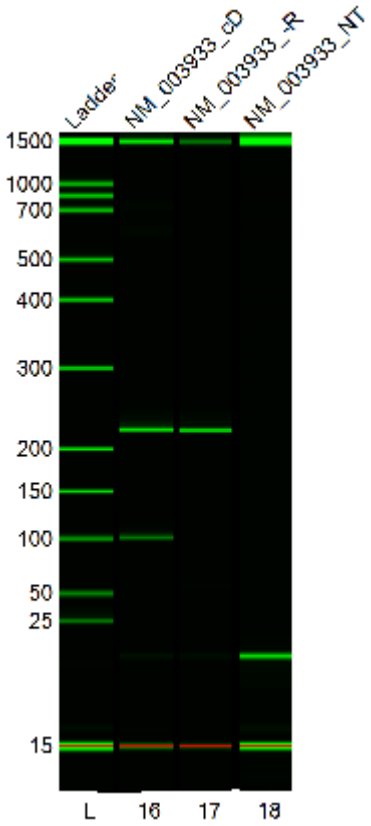
Cross Intron Assay Design

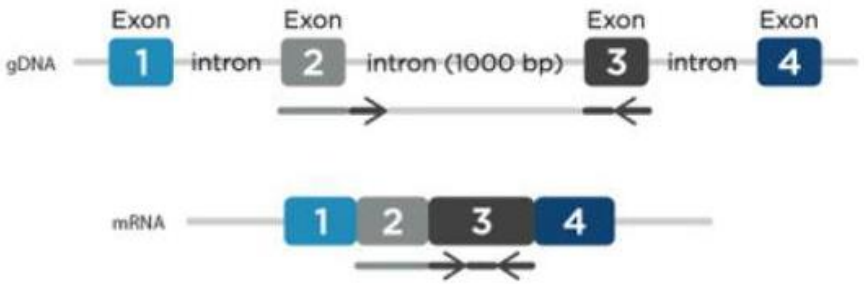
Sample Results



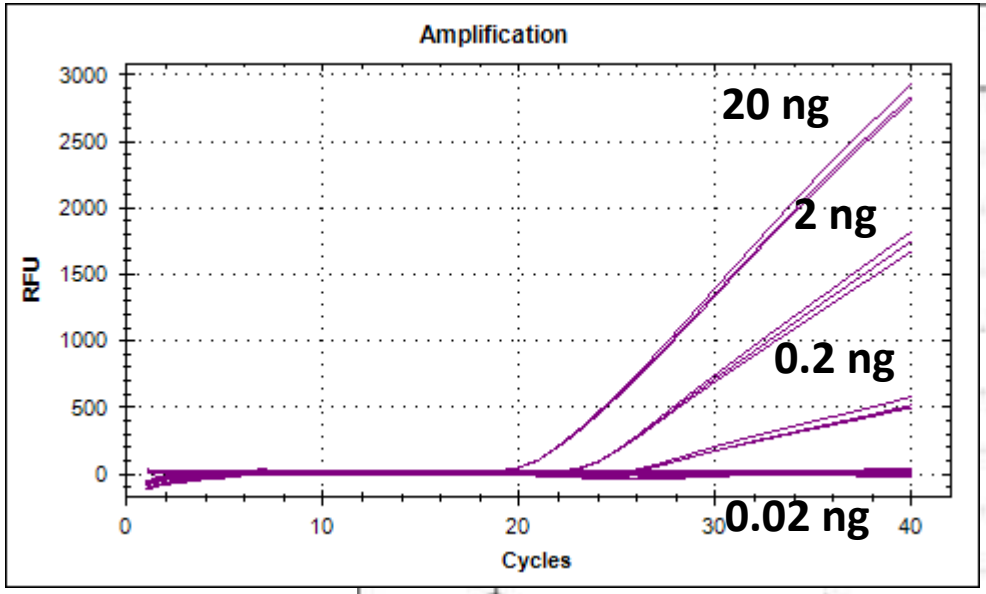
No Reverse Transcription







Case 5: Effect of Master Mix



- Real Time PCR Basic Training
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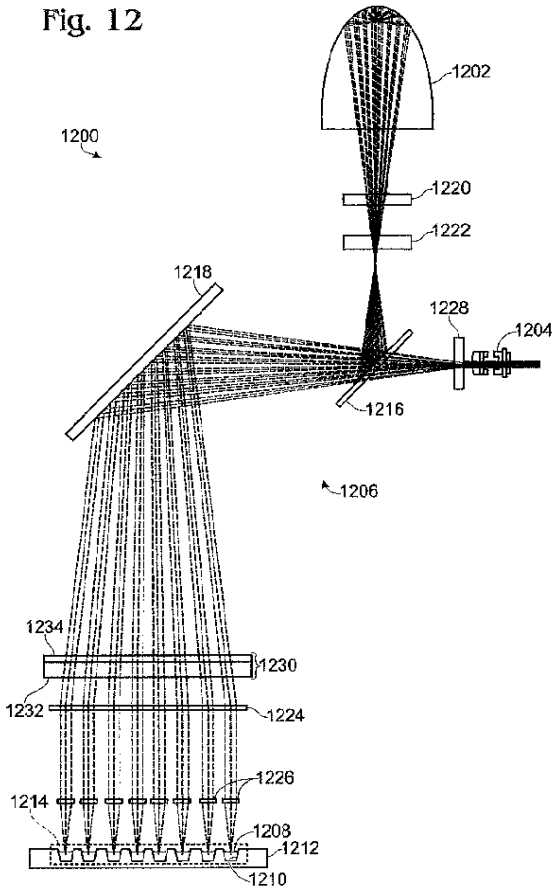
- Q&A

LightCycler® 480 Real-Time PCR System

Data Capture (Other Brand)



Report signal : SYBR Green 1

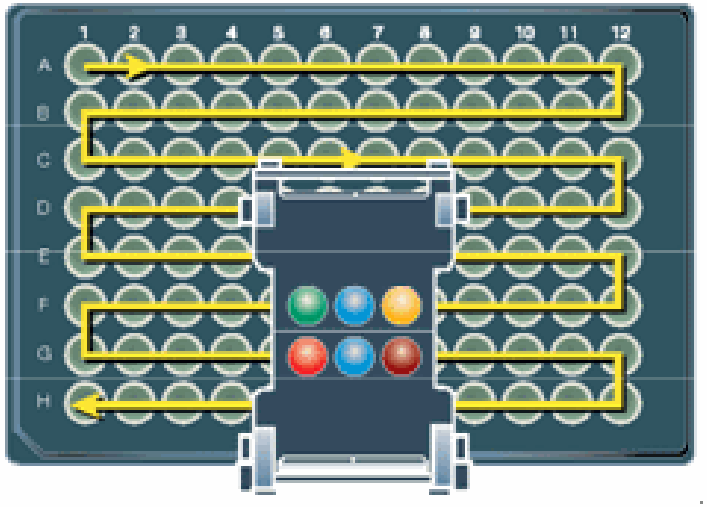
Reference signal : ROX

- 校正

(MeltDoctor™ HRM Calibration Plates)

Data Capture (Other Brand)

- 非同步
- 時間長

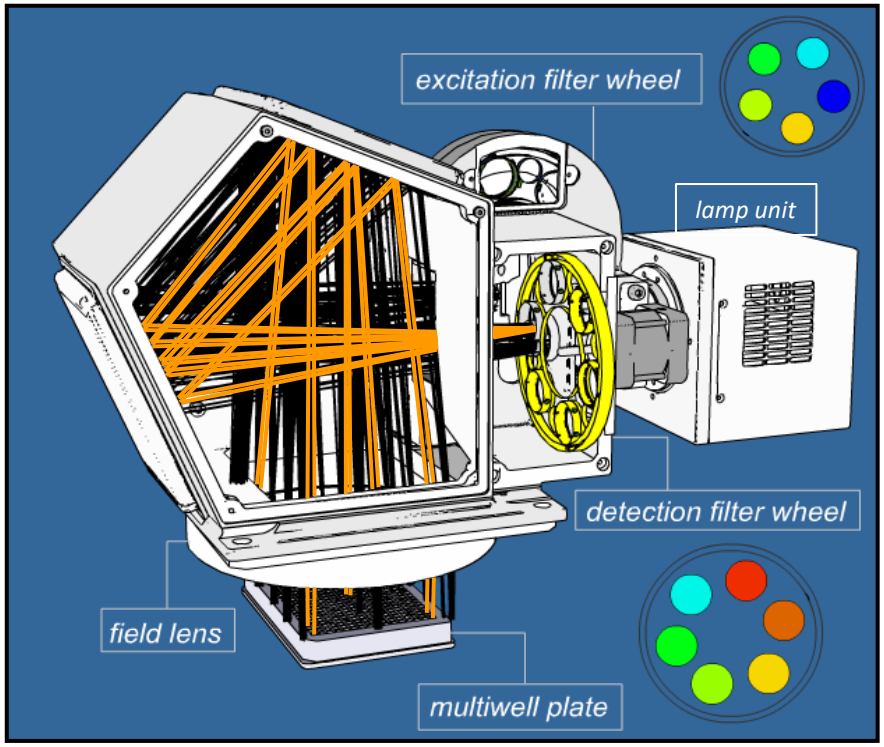


- 校正

(Melt Calibration Kit)

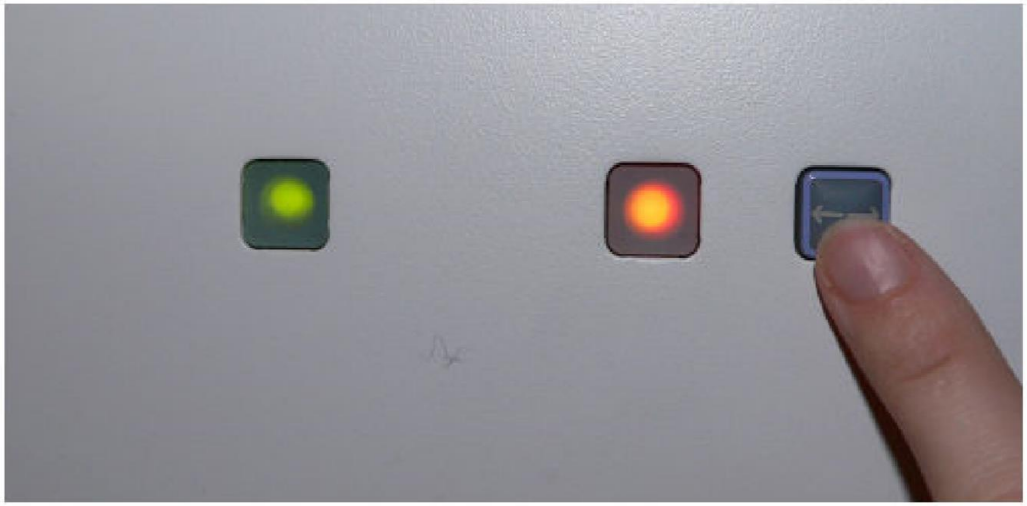
LightCycler® 480 System

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**

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):

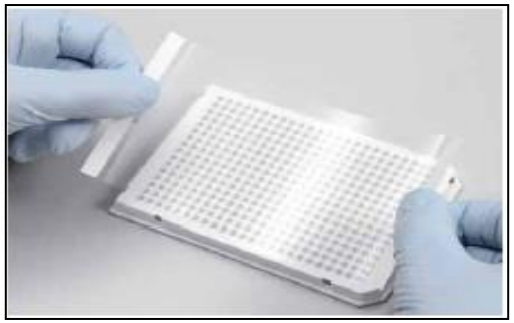
Place the multiwell plate into the loading frame of the loader with the flat edge pointing towards the instrument. (The short plate edge with beveled corners points away from the instrument.)

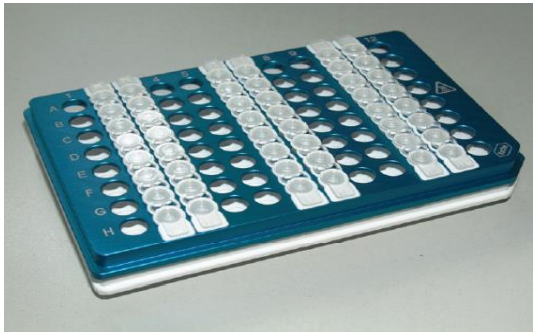
Press the plate loading push button again to retract the loader with the inserted multiwell plate into the instrument. You are now ready to start the run.

LightCycler® 480 System

Disposables

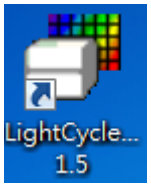
96-well plate for
10–100 µl





LightCycler® 8-Tube Strips (white and clear)

Open the software and Login to the data base



Instrument: No active instrument

Database:

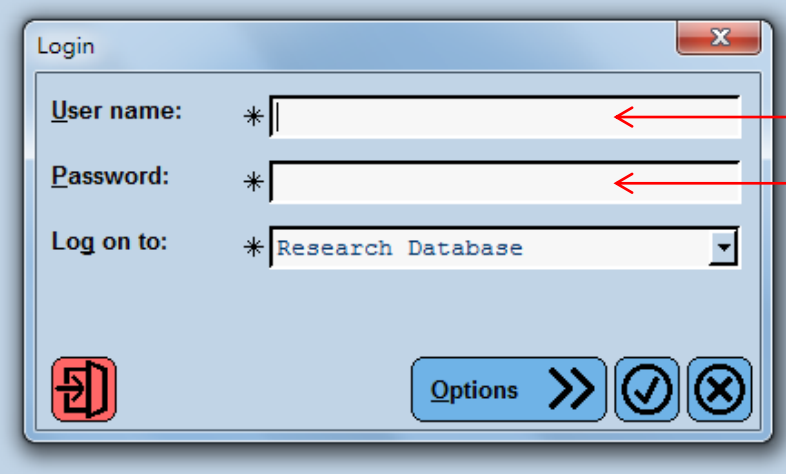
Window:

User:

For PC login

ID: operator

PW: **LC**480

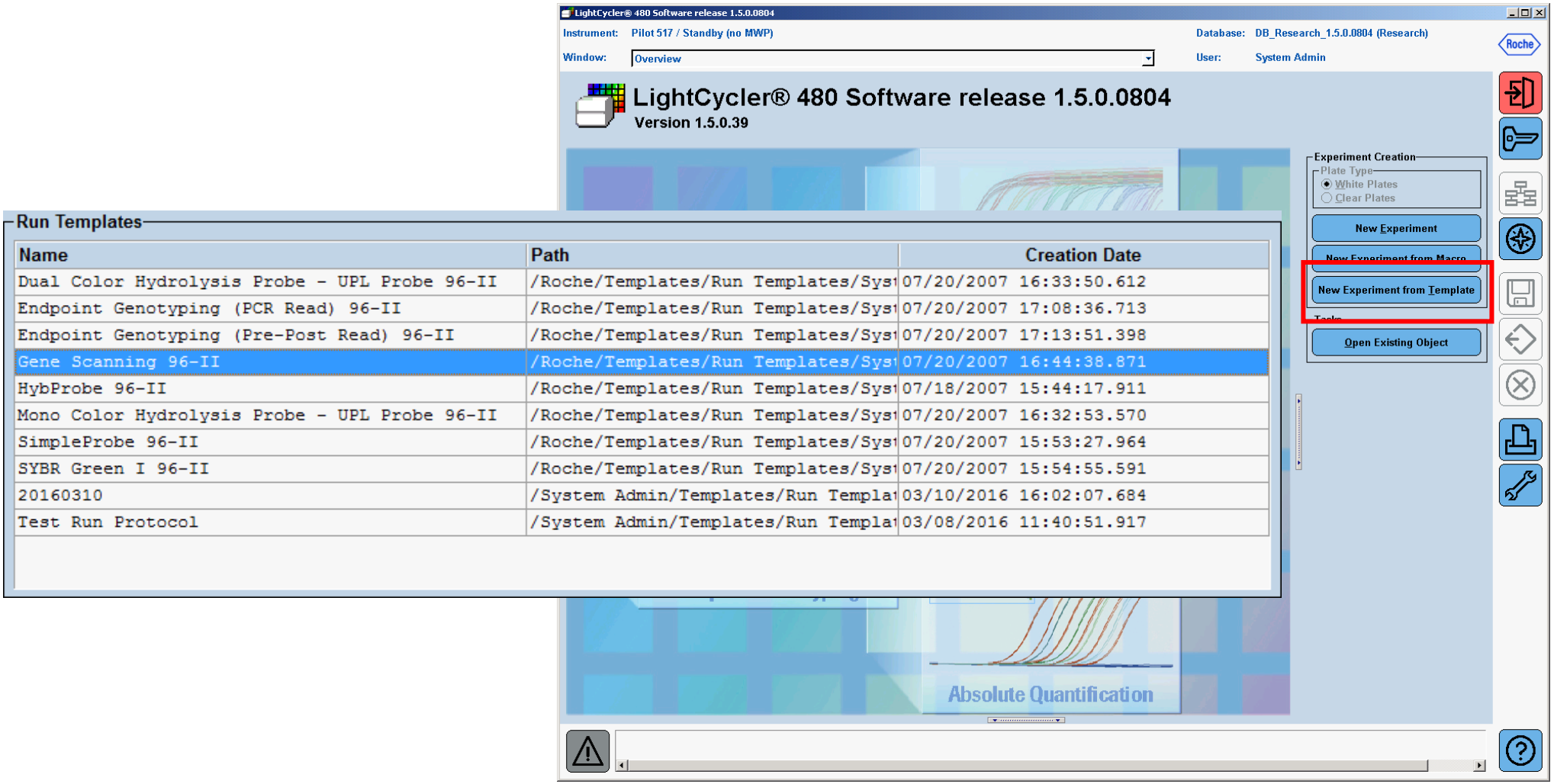


admin

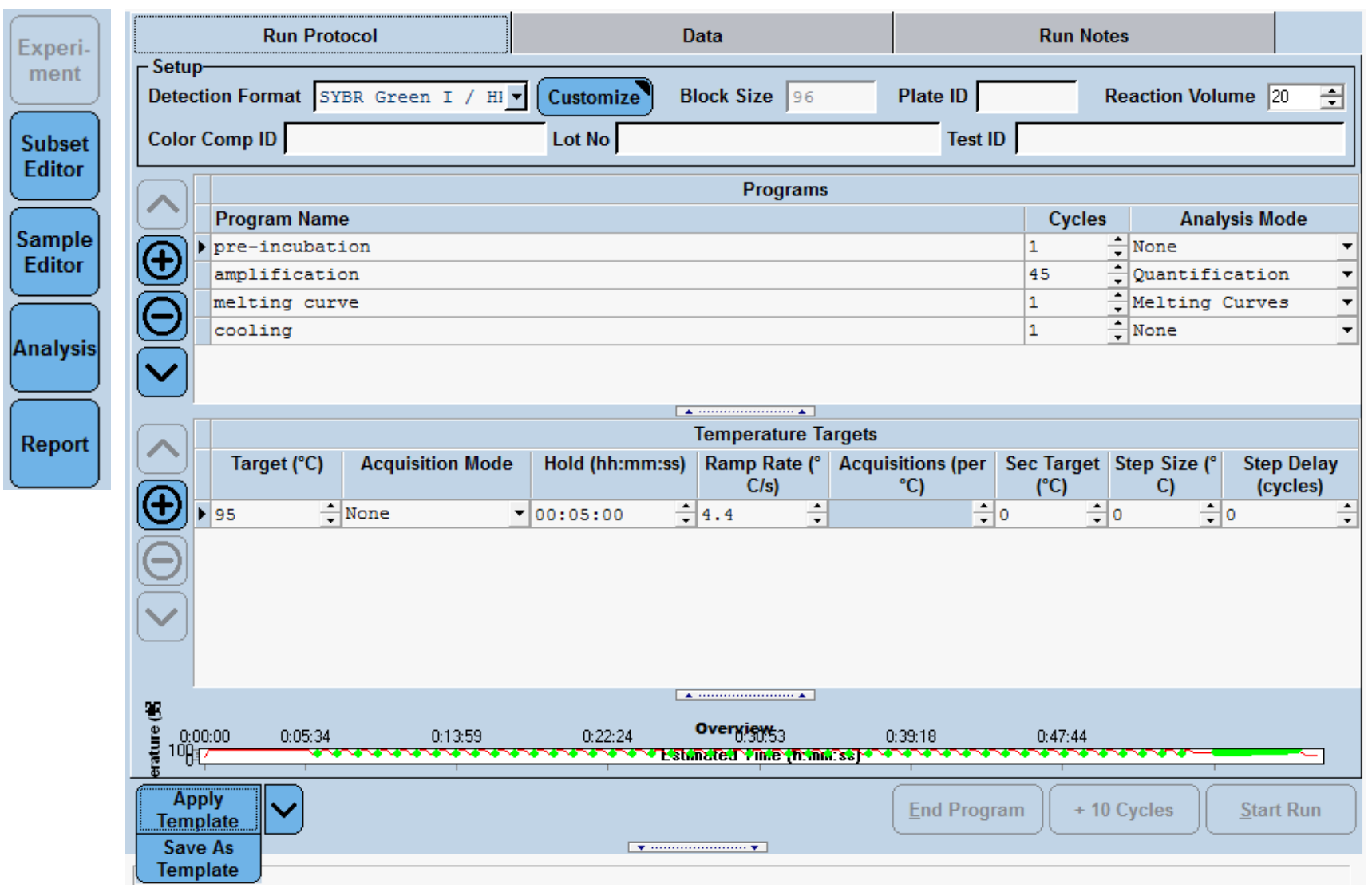
Light**C**ycler480

LightCycler® 480 Software Version 1.5

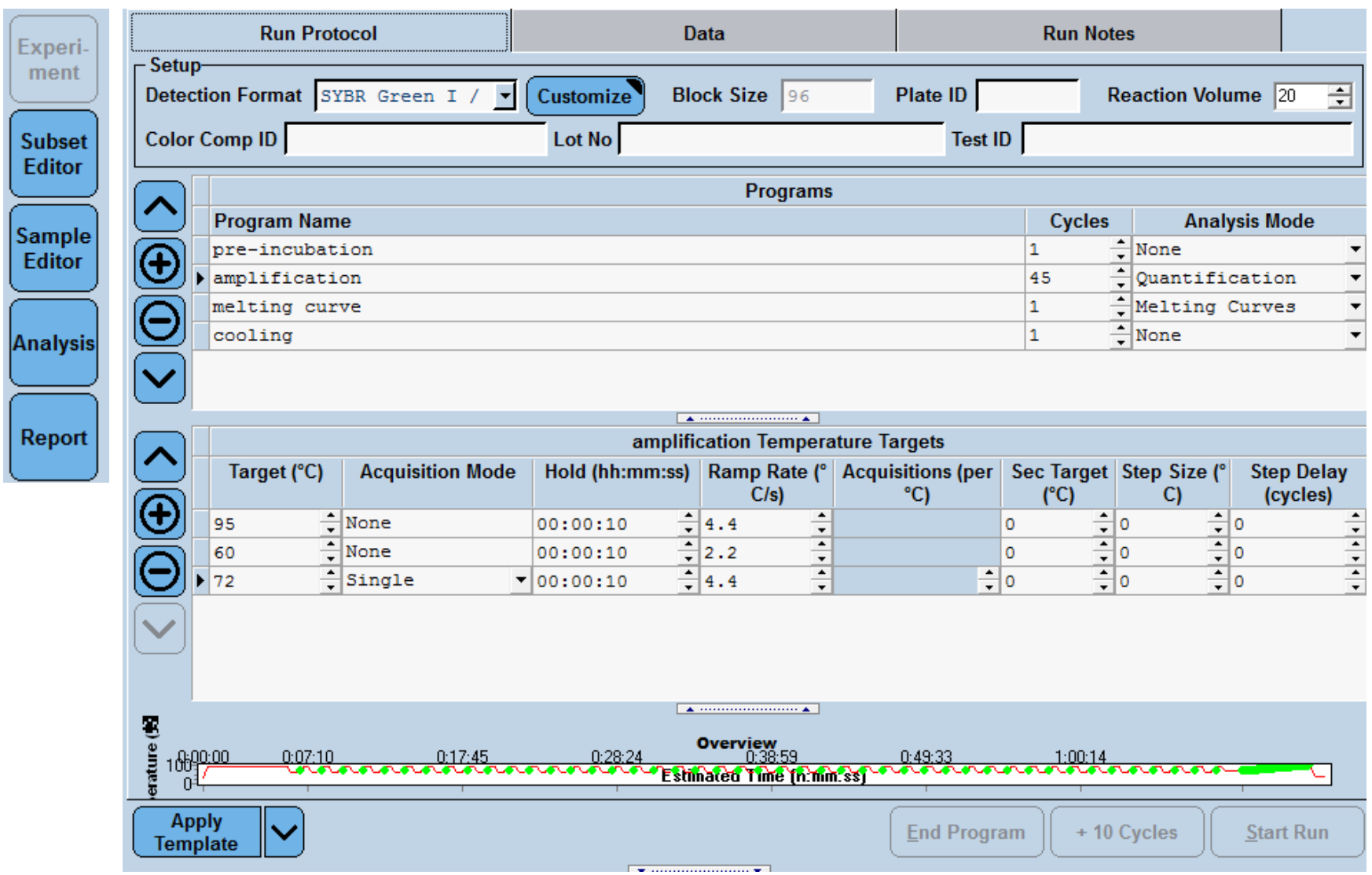
Front screen: New experiment from template



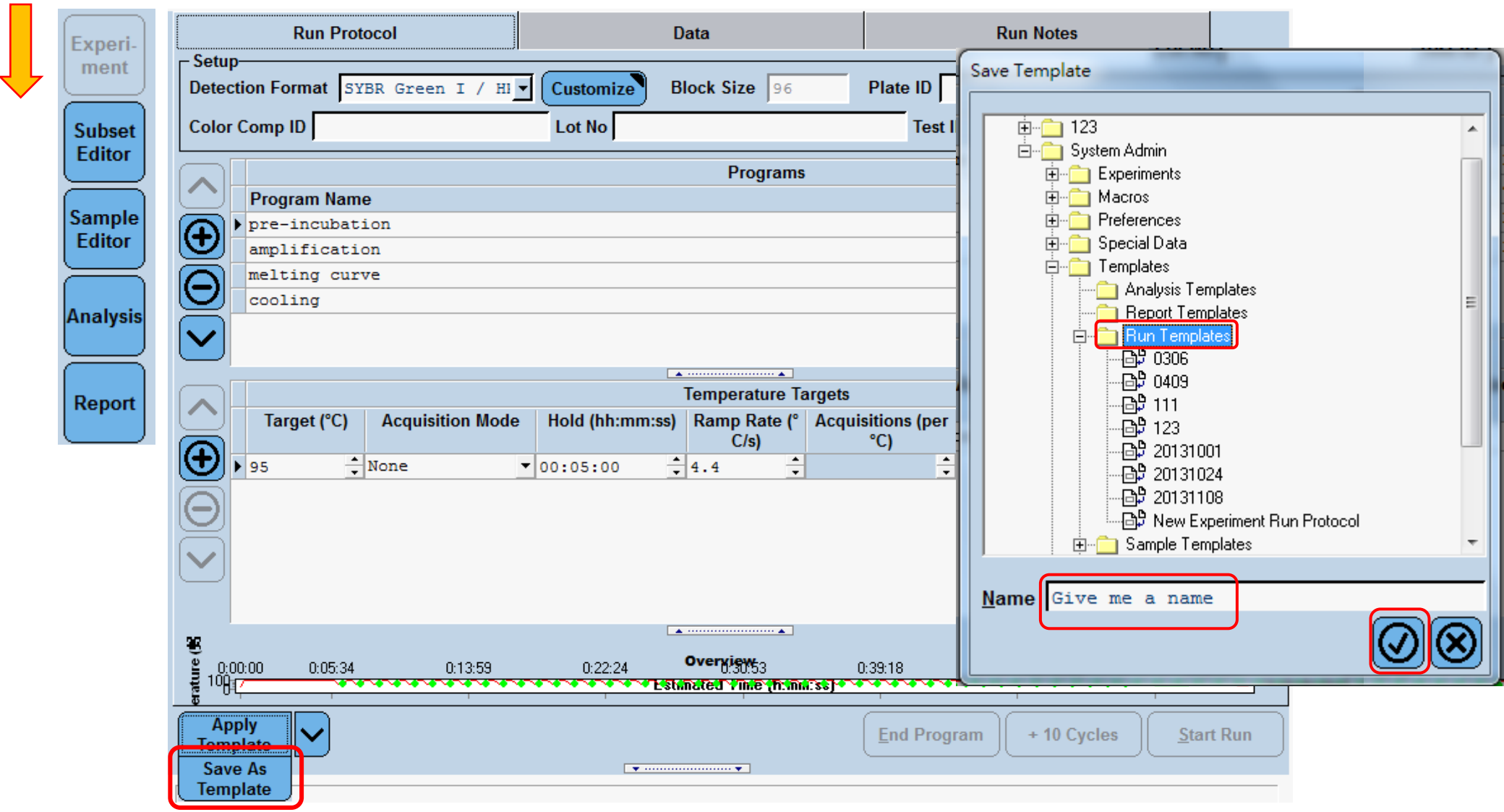
Start Run


Experi-ment
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
Program Name
Cycles
Analysis Mode
pre-incubation
1
None
amplification
45
Quantification
melting curve
1
Melting Curves
cooling
1
None
Temperature Targets
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
0:00:00
0:05:34
0:13:59
0:22:24
0:30:53
0:39:18
0:47:44
Estimated Time (h:mm:ss)
Apply Template
Save As Template
End Program
+ 10 Cycles
Start Run

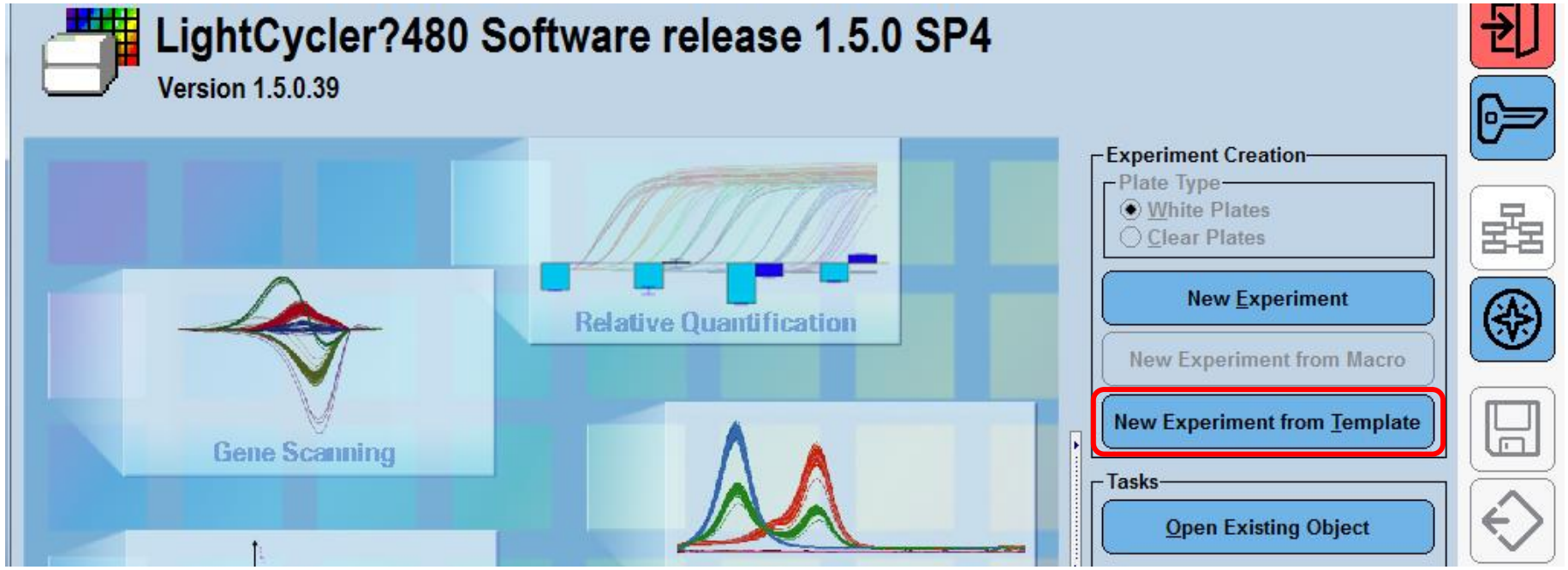
Start Run


Experi-ment
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
Program Name
Cycles
Analysis Mode
pre-incubation
1
None
amplification
45
Quantification
melting curve
1
Melting Curves
cooling
1
None
amplification Temperature Targets
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
0:00:00
0:07:10
0:17:45
0:28:24
0:38:59
0:49:33
1:00:14
100
0
Estimated Time (h:mm:ss)
Apply 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 / 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
0:00:00
0:05:34
0:13:59
0:22:24
0:30:53
0:39:18
Overview
Estimated Time (h:mm:ss)
Apply Template
Save As Template
End Program
+ 10 Cycles
Start Run
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

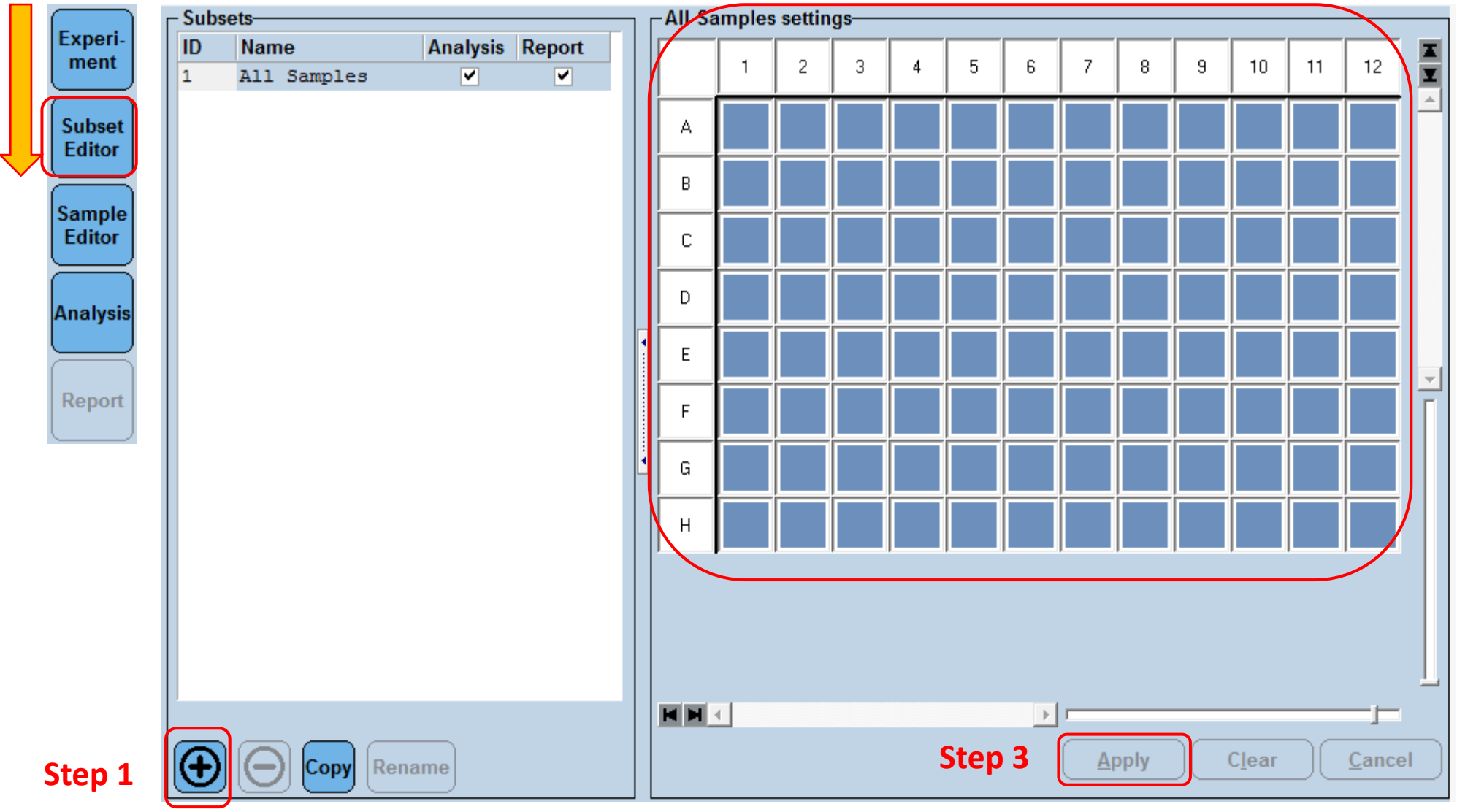
Start your experiment by your template



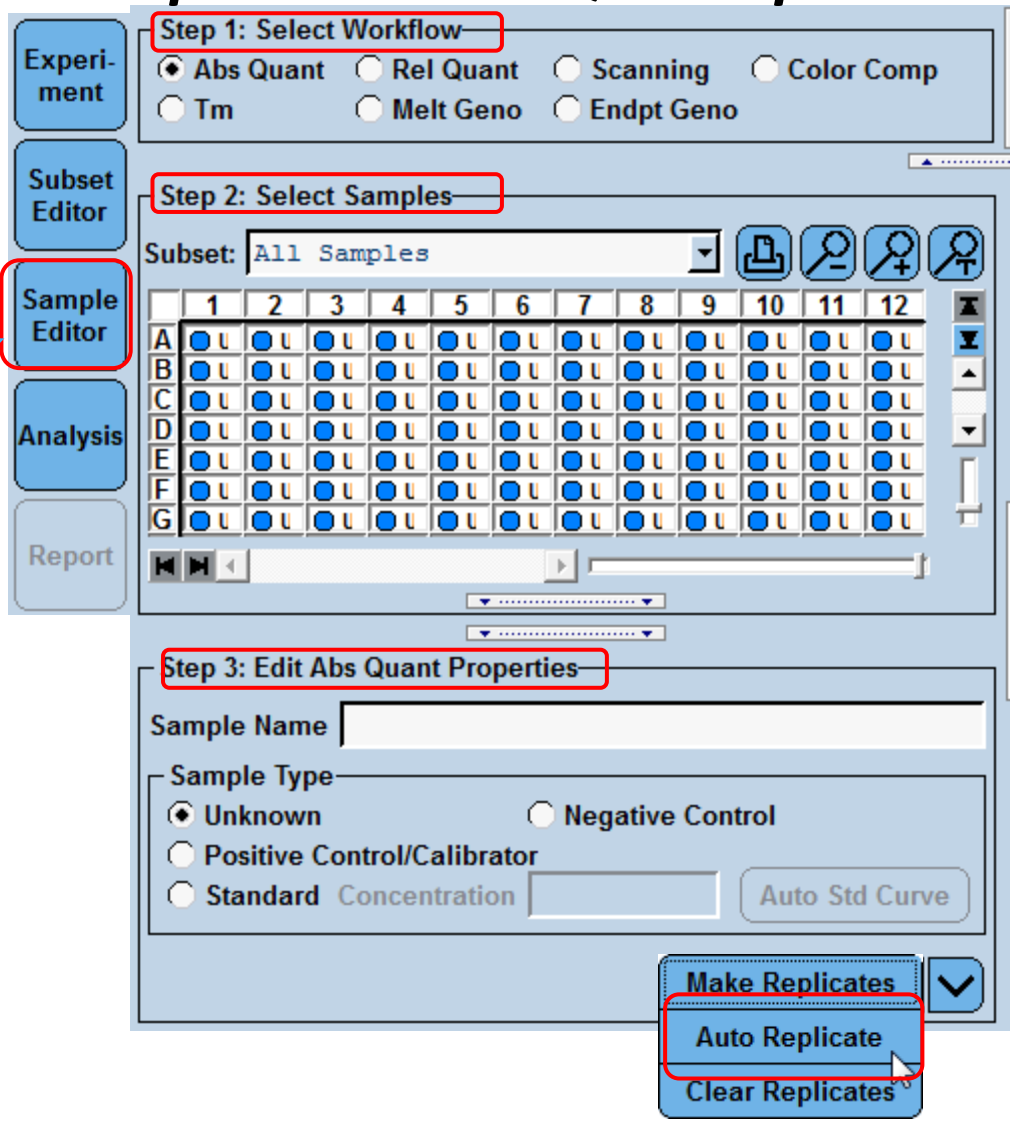
LightCycler480 Software:

1. New experiment from template
2. Subset & sample set up
3. Data analysis & report

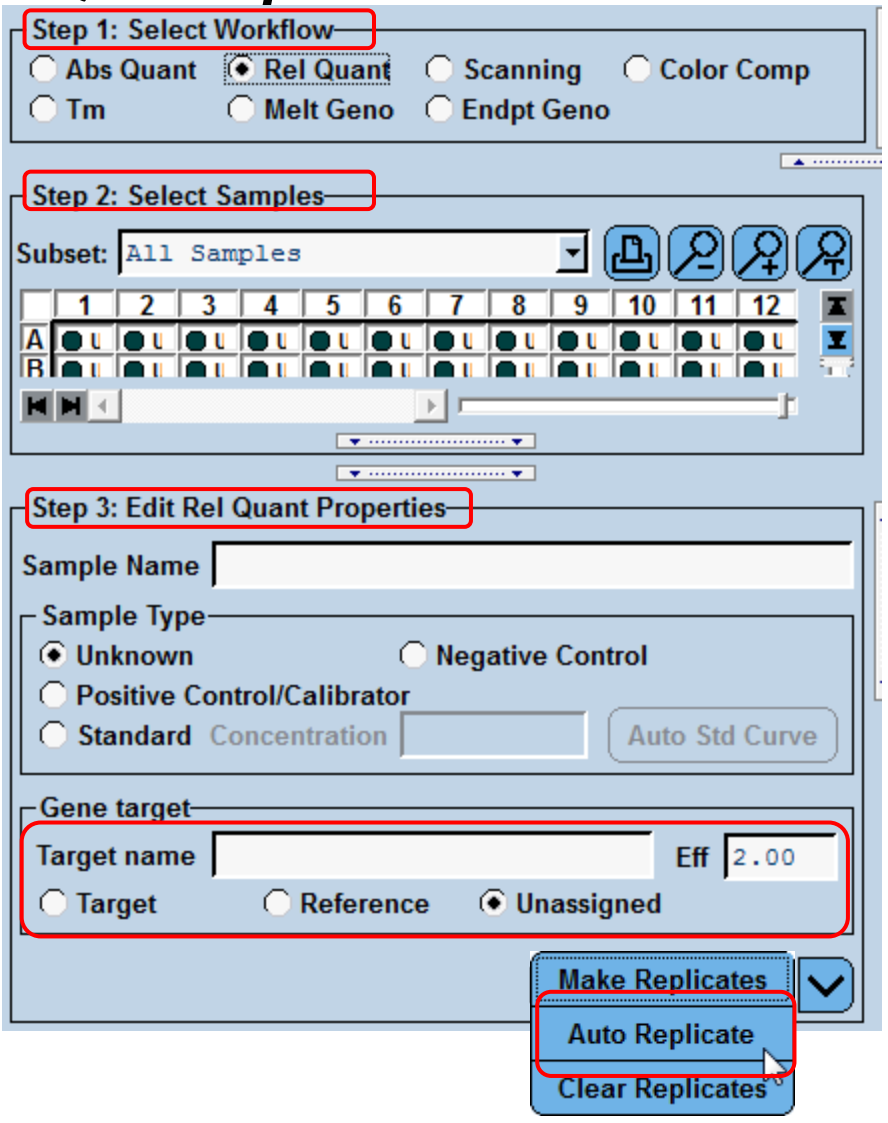
Subset


Step 2
Experi-ment
Subset Editor
Sample Editor
Analysis
Report
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
Step 1
Copy
Rename
Step 3
Apply
Clear
Cancel

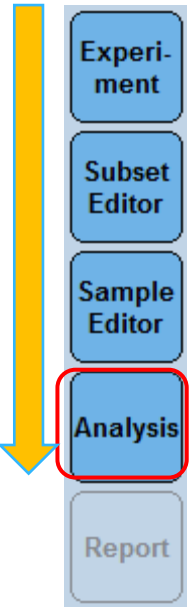
Sample Editor – AQ example

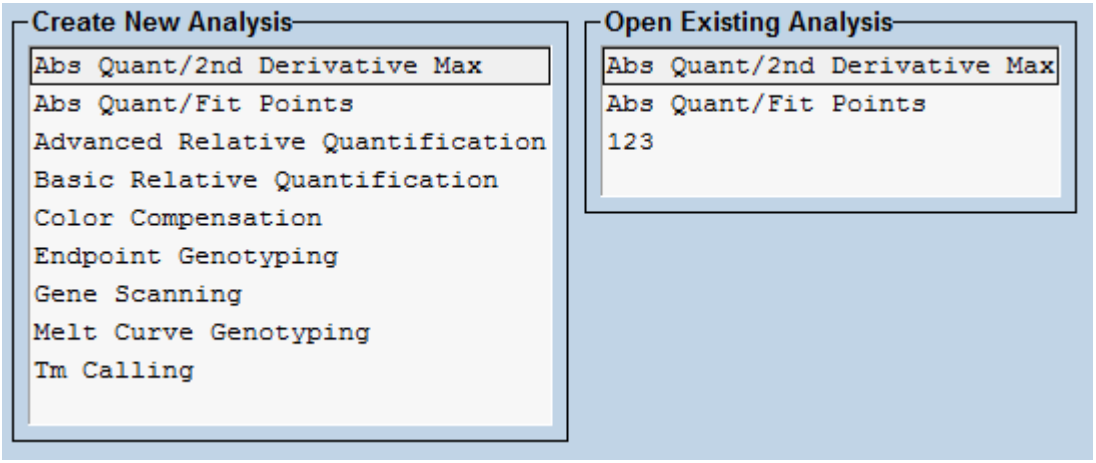


RQ example



Analysis

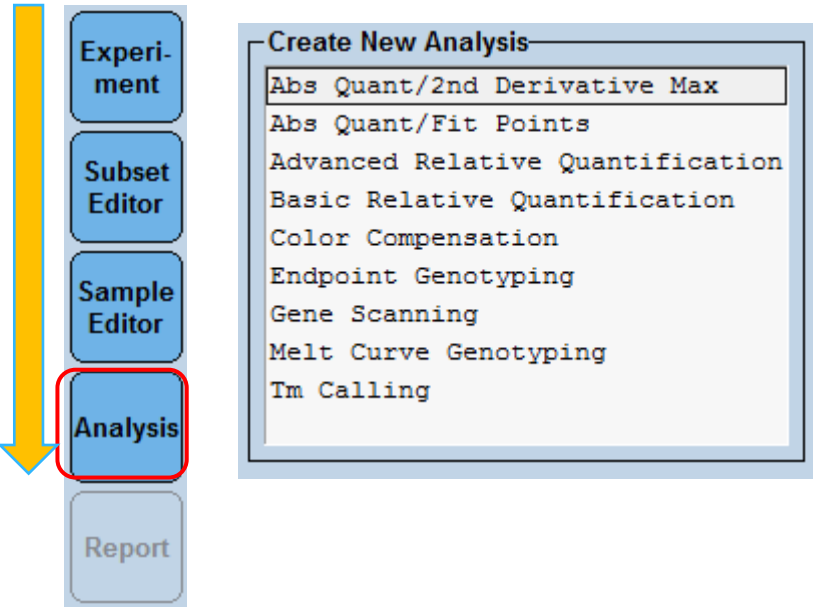


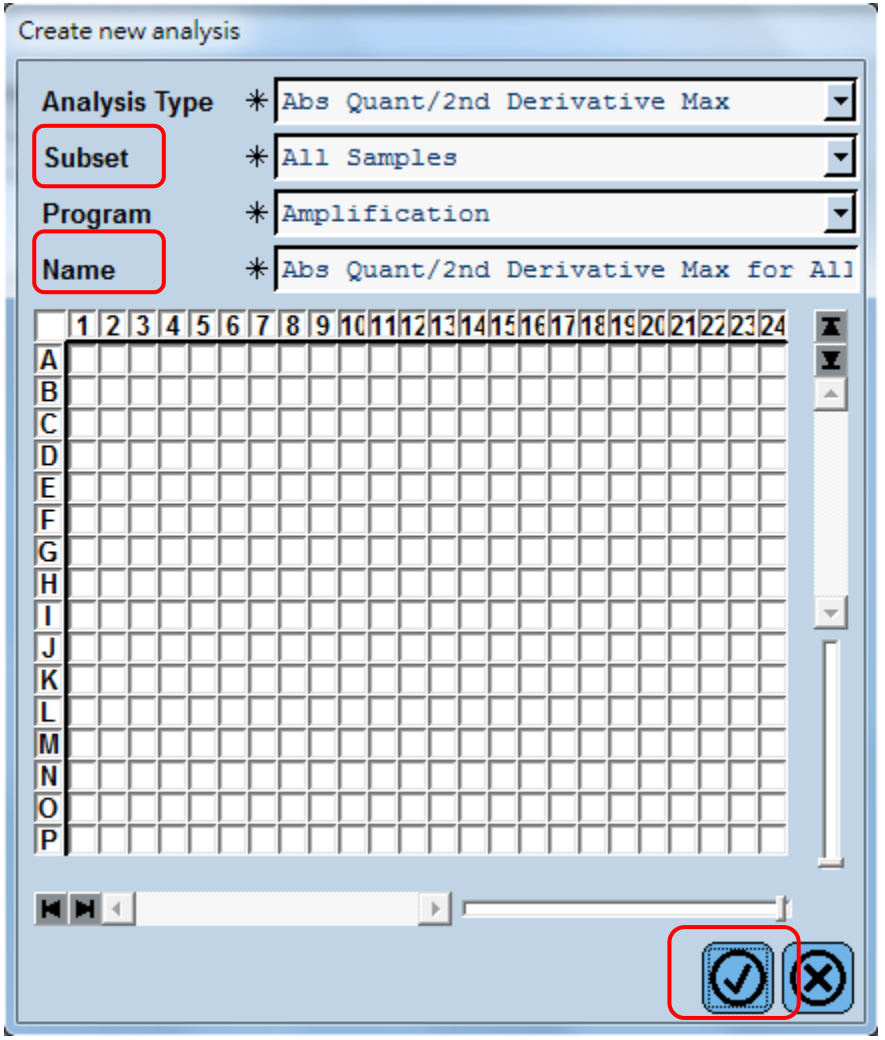


分析模式簡要說明：

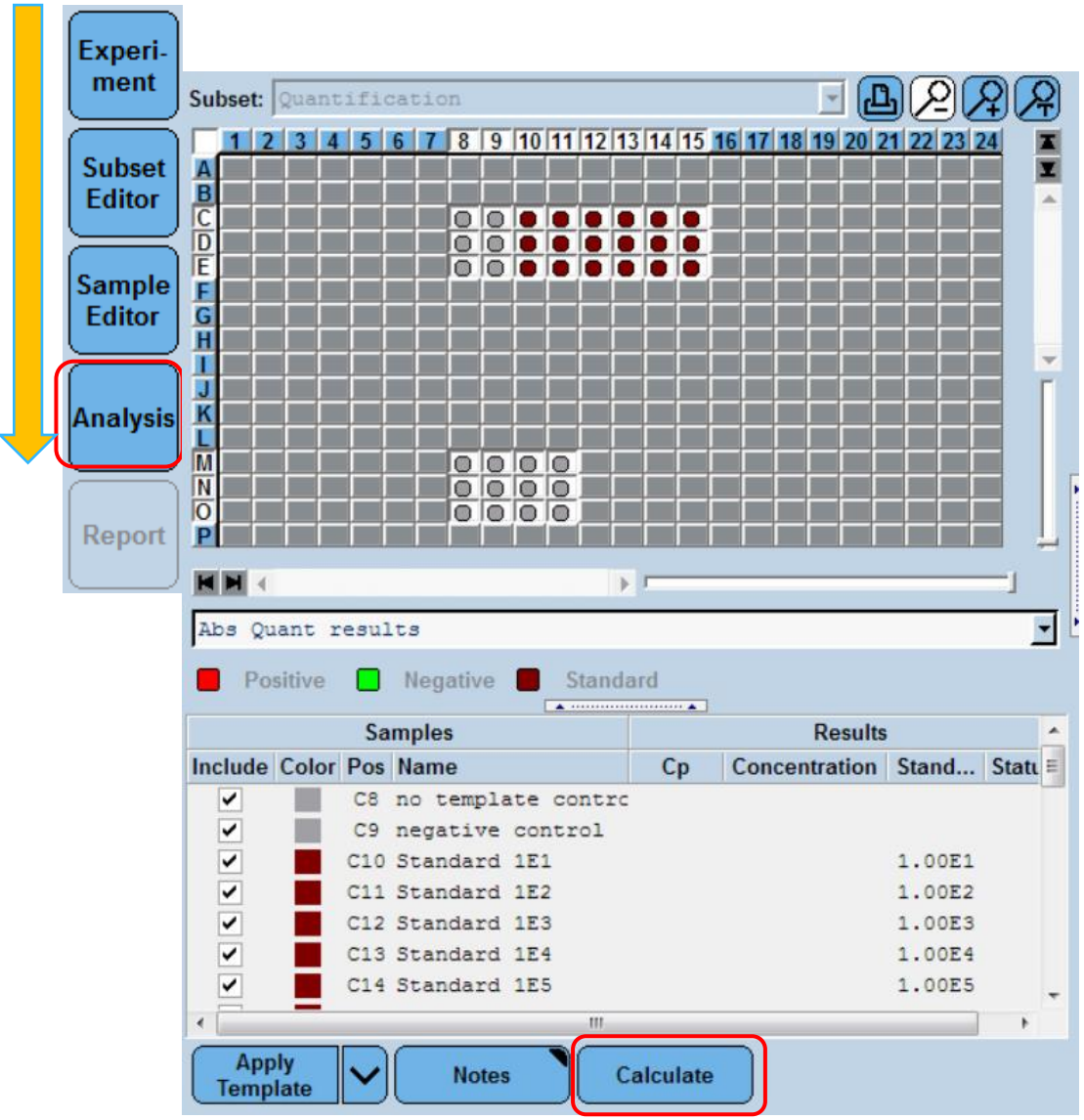
分析模式	說明
Abs Quant / 2nd Derivative Max	絕對定量 (2ndMax)
Abs Quant / Fit Points	絕對定量 (Fit Point)
Advanced Relative Quantification	進階相對定量(2ndMax or Fit Point)
Basic Relative Quantification	基礎相對定量 (Fit Point only)
Endpoint Genotyping	基因分型(適用於TaqMan probe)
Melt Curve Genotyping	基因分型(適用於Hyprobe)
Tm Calling	Tm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

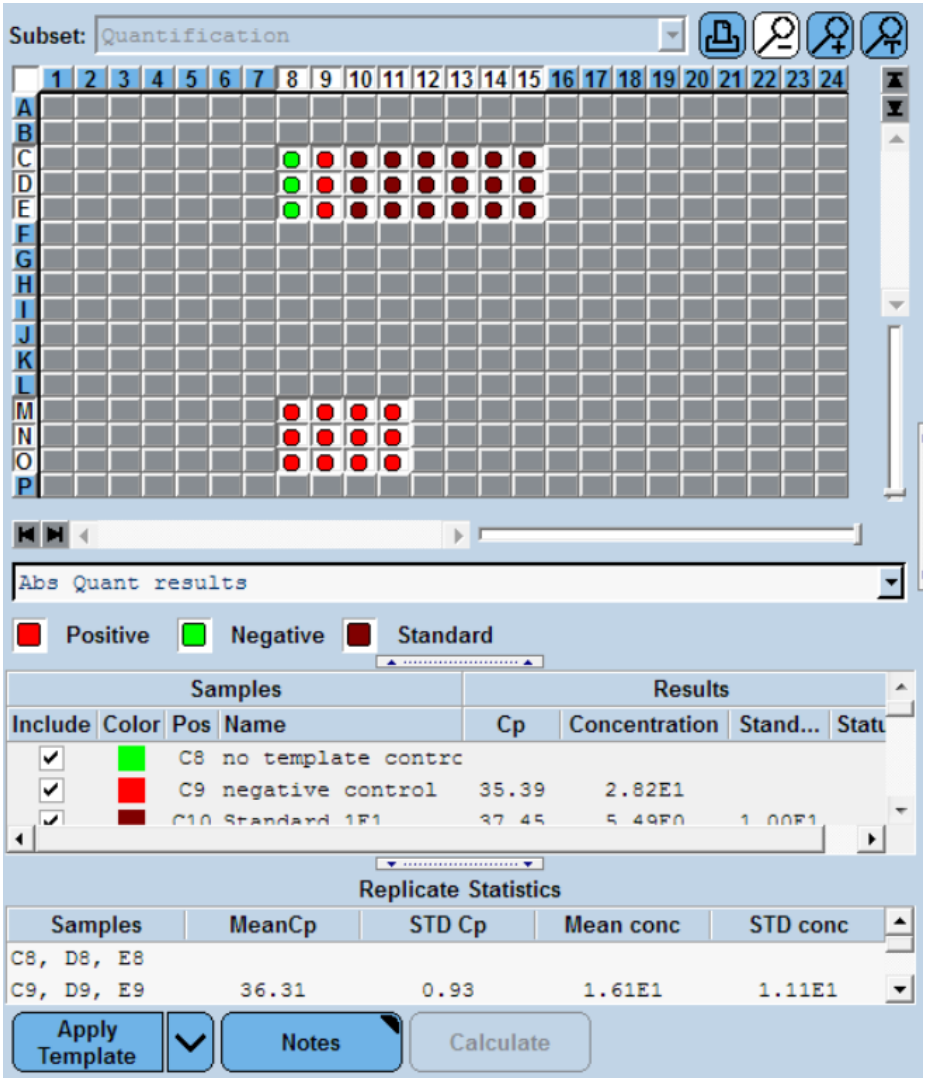
Analysis


Experi-ment
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

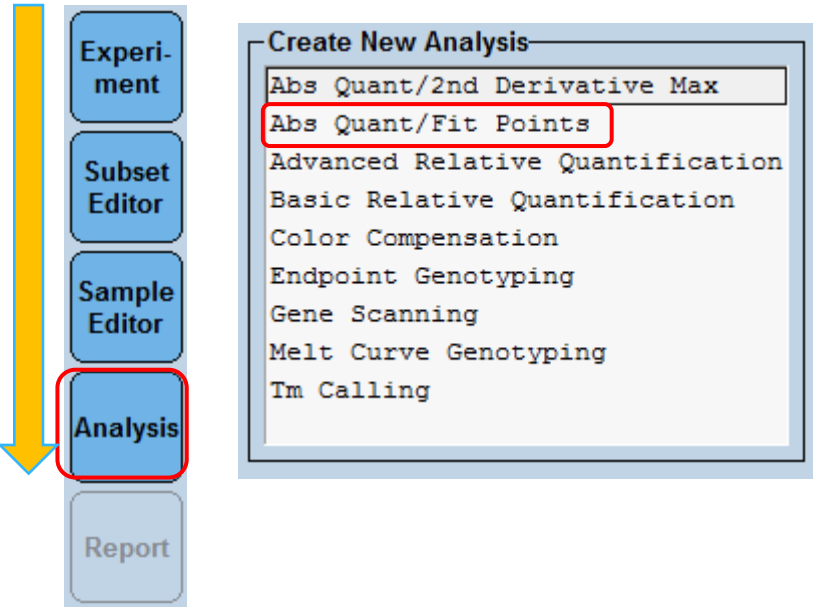

Create new analysis
Analysis Type
Abs Quant/2nd Derivative Max
Subset
All Samples
Program
Amplification
Name
Abs Quant/2nd Derivative Max for All
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
A B C D E F G H I J K L M N O P

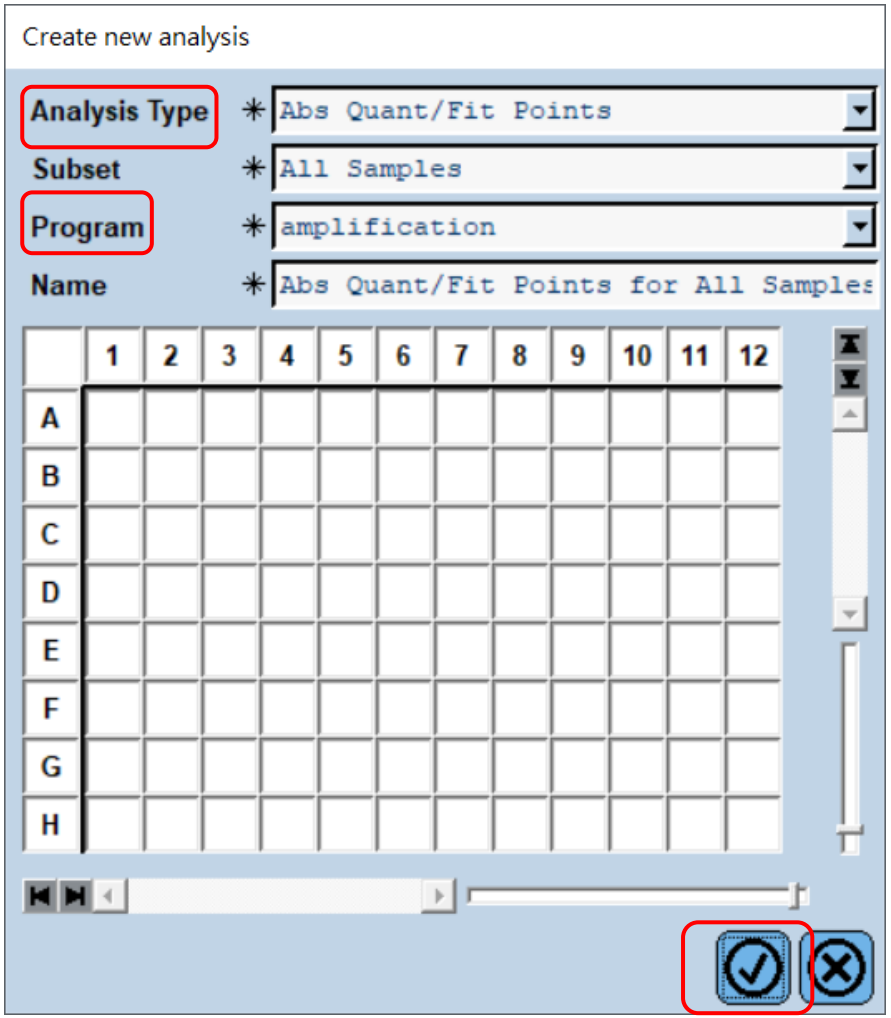
AQ Analysis


Experi-ment
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

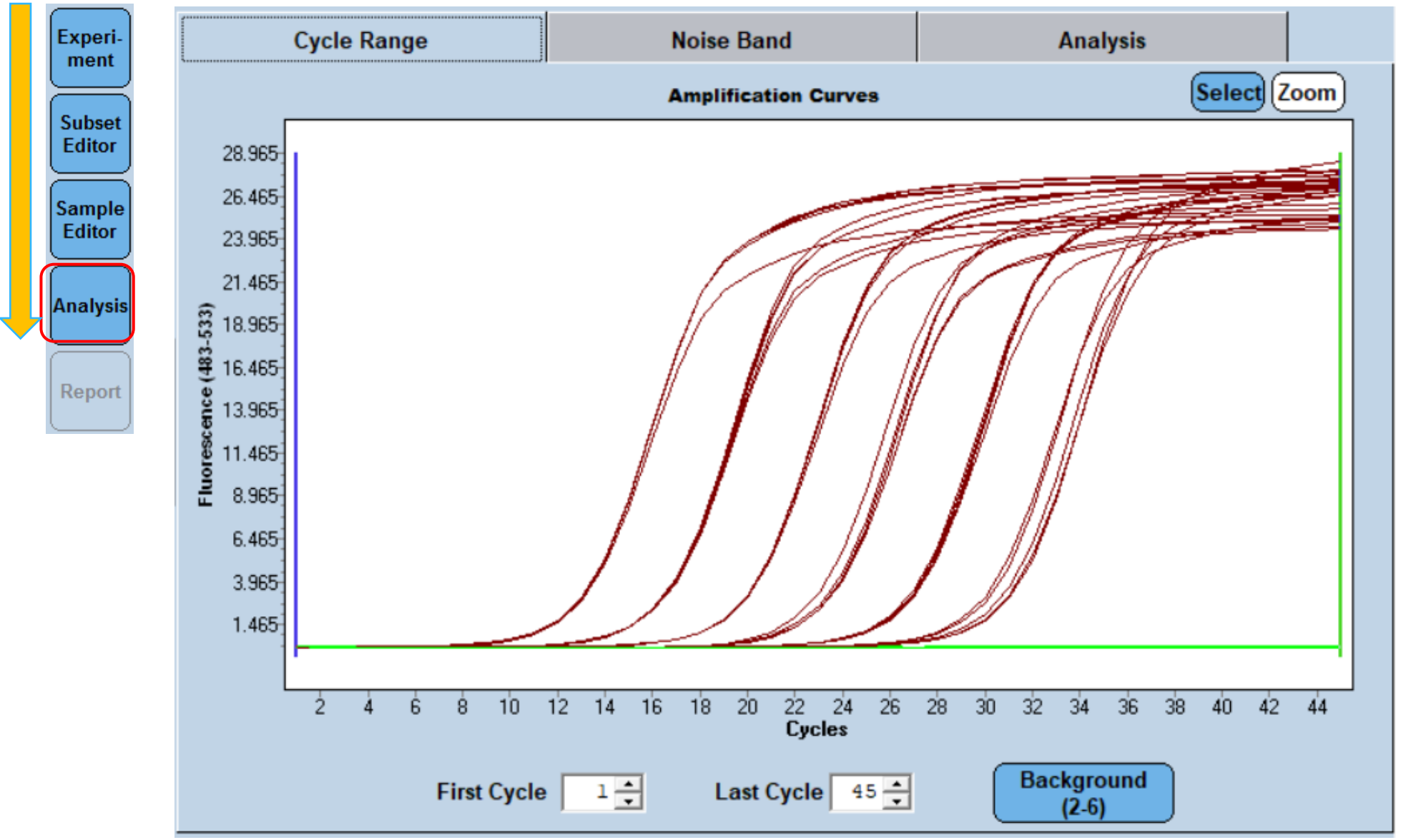

Subset: Quantification
Abs Quant results
Positive
Negative
Standard
Samples
Results
Include Color Pos Name Cp Concentration Stand... Statu
C8 no template contrc
C9 negative control 35.39 2.82E1
Replicate Statistics
Samples MeanCp STD Cp Mean conc STD conc
C8, D8, E8
C9, D9, E9 36.31 0.93 1.61E1 1.11E1
Apply Template
Notes
Calculate

Analysis

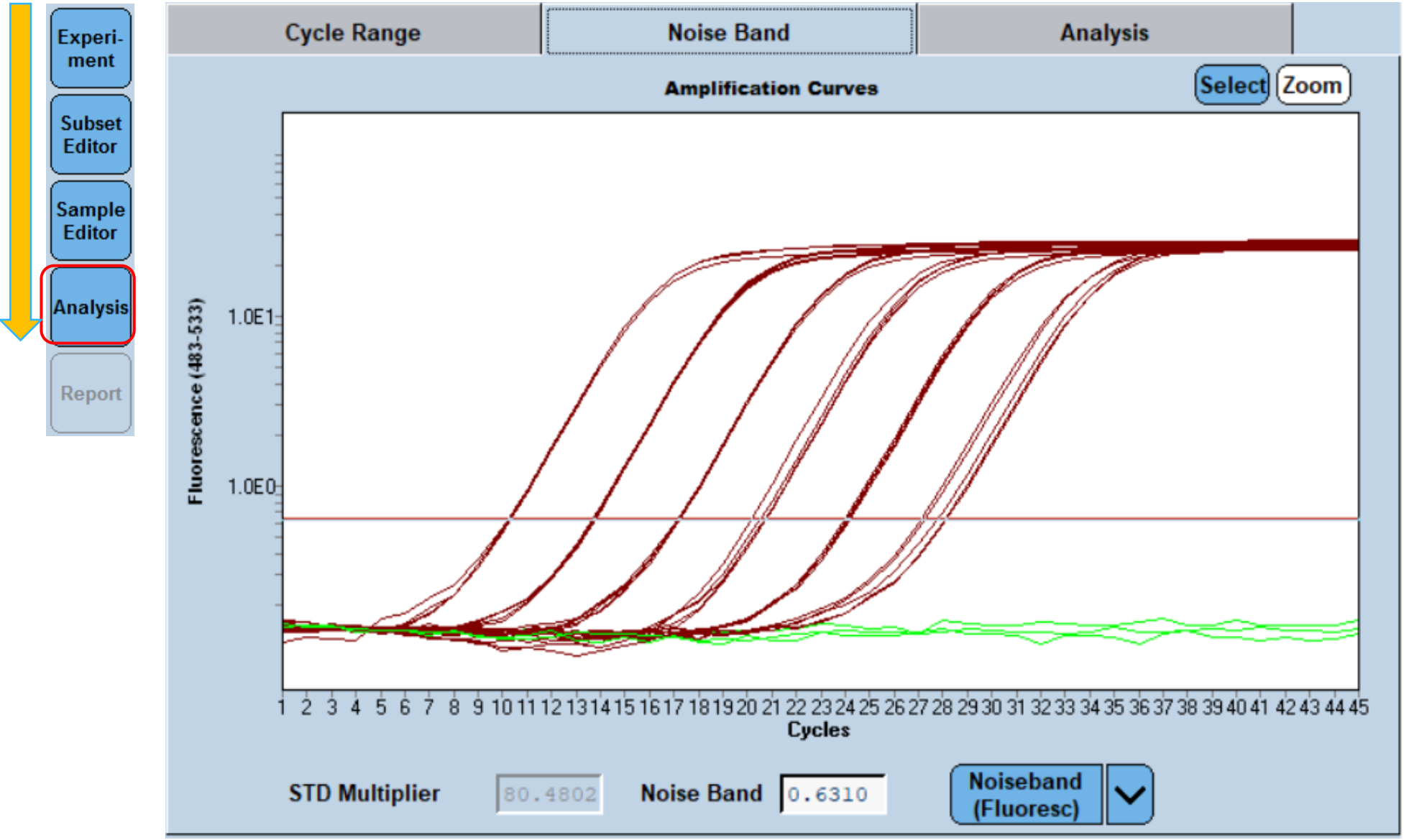

Experi-ment
Subset Editor
Sample Editor
Analysis
Report
Create New Analysis
Abs Quant/2nd Derivative Max
Abs Quant/Fit Points
Advanced Relative Quantification
Basic Relative Quantification
Color Compensation
Endpoint Genotyping
Gene Scanning
Melt Curve Genotyping
Tm Calling


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

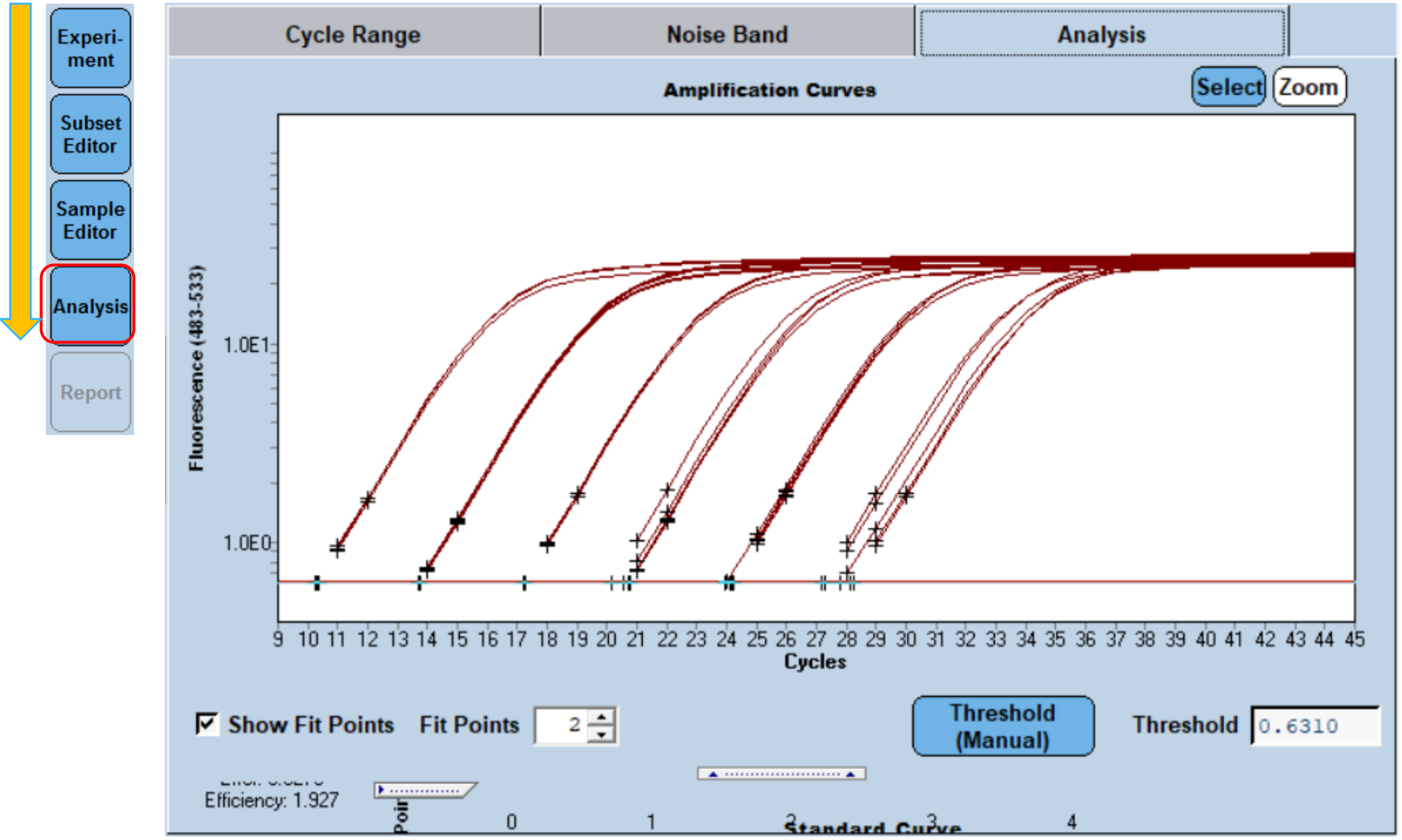
AQ Analysis


Experi-ment
Subset Editor
Sample Editor
Analysis
Report
Cycle Range
Noise Band
Analysis
Amplification Curves
Select
Zoom
Fluorescence (483-533)
28.965
26.465
23.965
21.465
18.965
16.465
13.965
11.465
8.965
6.465
3.965
1.465
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44
Cycles
First Cycle
1
Last Cycle
45
Background (2-6)

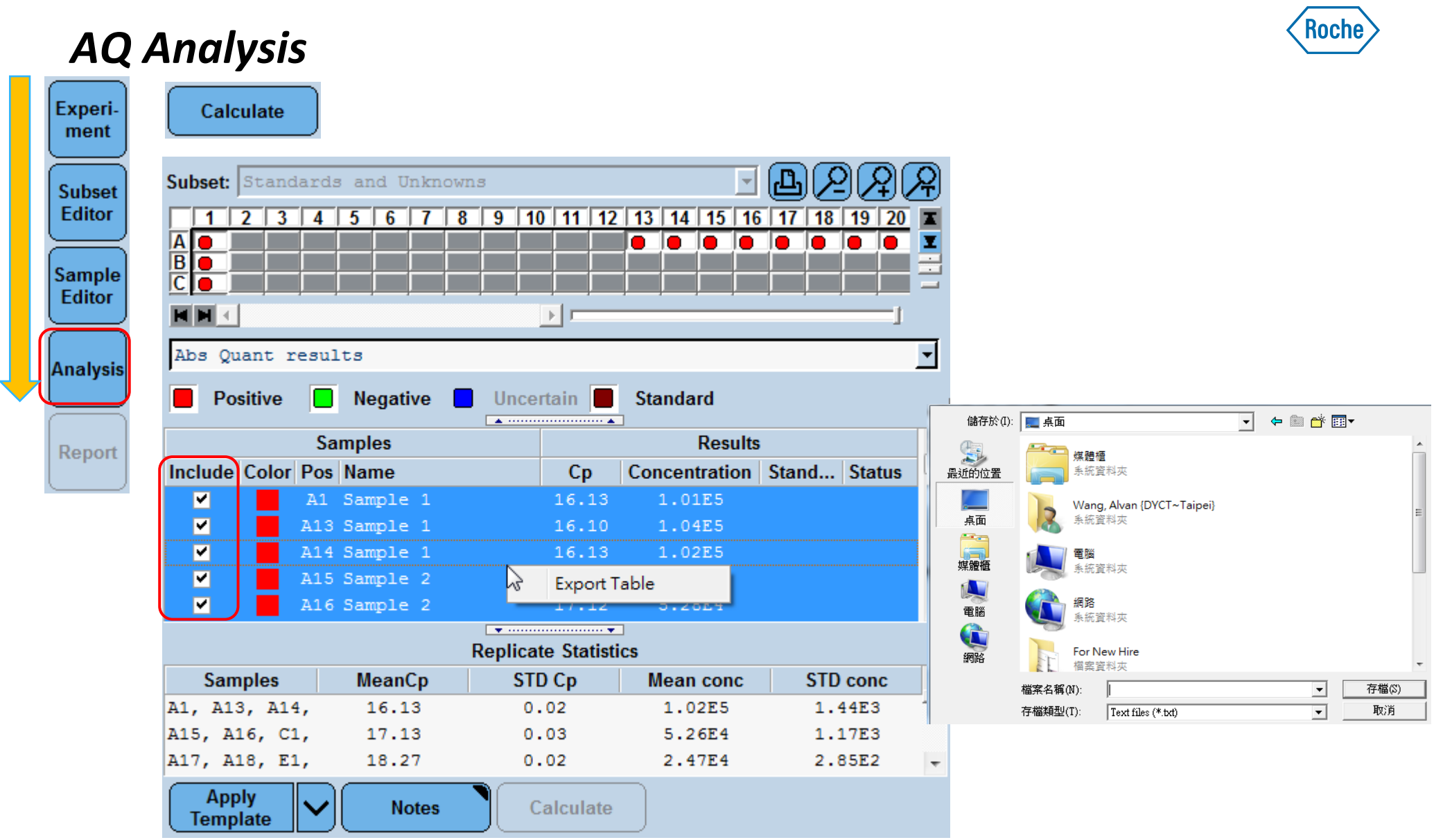
AQ Analysis


Experi-ment
Subset Editor
Sample Editor
Analysis
Report
Cycle Range
Noise Band
Analysis
Amplification Curves
Select
Zoom
Fluorescence (483-533)
1.0E1
1.0E0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
Cycles
STD Multiplier
80.4802
Noise Band
0.6310
Noiseband (Fluoresc)

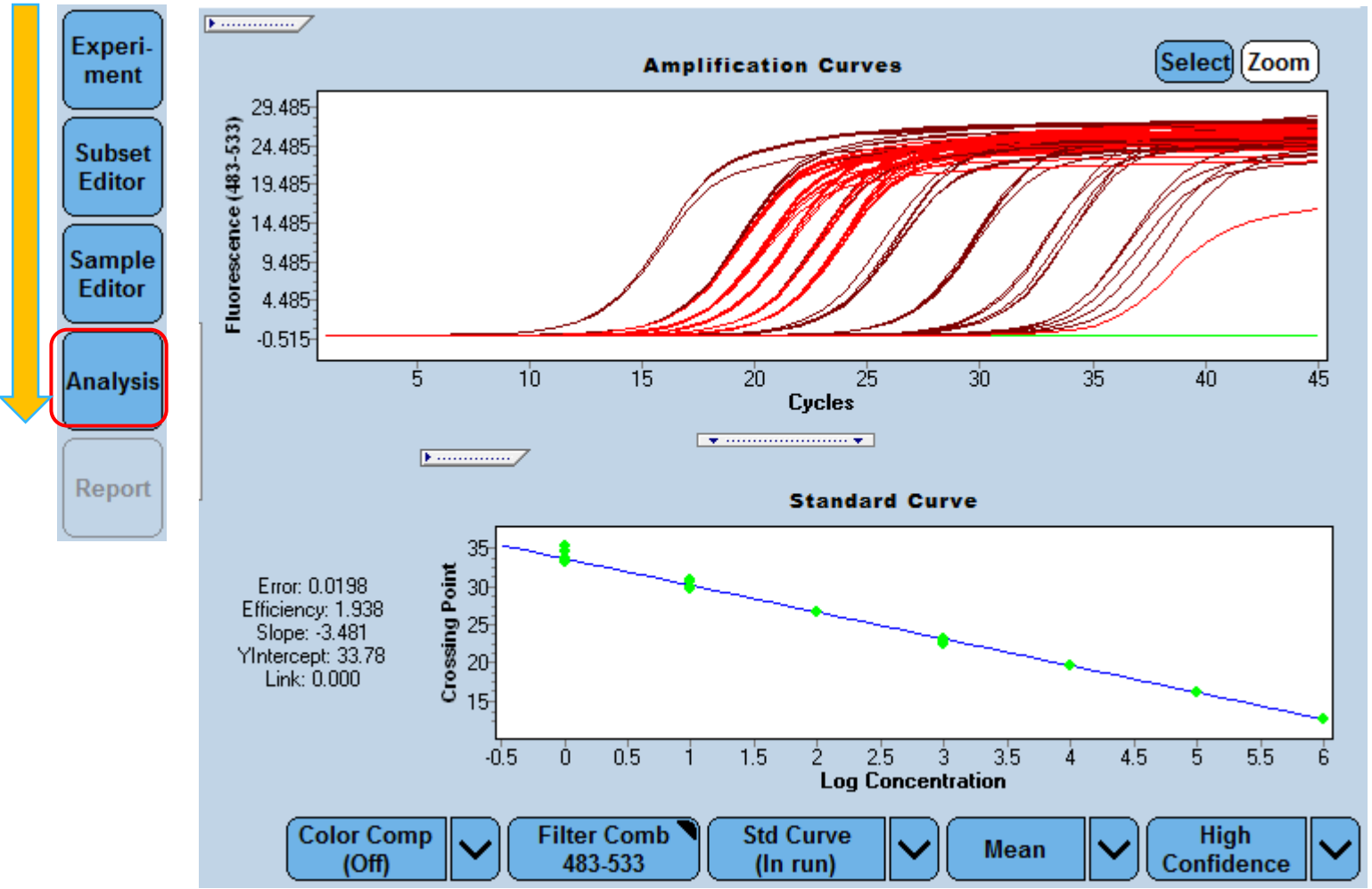
AQ Analysis


Experi-ment
Subset Editor
Sample Editor
Analysis
Report
Cycle Range
Noise Band
Analysis
Amplification Curves
Select
Zoom
Fluorescence (483-533)
1.0E1
1.0E0
9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
Cycles
Show Fit Points
Fit Points
2
Threshold (Manual)
Threshold
0.6310
Efficiency: 1.927
0
1
2
3
4
Standard Curve

AQ Analysis


Experi-ment
Subset Editor
Sample Editor
Analysis
Report
Calculate
Subset: Standards and Unknowns
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
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
儲存於(I): 桌面
最近的位置
桌面
媒體櫃
電腦
網路
媒體櫃 系統資料夾
Wang, Alvan (DYCT-Taipei) 系統資料夾
電腦 系統資料夾
網路 系統資料夾
For New Hire 檔案資料夾
檔案名稱(N):
存檔類型(T): Text files (*.txt)
存檔(S)
取消

AQ Analysis


Experi-ment
Subset Editor
Sample Editor
Analysis
Report
Amplification Curves
Select
Zoom
Fluorescence (483-533)
29.485
24.485
19.485
14.485
9.485
4.485
-0.515
5
10
15
20
25
30
35
40
45
Cycles
Standard Curve
Error: 0.0198
Efficiency: 1.938
Slope: -3.481
YIntercept: 33.78
Link: 0.000
Crossing Point
35
30
25
20
15
-0.5
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
Log Concentration
Color Comp (Off)
Filter Comb 483-533
Std Curve (In run)
Mean
High Confidence

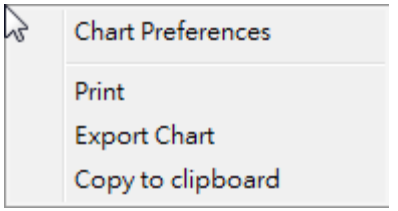


Chart Preferences
Print
Export Chart
Copy to clipboard

Providing the Standard Curve

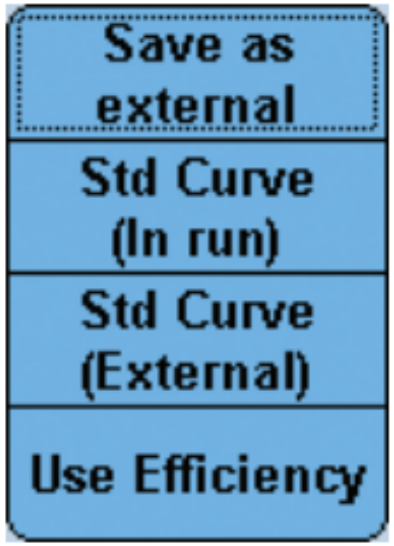
- Use a previously saved standard curve (called an external standard curve). An external standard curve can be loaded into experiments that do not have a standard curve, thus allowing quantitative analysis of those runs. This is especially suitable for applications where the same parameter is analyzed 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  .

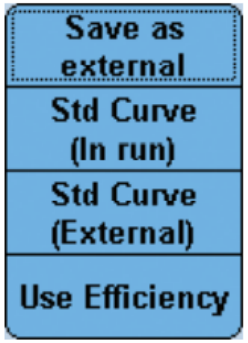
You can use the saved external standard curve in other quantification analyses for experiments that have the same experiment parameters as those used to create the standard curve.

To use an external standard curve

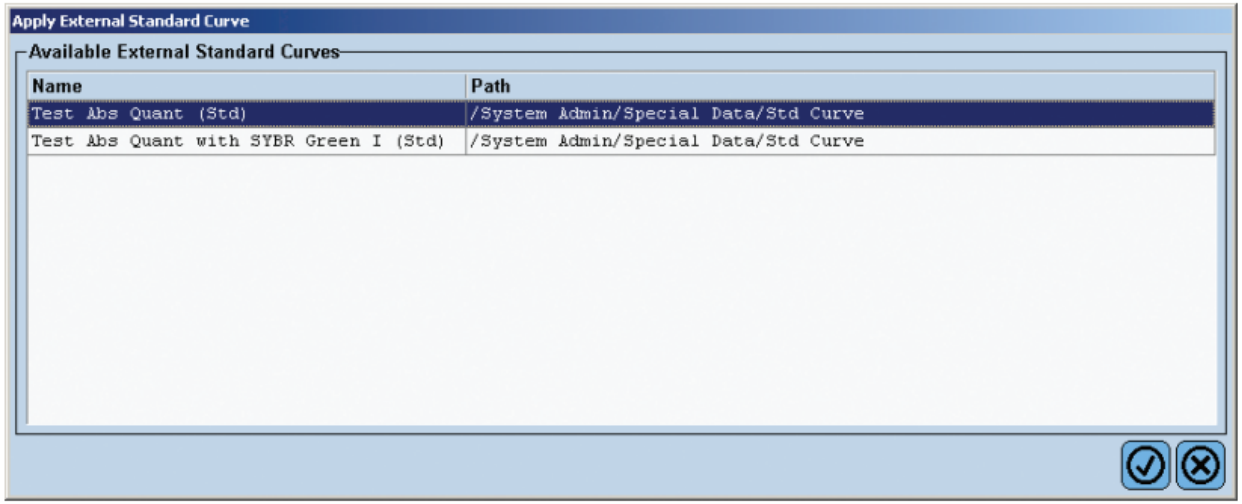
Select *Standard* as the sample type for the standard sample and specify the standard concentration.

For detailed information on the *Sample Editor* see section *Entering Sample Information*.

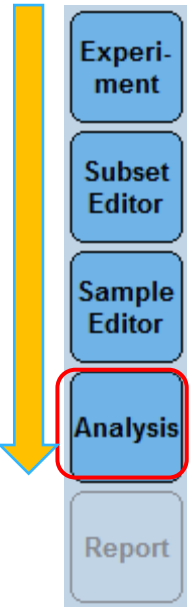
On the *Standards* multi-select button, select *Std Curve (External)*.

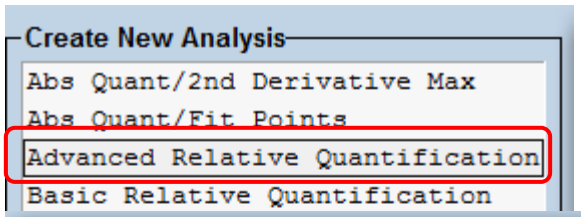


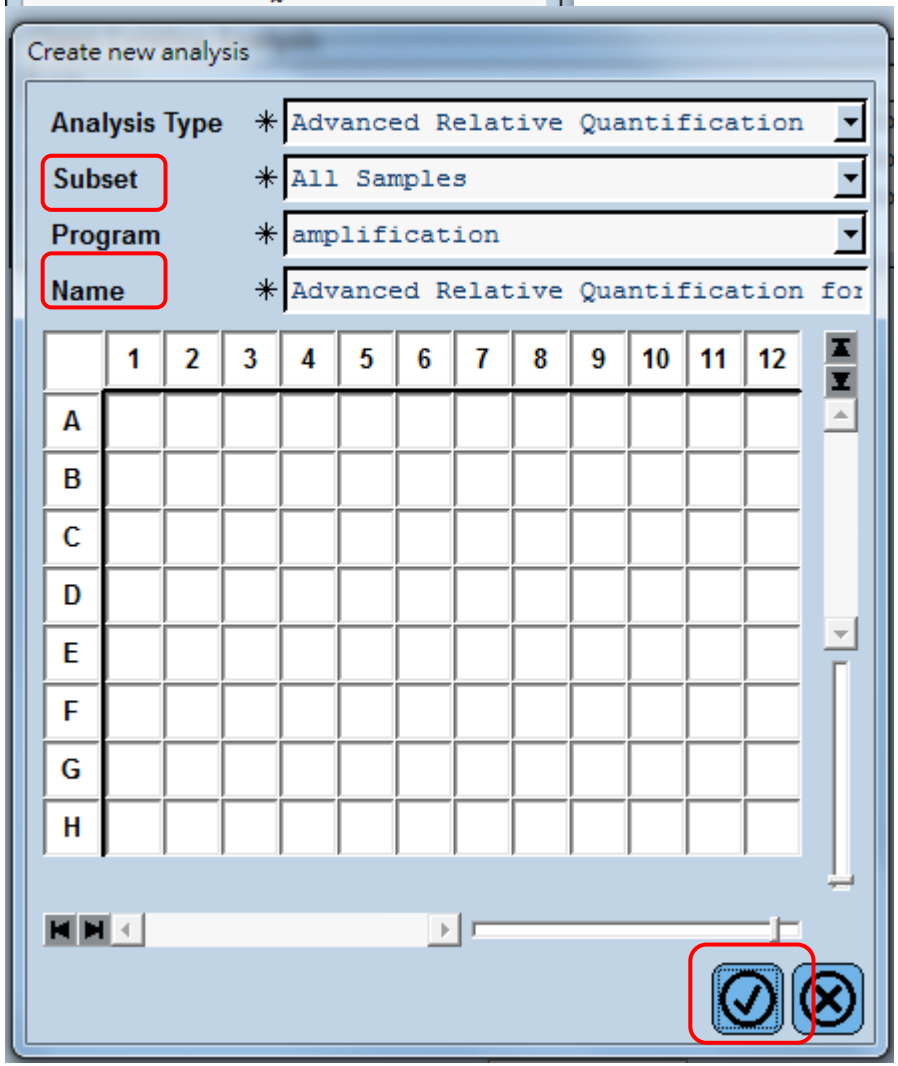
The *Apply External Standard Curve* dialog opens. Select an appropriate external standard curve object from the list:

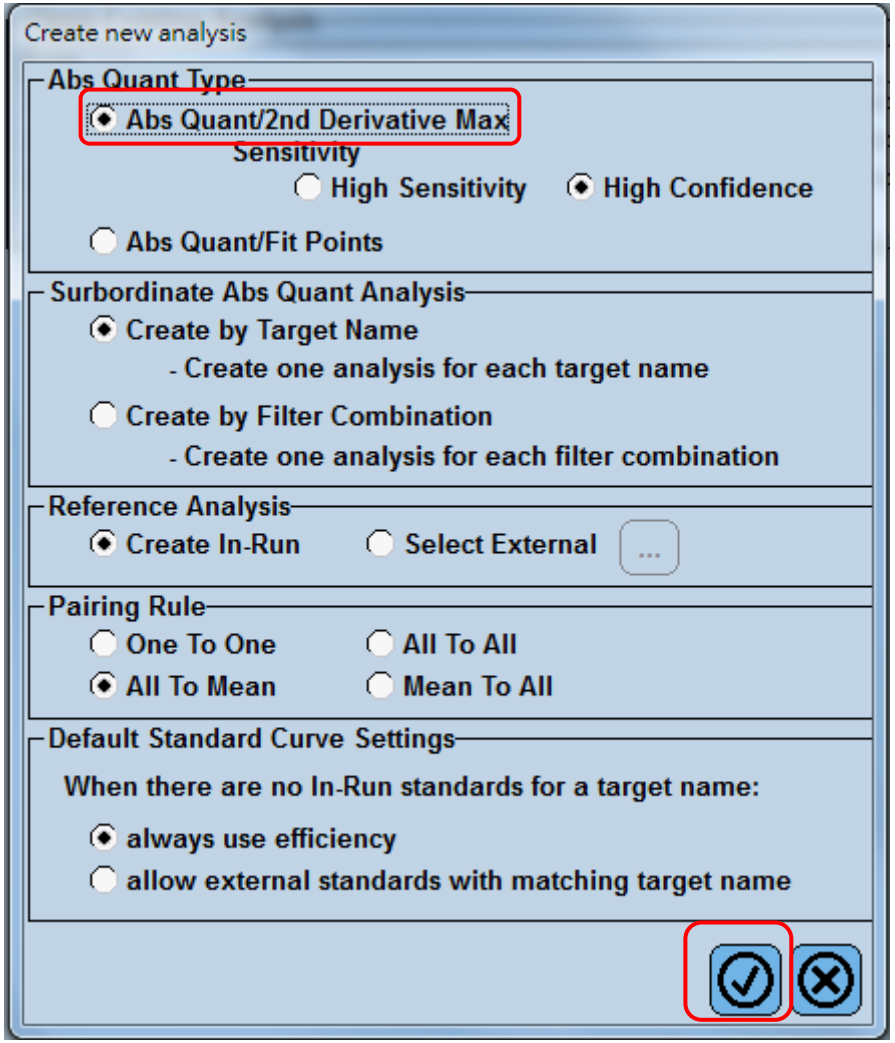


RQ Analysis


Experi-
ment
Subset
Editor
Sample
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
Surbordinate 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

Roche

Experi-ment

Subset Editor

Sample Editor

Analysis

Report

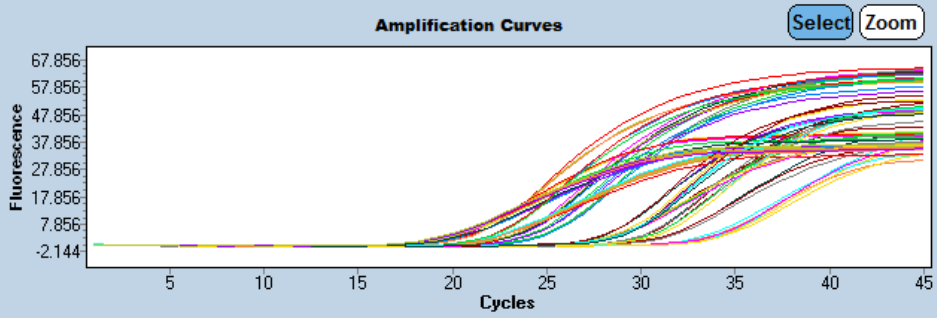
Calculate

Results | Manual Pairing | Target Name

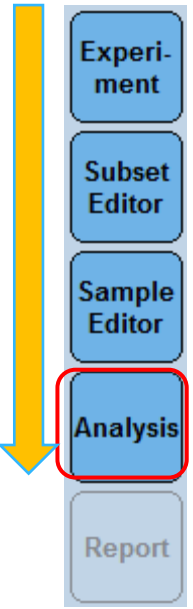
Bar Chart	Pairing	Sample Name	Target Name: Targets	Target Name: References	Target: Mean Cp	Reference: Mean Cp	Ratios: Target/Ref	Ratios: Normalized	Status
☑		calibrator	Target 1	Reference 1;Refere	22.43	24.14	3.266	1.000	
☑	A1/D1	sample 1	Target 1	Reference 1;Refere	23.76	26.31	5.859	1.794	
☑	A2/D2	sample 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	

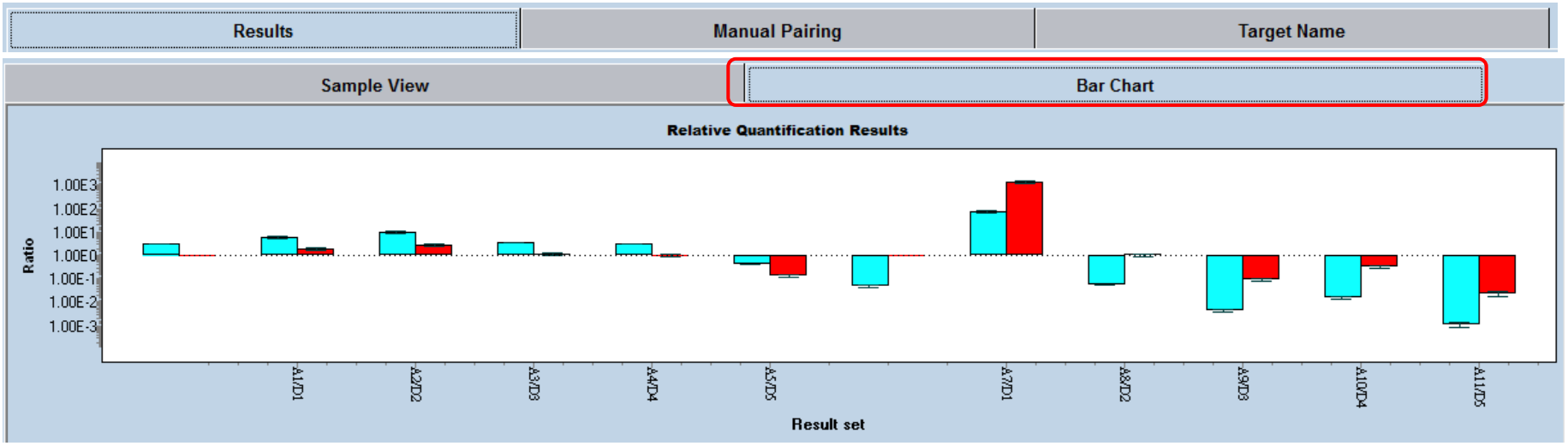
Sample View | Bar Chart

Samples for Pairing						Results
Color	Pos	Sample Name	Type	Filter Comb	Target Name	Cp
	A1	sample 1	Target Unkno	465-510	Target 1	23.87
	A2	sample 2	Target Unkno	465-510	Target 1	21.47
	A3	sample 3	Target Unkno	465-510	Target 1	24.27
	A4	sample 4	Target Unkno	465-510	Target 1	23.53
	A5	sample 5	Target Unkno	465-510	Target 1	25.19
	A7	sample 1	Target Unkno	465-510	Target 2	20.30
	A8	sample 2	Target Unkno	465-510	Target 2	28.87
	A9	sample 3	Target Unkno	465-510	Target 2	34.09
	A10	sample 4	Target Unkno	465-510	Target 2	31.45

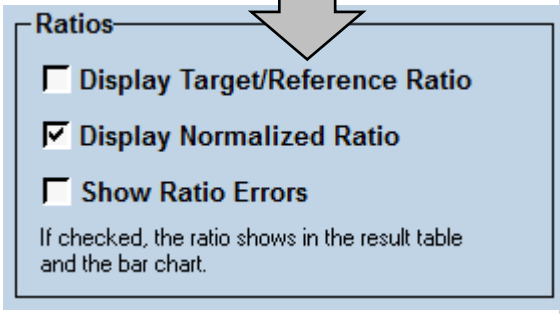


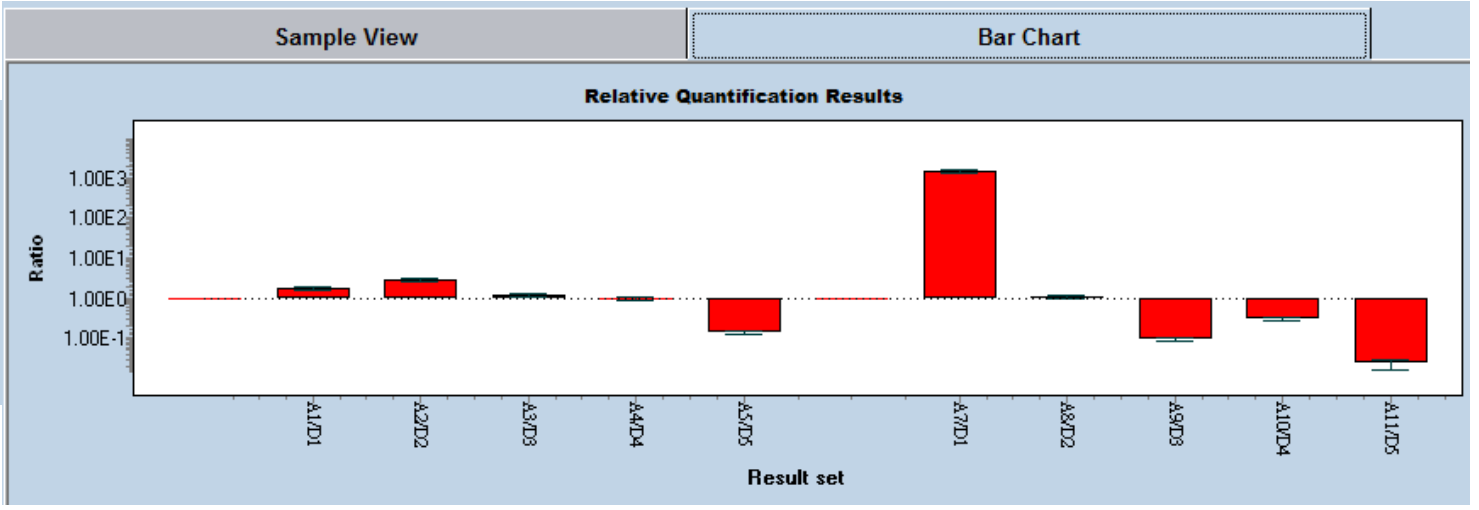
RQ Analysis


Experi-
ment
Subset
Editor
Sample
Editor
Analysis
Report

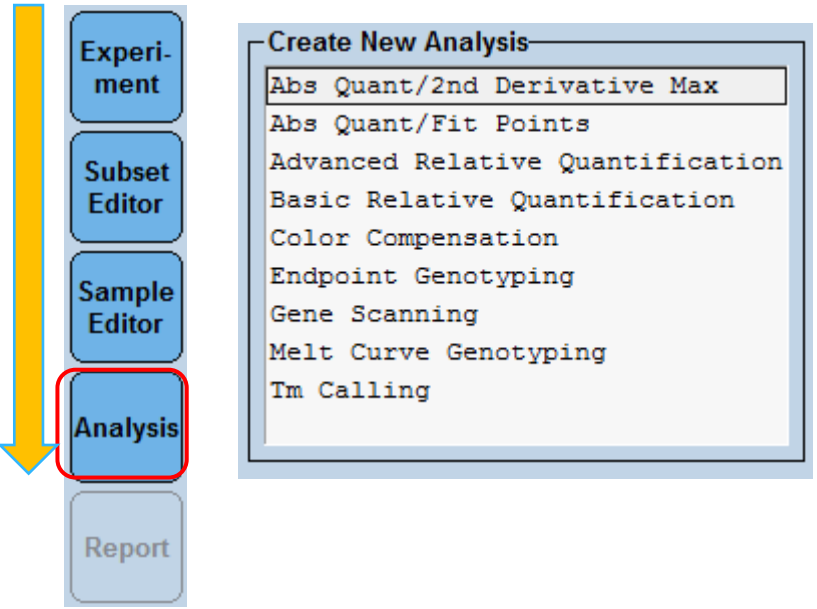

Results
Manual Pairing
Target Name
Sample View
Bar Chart
Relative Quantification Results
Ratio
1.00E3
1.00E2
1.00E1
1.00E0
1.00E-1
1.00E-2
1.00E-3
A1/D1
A2/D2
A3/D3
A4/D4
A5/D5
A7/D1
A8/D2
A9/D3
A10/D4
A11/D5
Result set

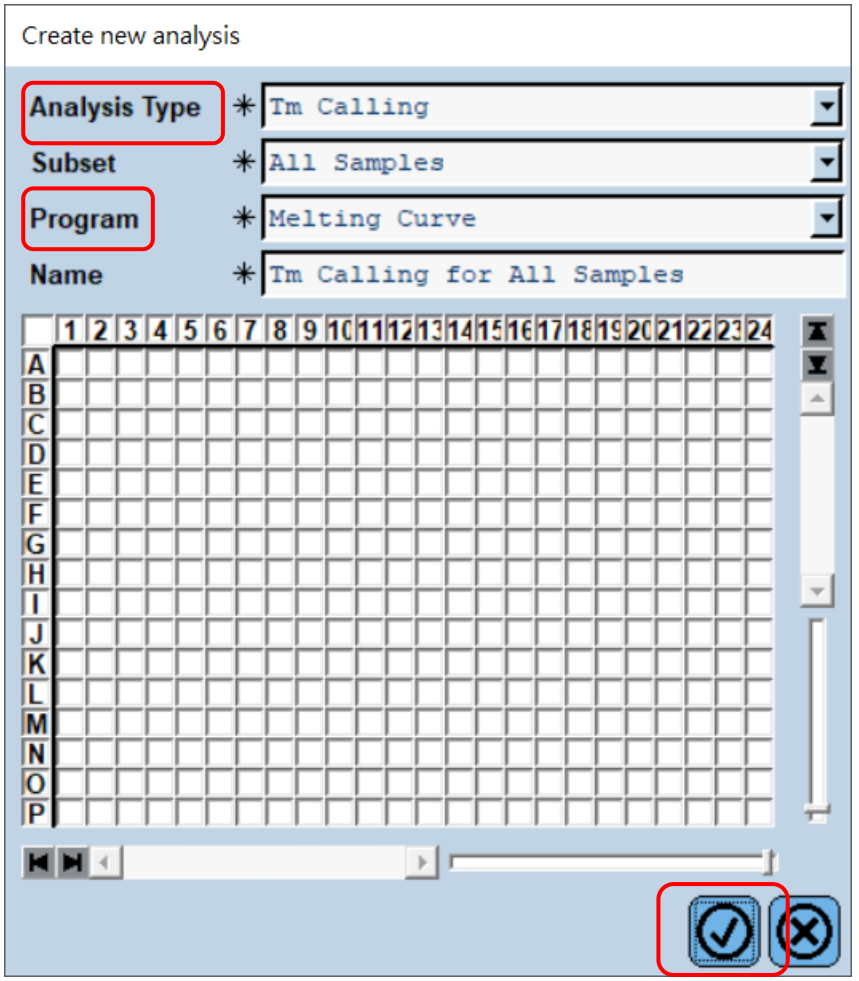

Settings


Ratios
Display Target/Reference Ratio
Display Normalized Ratio
Show Ratio Errors
If checked, the ratio shows in the result table
and the bar chart.

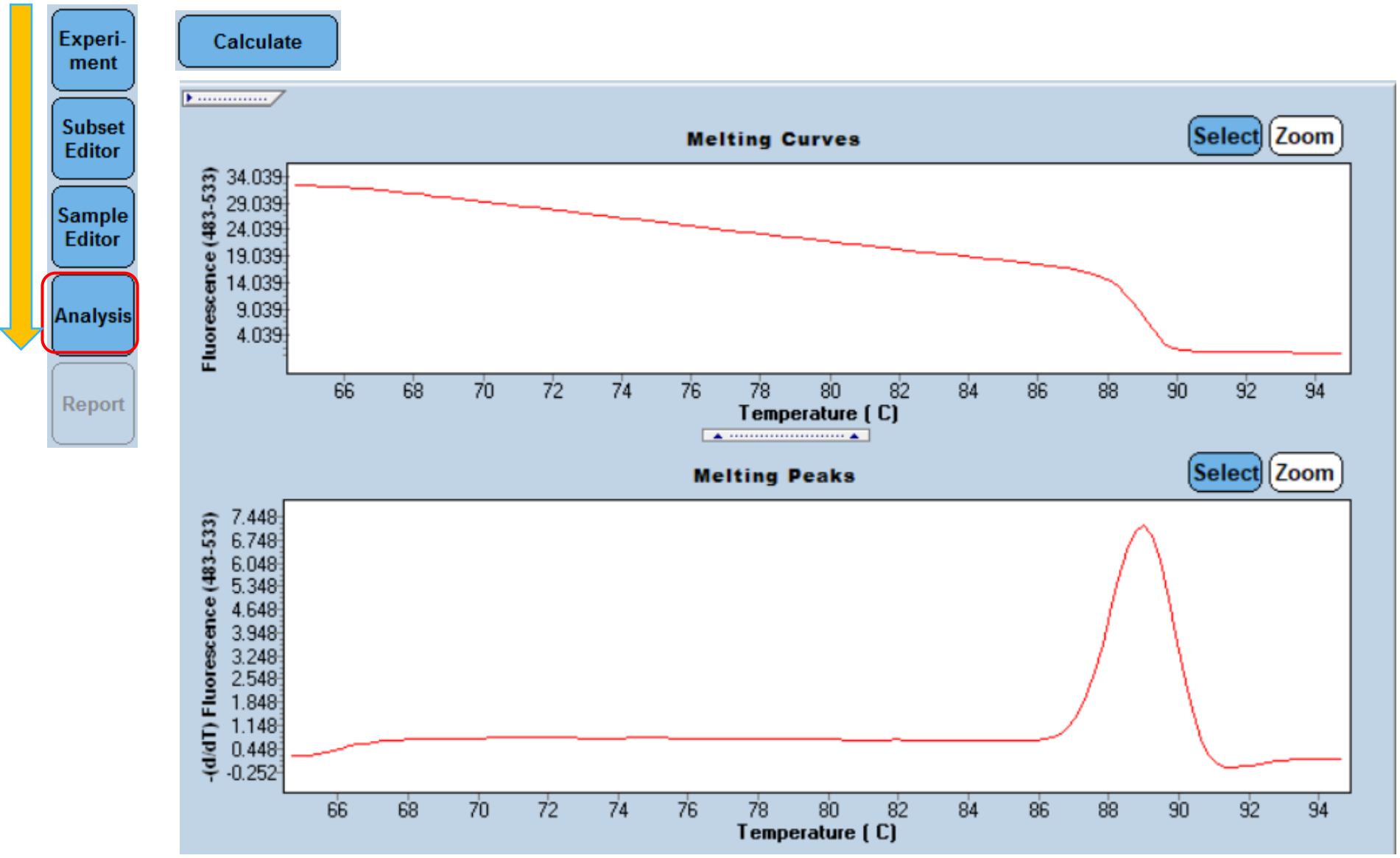

Sample View
Bar Chart
Relative Quantification Results
Ratio
1.00E3
1.00E2
1.00E1
1.00E0
1.00E-1
A1/D1
A2/D2
A3/D3
A4/D4
A5/D5
A7/D1
A8/D2
A9/D3
A10/D4
A11/D5
Result set

Analysis

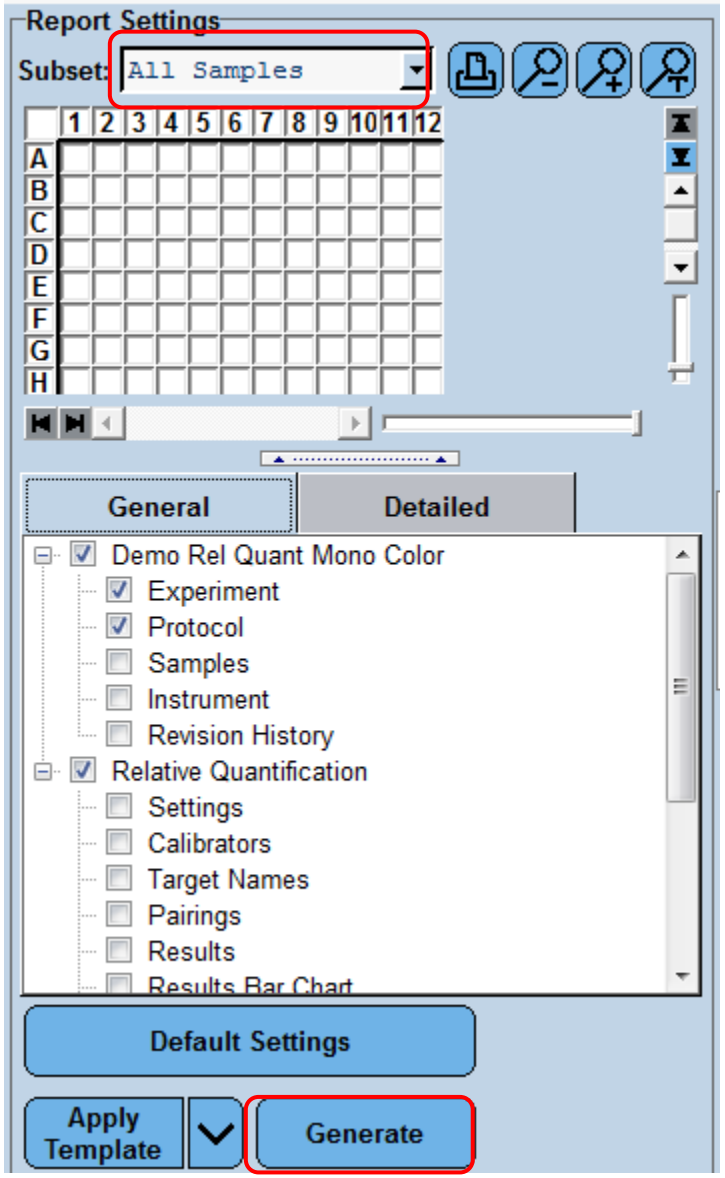

Experi-ment
Subset Editor
Sample Editor
Analysis
Report
Create New Analysis
Abs Quant/2nd Derivative Max
Abs Quant/Fit Points
Advanced Relative Quantification
Basic Relative Quantification
Color Compensation
Endpoint Genotyping
Gene Scanning
Melt Curve Genotyping
Tm Calling

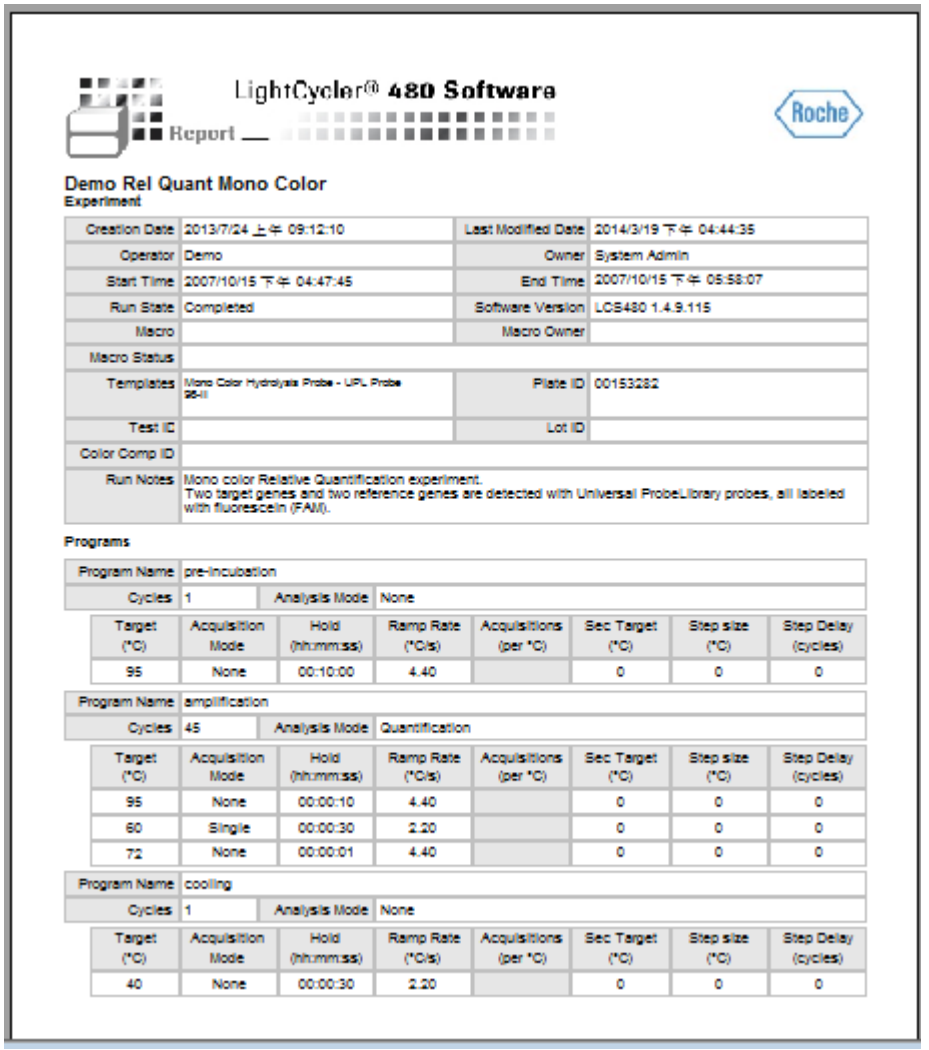

Create new analysis
Analysis Type
Tm Calling
Subset
All Samples
Program
Melting Curve
Name
Tm Calling for All Samples
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
A B C D E F G H I J K L M N O P

Tm calling/Melting curve



Report





LightCycler® 480 Software

Report

Roche

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-II	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


Log into a database


Show the overview


Show the navigator


Save changes to selected object

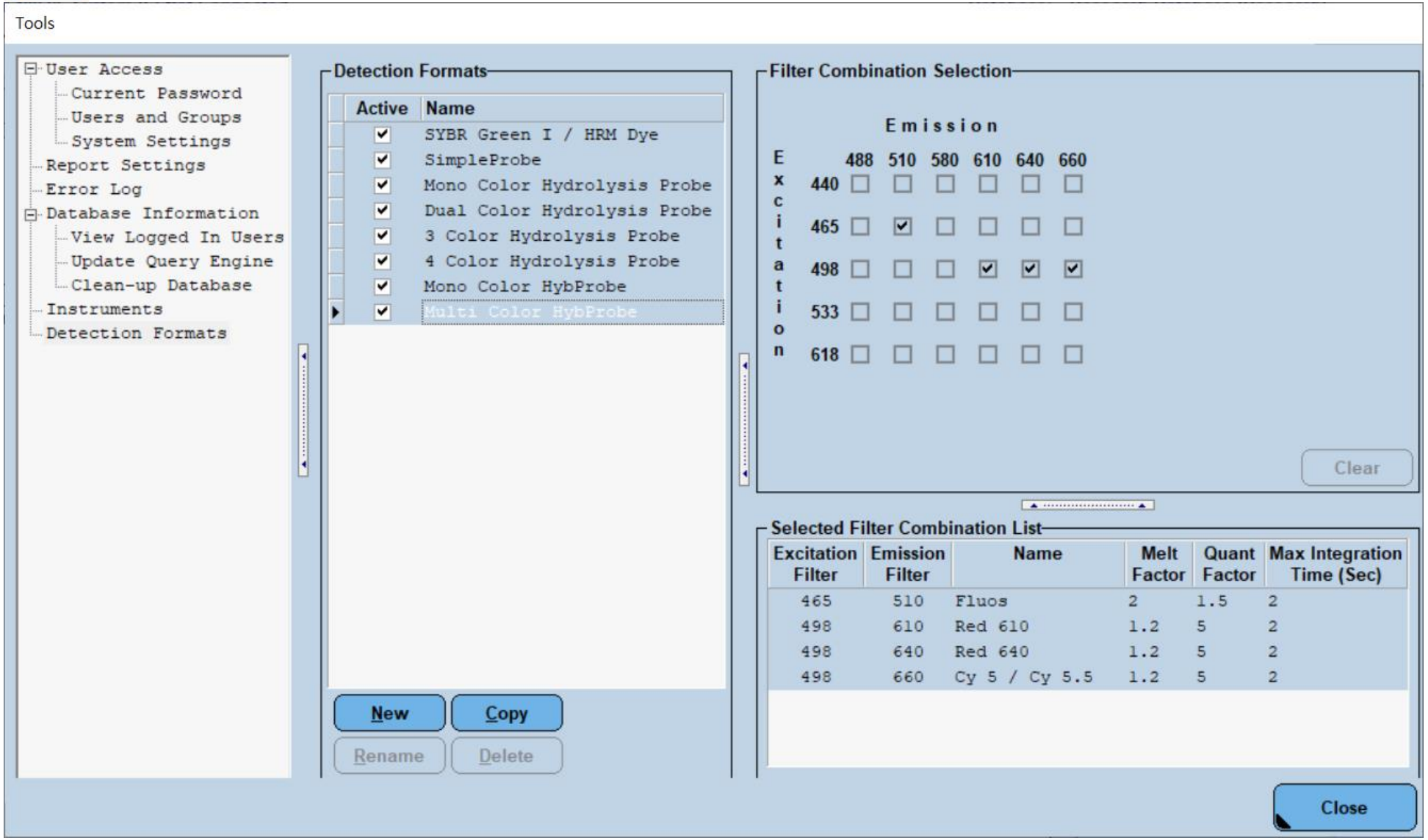

Export the selected object to a file


Close the selected object


Print the screen

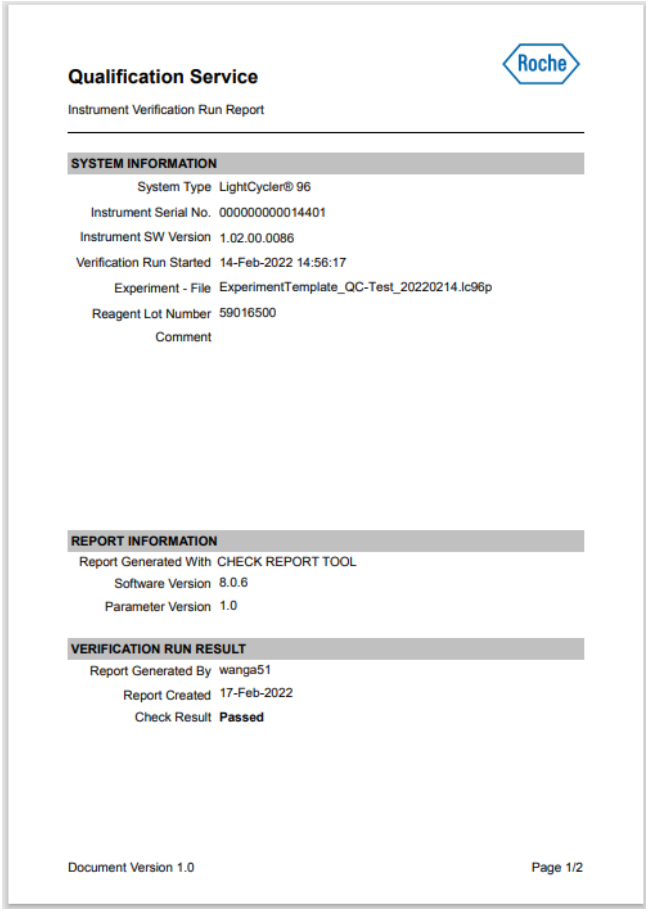

Open Tools

Create a new detection Format



- Real Time PCR Basic Training
- Troubleshooting cases sharing
- 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.	000000000014401
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	

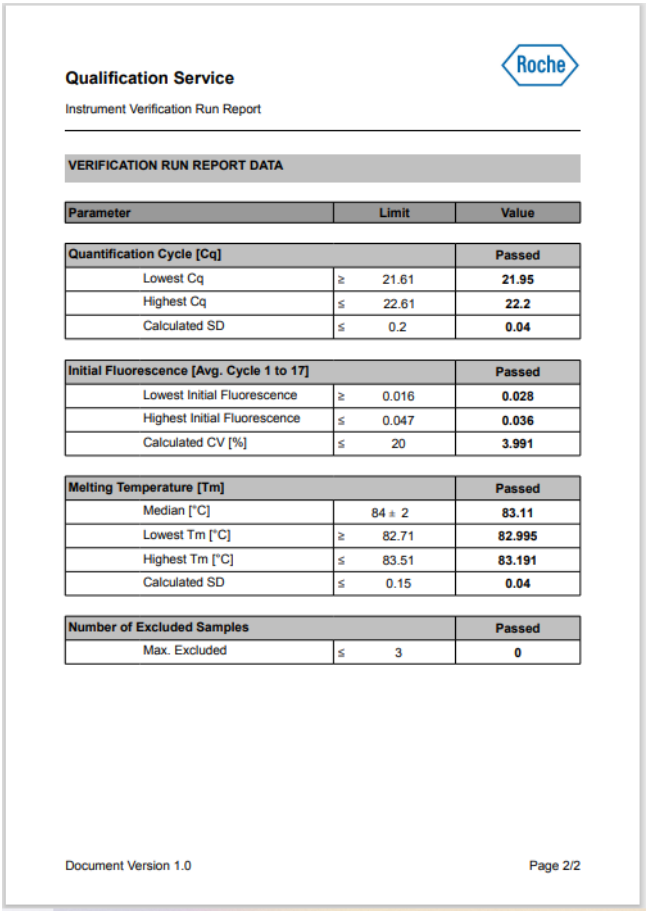
REPORT INFORMATION

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

Q³ 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.ixo
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

ıment Version 1.0 Page 2/3

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

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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.ixo

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:

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- Real Time PCR Basic Training
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Roche Digital LightCycler® System

A technological guide to the powerful new addition to our PCR ecosystem

It's time for a leap forward in digital PCR technology. The Roche Digital LightCycler® System is the digital PCR instrument of tomorrow. With a unique combination of 3 nanowell plate configurations, 6 advanced optical channels, and 5x concentrated DNA and RNA master mixes, it has the potential to help your lab to make the leap from publishing research to producing clinically viable assays.

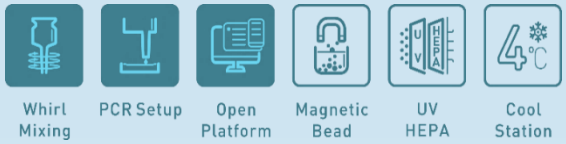
Digital LightCycler®

Automated Workflows From Sample to Result

MAGXTRACT 3200

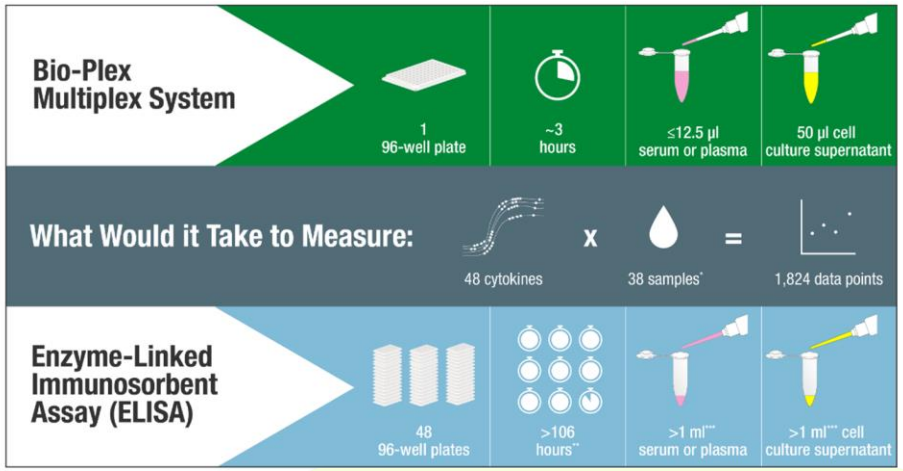
Automated Nucleic Acid purification and PCR Setup System

Beyond Nucleic Acid Purifications

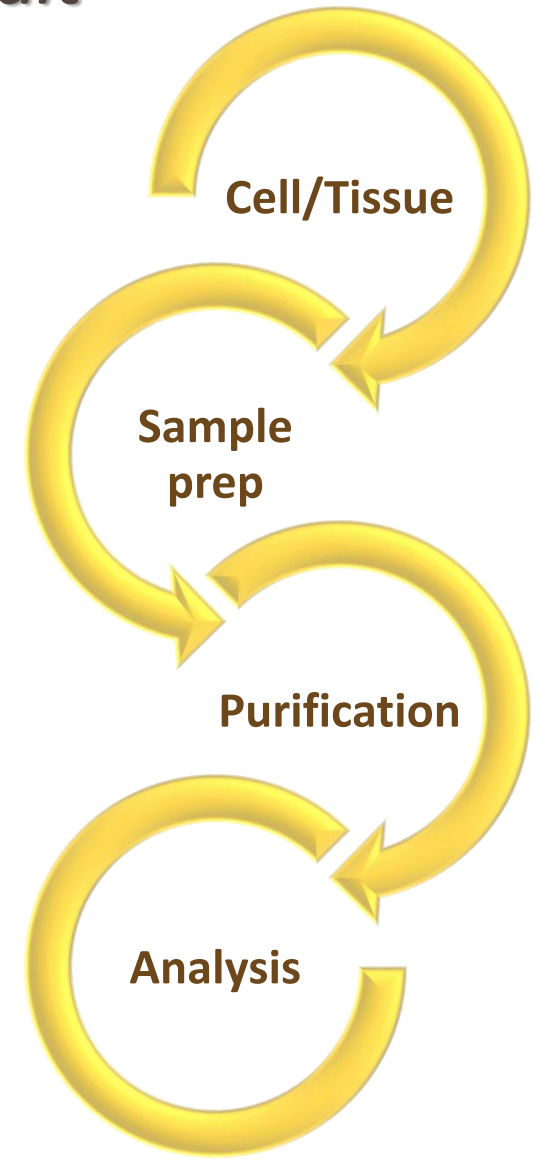


IVDR Registered

Comparison of Bio-Plex Multiplexing vs. ELISA



* Samples run in duplicate.
** Calculated as 2.2 hours required per plate.
*** Assumes 50 µl of sample used per well.



提供快速精準的代檢服務

Thank You

又鑫生物科技有限公司

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Sophia Lin

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