



ThermoFisher
S C I E N T I F I C

定量PCR數據分析之常見問題與Troubleshooting

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Field Application Scientist

The world leader in serving science

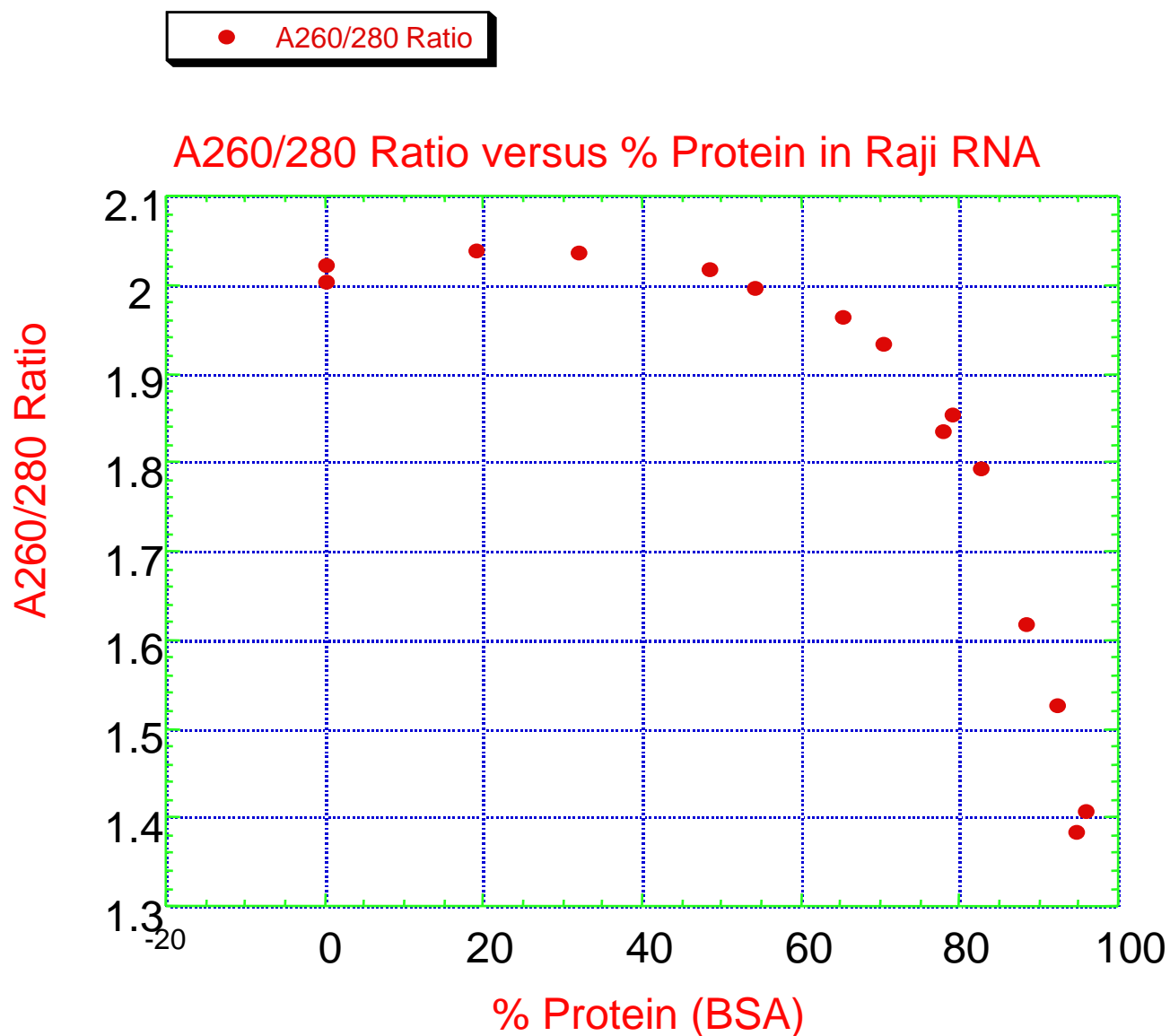
Troubleshooting and FAQ

- Pre-PCR
 - Primer design: bioinformatics
 - Sample Preparation
- Post-PCR
 - Software
 - Operations
 - Instrument
 - Reagents and Consumables

- Pre-PCR
 - Primer design: bioinformatics evaluations
 - Quality of sequence
 - RepeatMasker (<http://www.repeatmasker.org>)
 - BLAST in NCBI (<http://www.ncbi.nlm.nih.gov/BLAST/>)
 - Design tools
 - Custom TaqMan® Assay Design Tool (<https://www.thermofisher.com/order/custom-genomic-products/tools/cadt/>)
 - Primer Express® 3.0.1
 - Sample Preparation

- Pre-PCR
 - Primer design: bioinformatics
 - Sample Preparation
 - Purity of DNA/RNA
 - Quantity of DNA/RNA
 - Reverse Transcription for RNA

Protein Contamination Affecting A260/280



PCR Inhibitors

Sample related:

Heparin > 0.15 mg/ml

Hemoglobin > 1 mg/ml

Melanin, humic acids,
chlorophyll, polysaccharides

Extraction related:

SDS > 0.01% (w/v)

Phenol > 0.2% (v/v)

Ethanol > 1%

Sod. acetate > 5 mM

PCR additives:

DTT > 1 mM

DMSO > 5 %

EDTA > 50 mM

Mercaptoethanol

標準曲線檢查樣本品質

- 以一個高濃度的樣本(cDNA or gDNA)作為起始樣本
- 對樣本進行梯度稀釋
- 每個稀釋度重複三次進行絕對定量實驗
- 檢查標準曲線的線性
 - 所有的點都應該落在同一條線上 (R^2)
 - PCR efficiency

Troubleshooting and FAQ

- Pre-PCR
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Troubleshooting and FAQ: Error Messages

- “Java.lang.IllegalArgumentException: One of the raw spectra is null”
 - Illegal characters (e.g. ~)
 - Double-clicking “Start Run” button
 - Interrupting analysis or saving process (e.g. closing experiment or unplugging USB before process completes)
- “Cannot calculate pure dye matrix”
 - Run file has lost raw data (e.g. run was aborted)
- “Analysis failed due to GExSession doesn't exist in context”
 - Data collection was turned off during cycling stage

Run Method: Data Collection

StepOne™ Software v2.3

File Edit Instrument Analysis Tools Help

New Experiment Open Save Close Send Experiment to Instrument... Download Experiment from Instrument... Export... Print Report...

Experiment: **Untitled 1** Type: **Comparative Ct ($\Delta\Delta Ct$)** Reagents: **SYBR® Green Reagents** **START RUN**

Run Method

Review the reaction volume and the thermal profile for the default run method. If needed, edit the default run method or select a run method from the library.

Graphical View Tabular View

Reaction Volume Per Well: 20 μ L

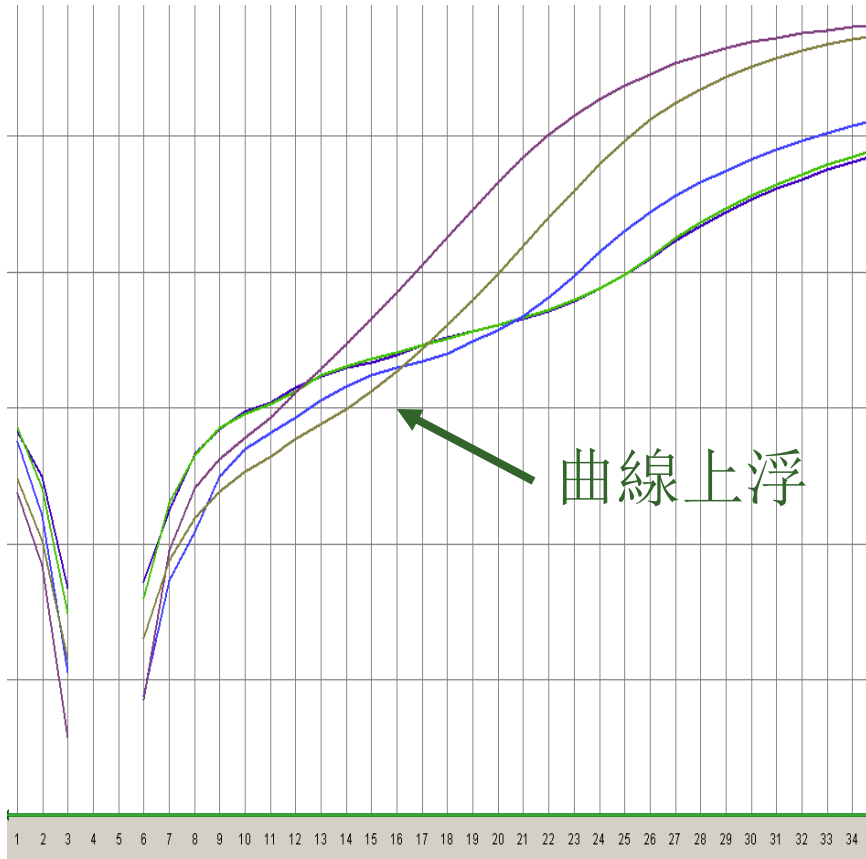
Add Stage Add Step Delete Selected (nothing to Undo) (nothing to Redo) Collect Data Open Run Method Save Run Method... Revert to Defaults

Stage	Temperature (°C)	Time	Notes
Holding Stage	95.0	10:00	100%
	95.0	00:15	100%
Cycling Stage	95.0	00:15	100%
	60.0	01:00	100%
	60.0	01:00	100%
Melt Curve Stage	95.0	00:15	100%
	60.0	01:00	100%
	95.0	00:15	100%

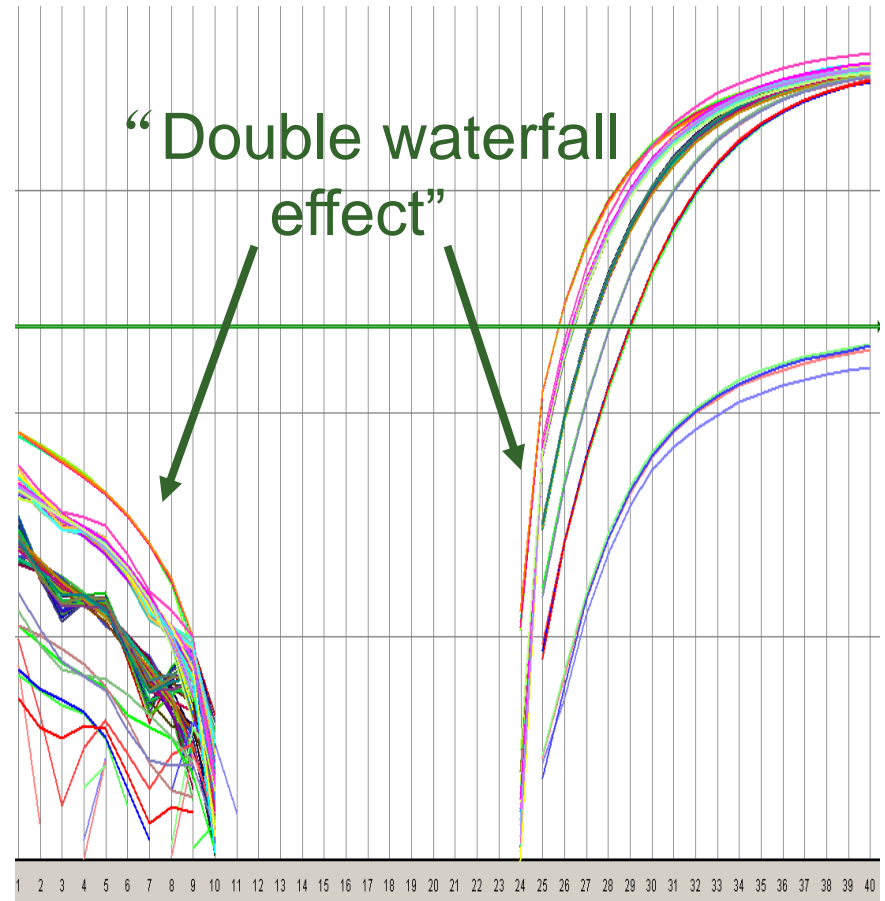
Legend: Data Collection On Data Collection Off AutoDelta On AutoDelta Off

Troubleshooting and FAQ: “Weird” Amplification Plots

- Baseline設置太低

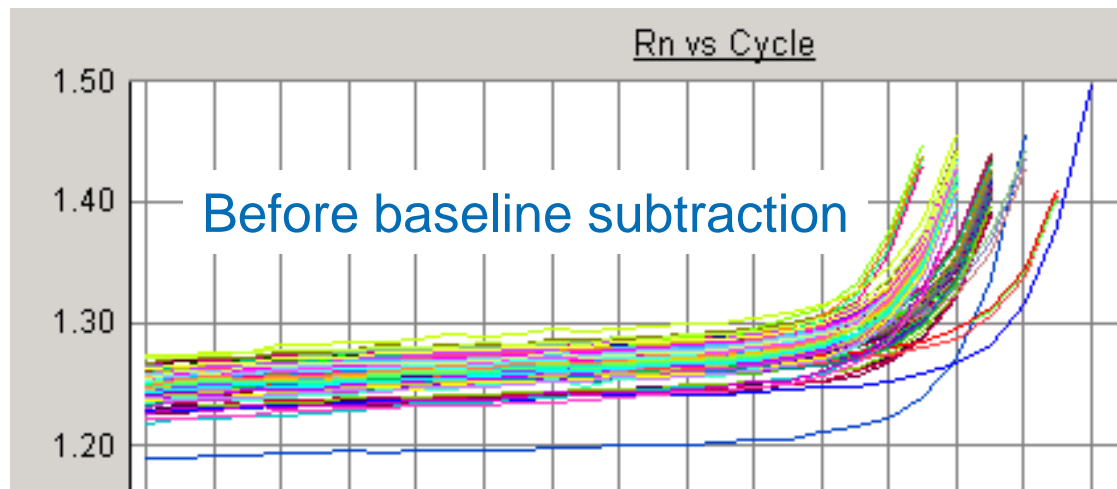


- Baseline設置太高

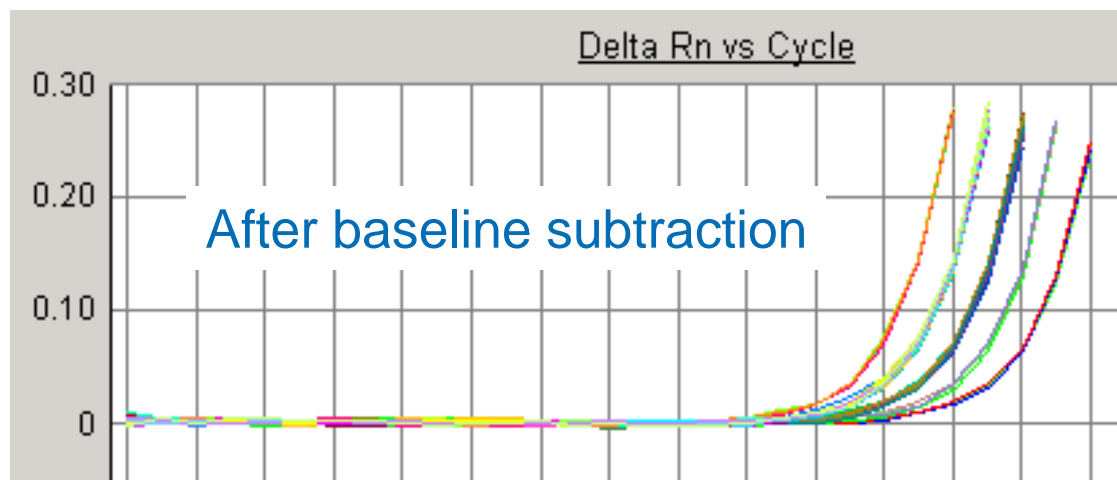


Baseline的作用

R_n



ΔR_n



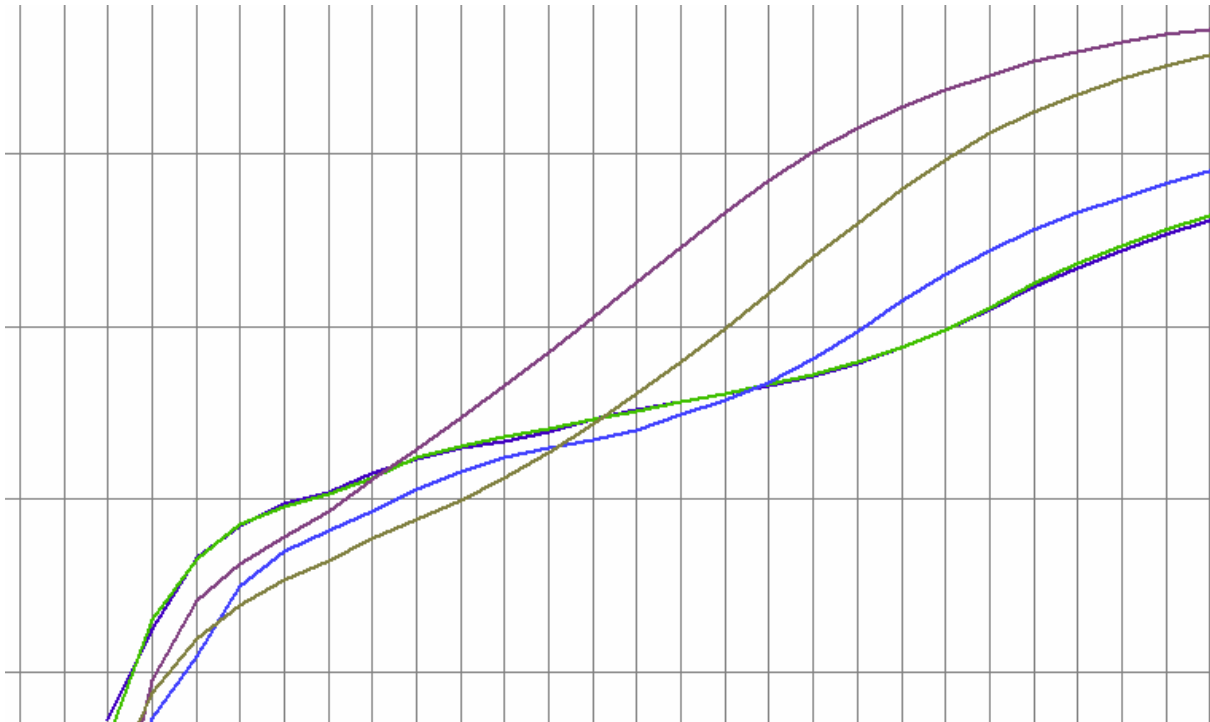
軟體自動設置baseline

- 軟體為每個well單獨設置baseline
- 使每條擴增曲線都能有最佳的baseline設置
- 同時包含高濃度和低濃度樣品的實驗也能準確設置baseline

The screenshot displays the StepOne Software v2.3 interface. The main window shows an "Amplification Plot" for a "96-Well Comparative" experiment using "TaqMan® Reagents". The plot shows ΔRn on the y-axis (log scale from 0.0001 to 10) versus Cycle on the x-axis (from 2 to 40). A horizontal pink line indicates the baseline at $\Delta Rn = 0.564853$. The "Options" section at the bottom of the plot is highlighted with a red box, showing "Target: BCL2", "Threshold: Auto", and "Auto Baseline" . The "Analysis Settings" button in the top right is also highlighted with a red box. The "View Plate Layout" section on the right shows a 96-well plate grid with wells A1-A12, B1-B12, C1-C12, and D1-D12, each containing a sample name and color-coded well type (e.g., N HPI, U H, U C, U B, U P). The status bar at the bottom indicates "Wells: U 36 Unknown N 12 Negative Control" and "48 Empty".

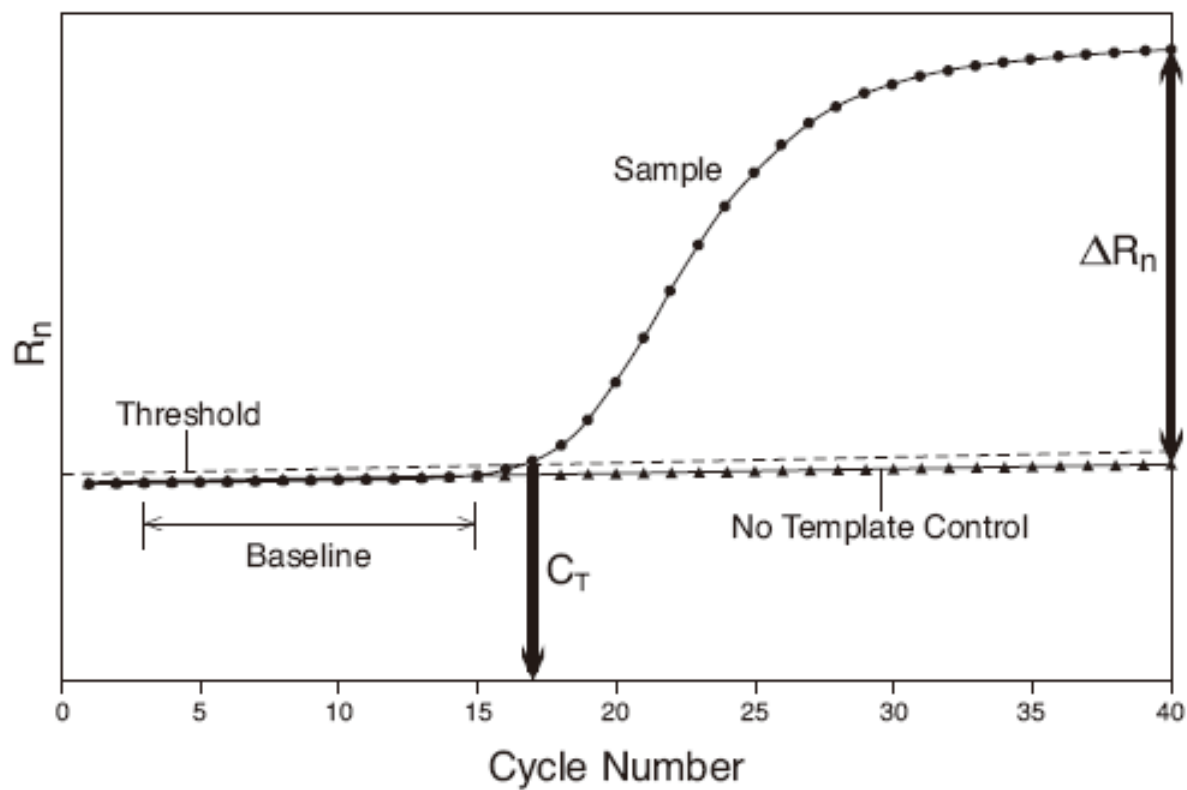
自動baseline設置會出錯嗎？

- Yes . . .
 - 如果試劑的背景值太高, 有時會被軟體錯誤地識別為擴增的信號。導致某些樣本的baseline範圍設置過小。
 - 解決方案: 手動設置baseline

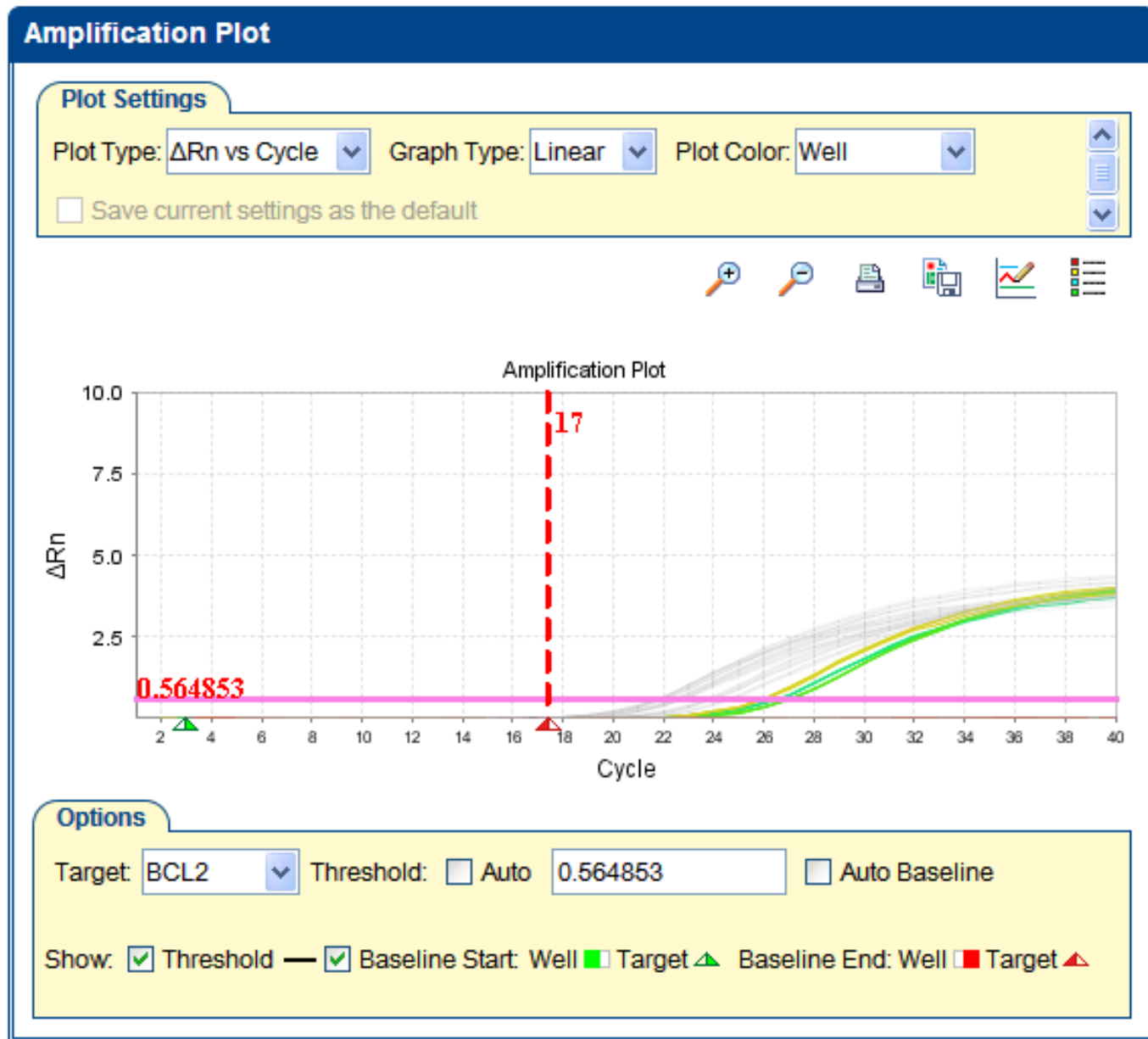


如何正確手動設置baseline?

- 將Y軸轉為線性 (Linear)
- Baseline需設置在訊號上升之前



如何正確手動設置baseline?



如何正確手動設置baseline?

Tools Help

Save Close Send Experiment to Instrument... Download Experiment from Instrument... Export... Print Report...

Experiment: **96-Well Comparativ...** Type: **Comparative Ct ($\Delta\Delta Ct$)** Reagents: **TaqMan® Reagents** Analyze Analysis Settings ?

Amplification Plot View Plate Layout View Well Table

Analysis Settings for 96-Well Comparative CT Example

CT Settings Flag Settings Relative Quantitation Settings **Advanced Settings**

I Review analysis settings for the wells in this experiment. To use different settings, select the well(s) from the table, deselect "Use Ct Settings Defined for the Target," then change the settings that are displayed.

Select a Well and Target

Well	Target	Baseline	Baseline Start	Baseline End
B1	HPRT1	Manual	3	17
B2	HPRT1	AUTO	3	17
B3	HPRT1	AUTO	3	17
B4	CDKN1B	AUTO	3	17
B5	CDKN1B	AUTO	3	17
B6	CDKN1B	AUTO	3	17
B7	BCL2	AUTO	3	19
B8	BCL2	AUTO	3	19
B9	BCL2	AUTO	3	19
B10	PGK1	AUTO	3	15

Baseline Settings for Well B1, HPRT1

Baseline Settings to Use: Use Ct Settings Defined for Target

Automatic Baseline

Baseline Start Cycle: 3 End Cycle: 17

48 Empty

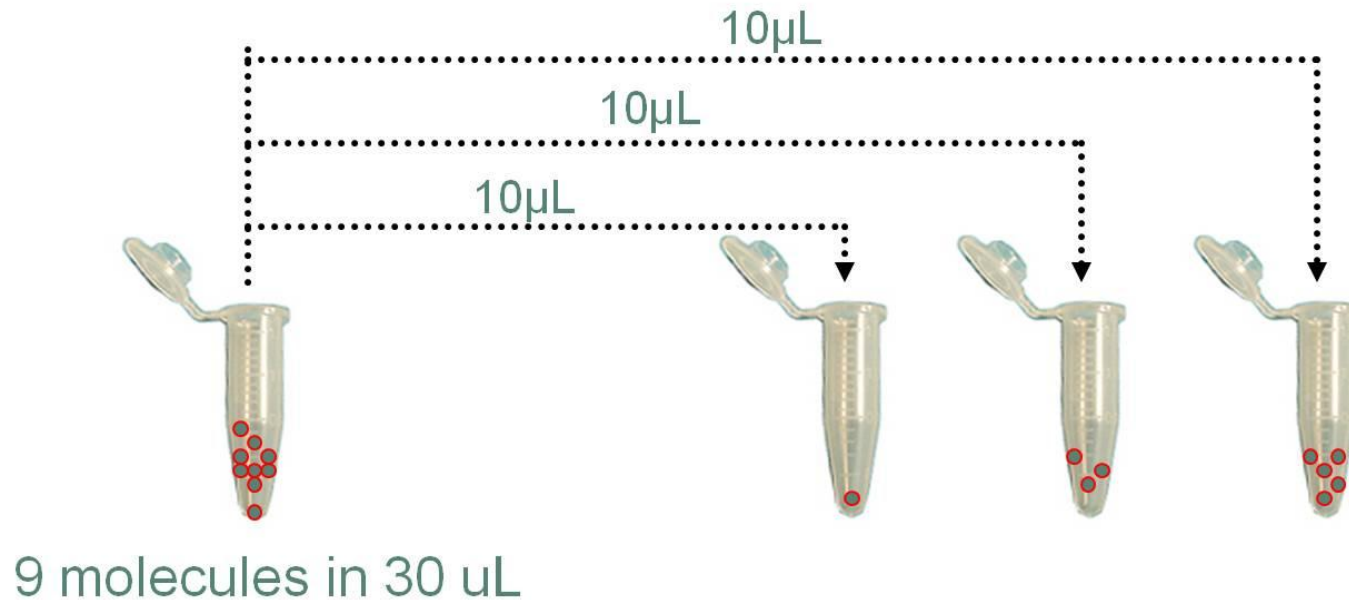
Samples Used: 3 Targets Used: 4

Revert to Default Analysis Settings Apply Analysis Settings Cancel

- Pre-PCR
 - Primer design: bioinformatics
 - Sample Preparation
- Post-PCR
 - Software
 - Operations
 - Instrument
 - Reagents and Consumables
 - High standard deviations in replicates
 - Amplification in NTC

Troubleshooting: High Standard Deviations in Replicates

- 加樣誤差(建議每次pipetting體積至少 $5\mu\text{L}$)
- 沒有將試劑和樣品充分混勻
- 低拷貝 (low copy) 的樣品: Poisson distribution

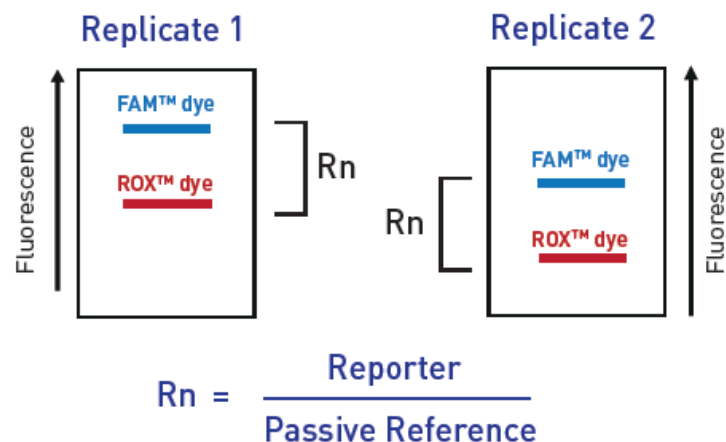


每個反應管均勻的分配到3個templates的幾率有多高?

Passive Reference: ROX™ Dye

- 哪些原因會影響螢光強度的一致性？

- Reaction是否充分混和
- 蒸發
- 氣泡
- Pipetting 誤差
- Condensation
- Plastic consumables



- ROX™ dye normalizes for non-PCR related fluorescence variations (volume, sample...) → 提高重複性
- Only the ROX dye can dynamically correct for fluorescence fluctuations during the PCR reaction
- It is impossible to apply the Ct-calling algorithm of software to correct any variables listed above during data analysis
- An excellent troubleshooting tool

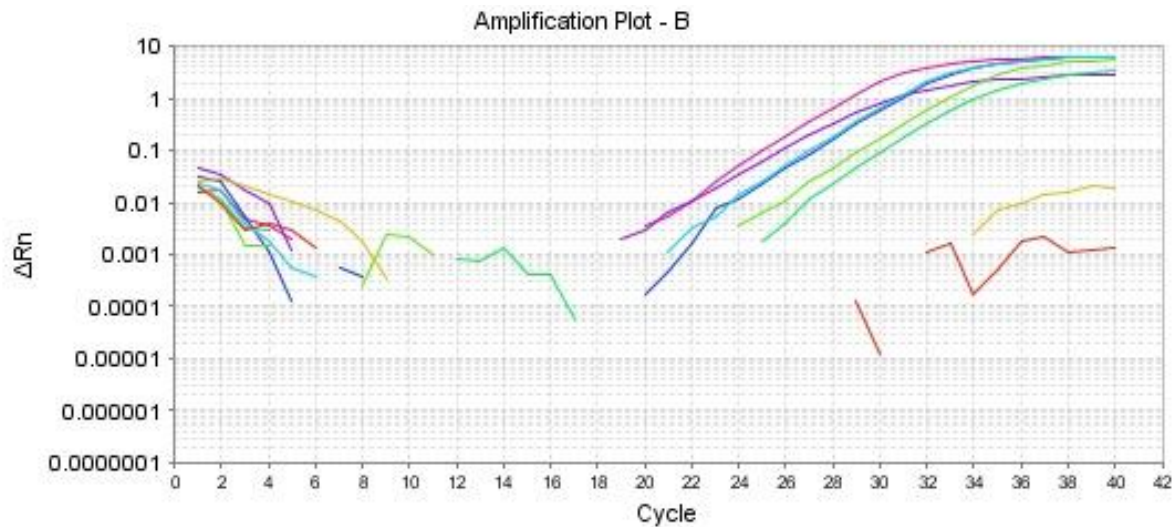
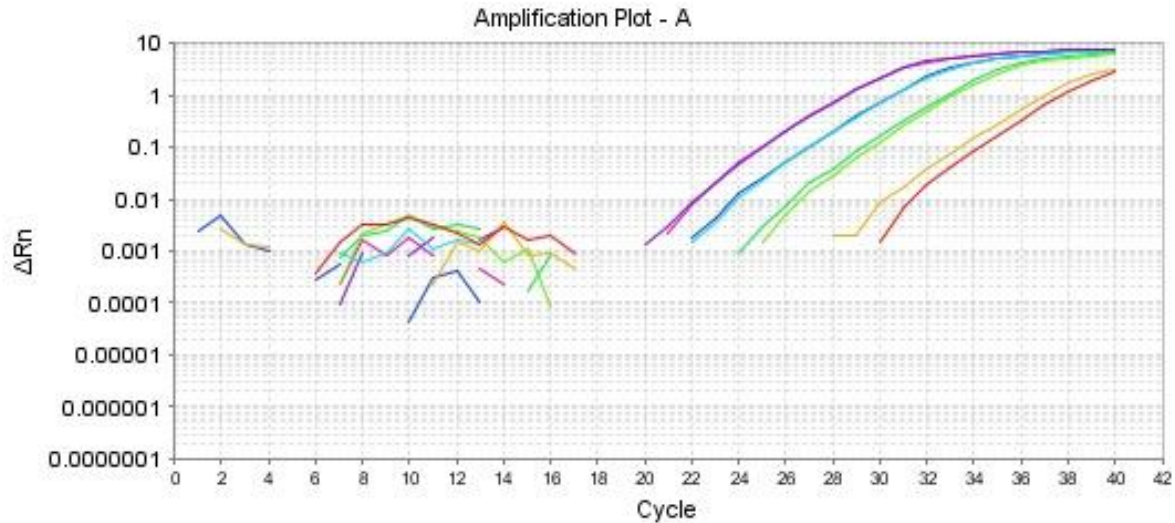
ROX™ Dye Normalization

- Normalization to ROX™ dye is performed automatically by SO software
- Some non-AB PCR master mixes do not contain ROX™ dye; if these are used, the passive reference option should be turned off prior to data analysis

The screenshot shows the StepOne™ Software v2.3 interface. The main window is titled 'Experiment: 96-Well Comparative C...' and 'Type: Comparative Ct (ΔΔ)'. The 'Assign Targets and Samples' tab is active, displaying instructions for setting up unknowns and negative controls. A table lists 'Assign' and 'Sample' columns with 'Kidney' and 'Lung' entries. Below this, a section titled 'Assign sample(s) of selected well(s) to biological group.' contains another table with 'Assign' and 'Biological Group' columns. The 'Passive quantitation settings.' section is highlighted with a red box, showing 'Reference Sample: Kidney' and 'Endogenous Control: PGK1'. A dropdown menu is open, showing options: ROX, FAM, JOE, NED, SYBR, TAMRA, VIC, None, and ROX (selected). The text 'dye to use as the passive reference.' is visible below the dropdown. The bottom status bar shows 'Home 96-Well Compa...CT Example.eda'.

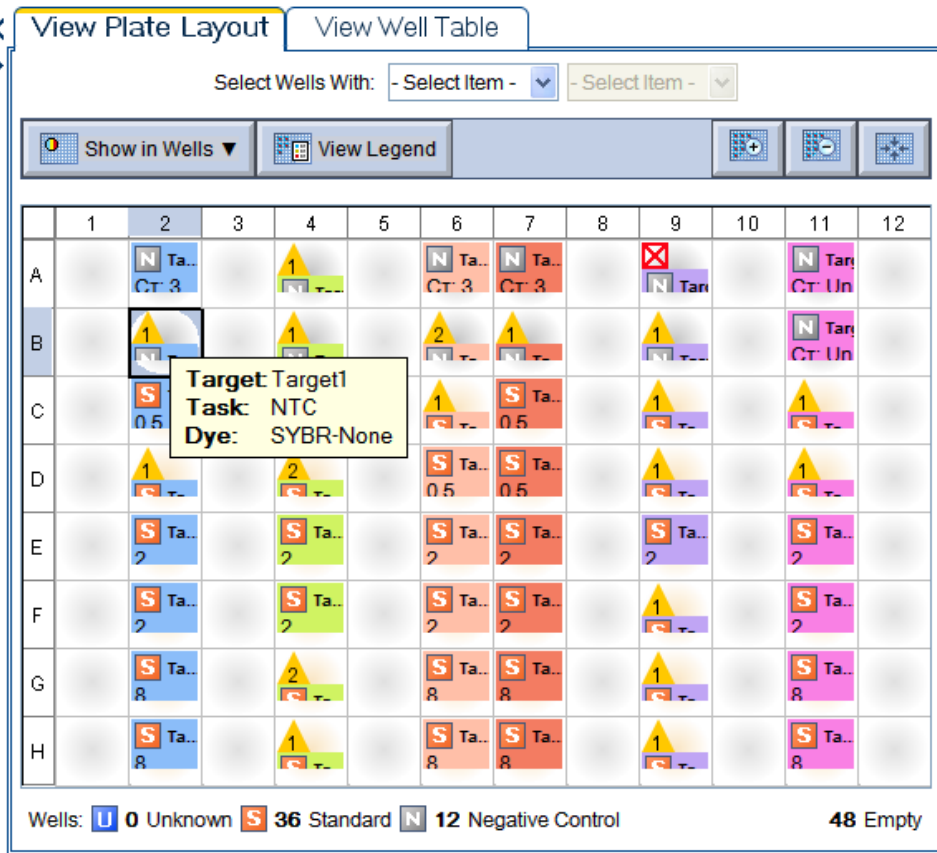
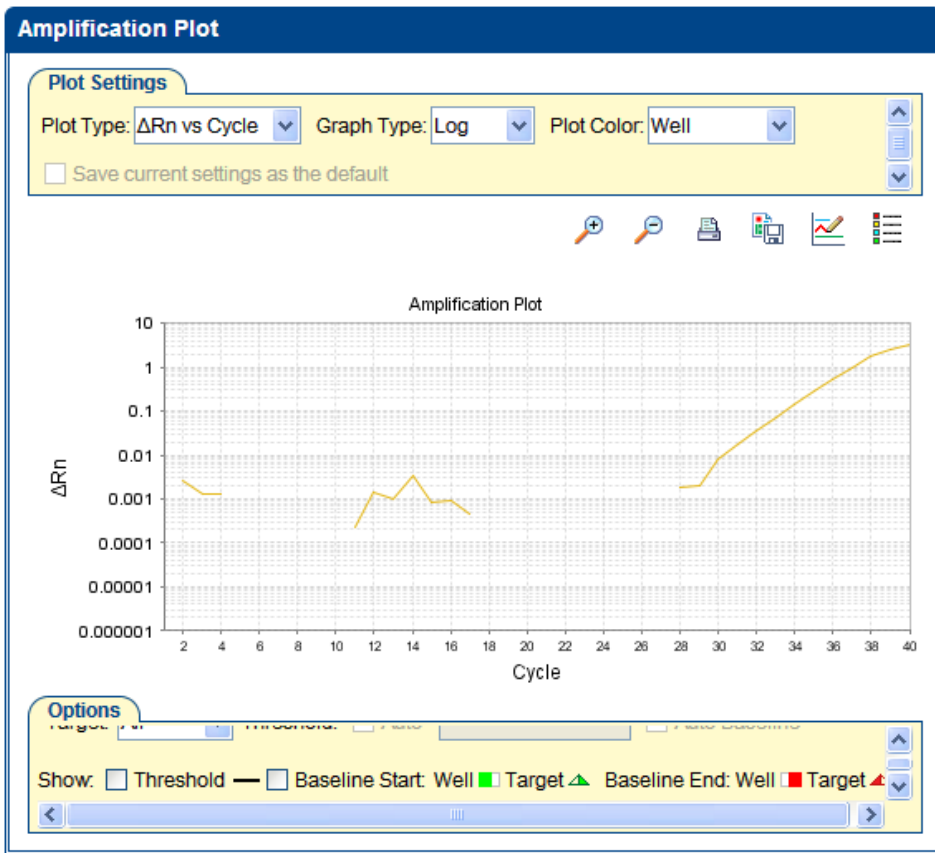
Troubleshooting and FAQ: Consumables

- Exact same reactions using 8-tube strips from different providers on the same run



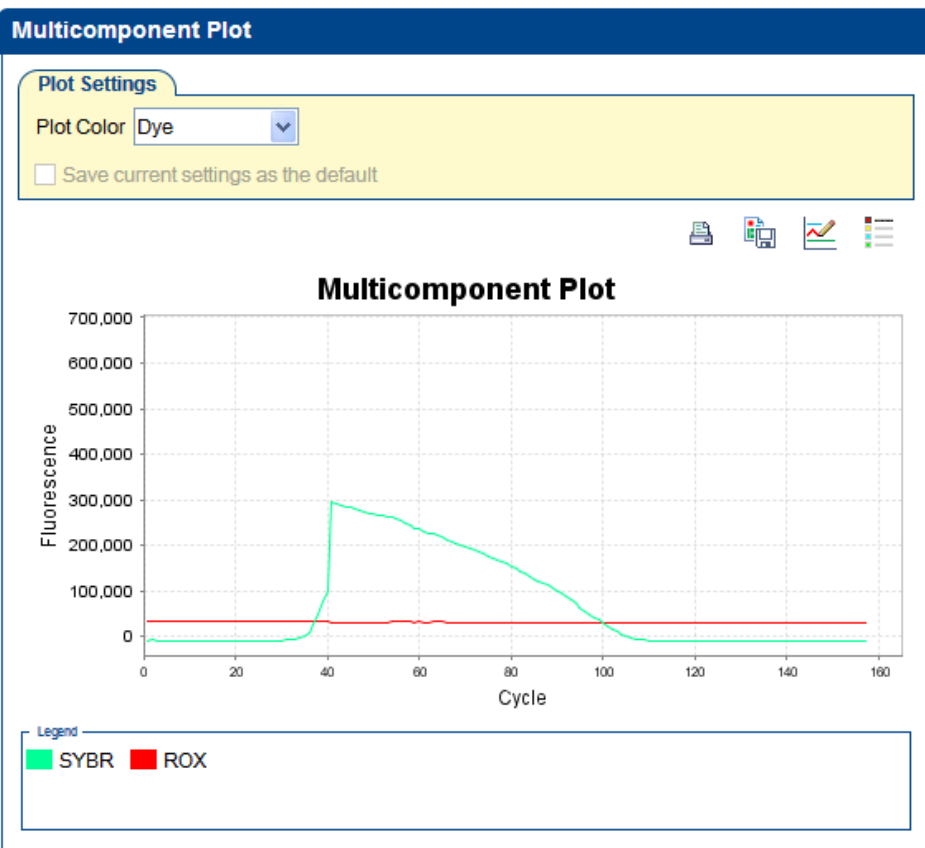
Troubleshooting and FAQ: Amplification in NTC

- Contaminations
- Primer dimer (SYBR green experiments)



Analysis Summary: Total Wells in Plate: 96 | Wells Set Up: 48 | Wells Omitted Manually: 1 | Wells Flagged: 19 | Wells Omitted by Analysis: 0 | Samples Used: 3 | Targets Used: 6

從Multicomponent Plot 確認Report Dye是否有上升



View Plate Layout View Well Table

Select Wells With: - Select Item - - Select Item -

Show in Wells View Legend

	1	2	3	4	5	6	7	8	9	10	11	12
A		N Ta... CT: 3		1		N Ta... CT: 3	N Ta... CT: 3		⊗		N Tar... CT: Un	
B		1		1		2	1		1		N Tar... CT: Un	
C		S Ta... 0.5		1		S Ta... 0.5	S Ta... 0.5		1		S Ta... 0.5	
D		1		1		S Ta... 0.5	S Ta... 0.5		1		S Ta... 0.5	
E		S Ta... 2		S Ta... 2		S Ta... 2	S Ta... 2		S Ta... 2		S Ta... 2	
F		S Ta... 2		S Ta... 2		S Ta... 2	S Ta... 2		1		S Ta... 2	
G		S Ta... 8		2		S Ta... 8	S Ta... 8		1		S Ta... 8	
H		S Ta... 8		1		S Ta... 8	S Ta... 8		1		S Ta... 8	

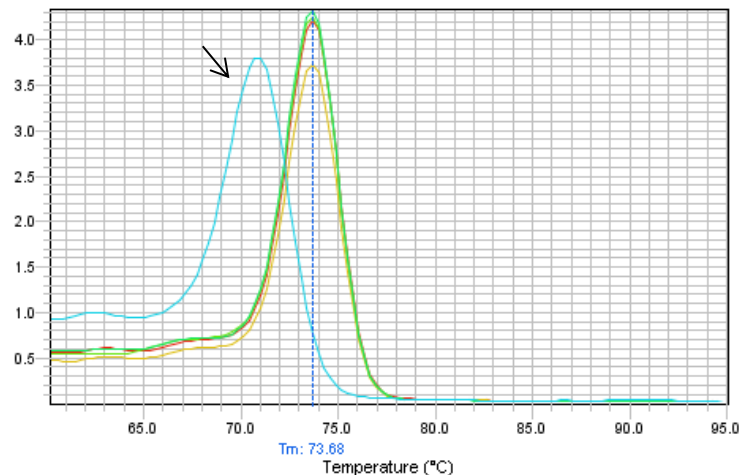
Wells: U 0 Unknown S 36 Standard N 12 Negative Control 48 Empty

Target: Target1
 Task: NTC
 Dye: SYBR-None

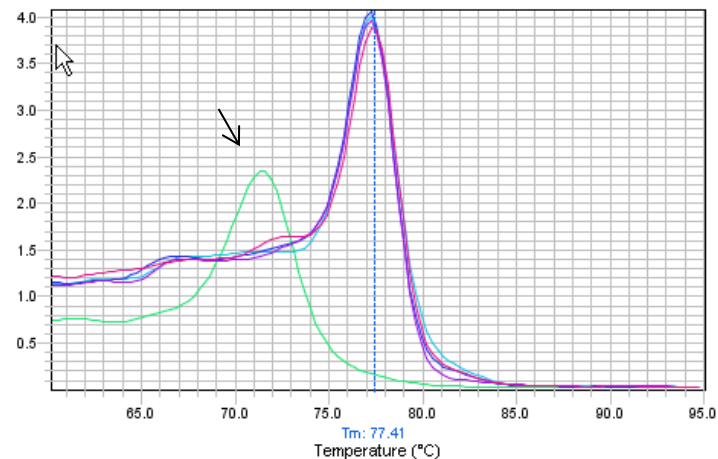
Analysis Summary: Total Wells in Plate: 96 Wells Set Up: 48 Wells Omitted Manually: 1 Wells Flagged: 19 Wells Omitted by Analysis: 0 Samples Used: 3 Targets Used: 6

從Melt Curve確認是因為Primer Dimer或是有汙染

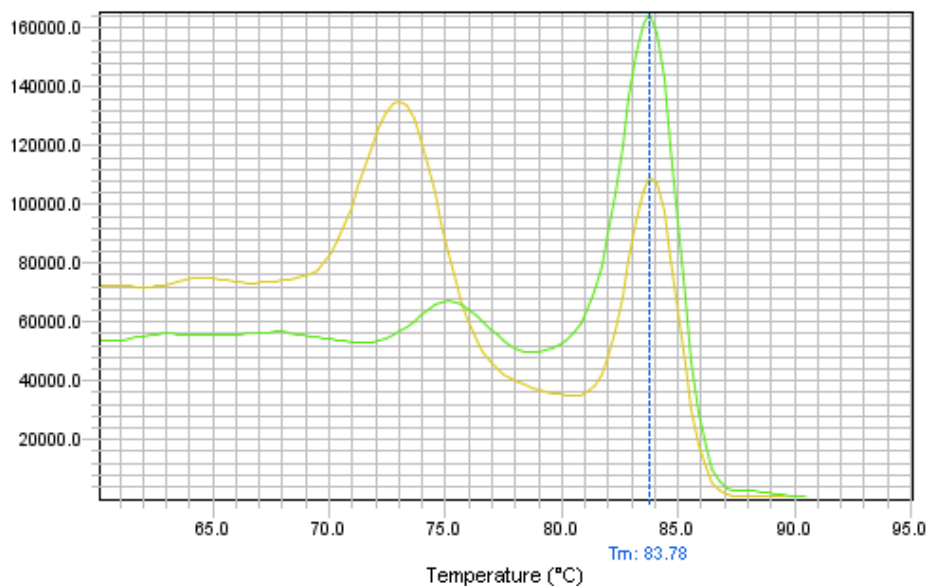
Melt Curve



Melt Curve



Melt Curve



A	N Ta.. CT 3	1	N Ta.. CT 3	N Ta.. CT 3	N Ta.. CT 3
B	1	1	2	1	1
C	S Ta.. 0.5	1	1	1	1
D	1	2	S Ta.. 0.5	S Ta.. 0.5	S Ta..
E	S Ta.. 2	S Ta.. 2	S Ta.. 2	S Ta.. 2	S Ta.. 2
F	S Ta.. 2	S Ta.. 2	S Ta.. 2	S Ta.. 2	1
G	S Ta.. 8	2	S Ta.. 8	S Ta.. 8	1
H	S Ta.. 8	1	S Ta.. 8	S Ta.. 8	1

Target: Target3
Task: NTC
Dye: SYBR-None

Wells: U 0 Unknown S 36 Standard N 12 Negative Control



ThermoFisher
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Introducing Thermo Fisher Cloud

蔡如芸 (Judy Tsai, Ph.D.)
Field Application Scientist

The world leader in serving science

Analysis Modules - Compatible Instrument Systems

Real-Time PCR System	Supported software version(s)	File extension
Applied Biosystems™ 7900HT Fast Real-Time PCR System	v2.4 or later	*.sds
Applied Biosystems™ 7500 Real-Time PCR System Applied Biosystems™ 7500 Fast Real-Time PCR System	v2.0.5 or later	*.eds
Applied Biosystems™ StepOne™ and StepOnePlus™ Real-Time PCR Systems	v2.0.1, v2.1, or later	
Applied Biosystems™ ViiA™ 7 Real-Time PCR System	v1.1 or later	
Applied Biosystems™ QuantStudio™ 12K Flex Real-Time PCR System	v1.1.1 or later	
Applied Biosystems™ QuantStudio™ 6 Flex Real-Time PCR System	v1.0 or later	
Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System		

Thermo Fisher Cloud Storage

- Start with **10 GB free**, purchase more as you need it
- How much storage do I need?

PCR	Plates per day	Storage needed annually
96 well	5	1.3 Gb
	10	2.5 Gb
	20	5 Gb
384 well	5	5 Gb
	10	10 Gb
	20	20 Gb
OpenArray®	5	90 Gb
	10	180 Gb
	20	360 Gb
Sanger	Plates per day	Storage needed annually
96 well	1	10 Gb
	2	20 Gb
	4	40 Gb
	8	80 Gb
	12	120 Gb

- Terms and Conditions for Thermo Fisher Cloud Storage Plans
 - <http://www.thermofisher.com/tw/en/home/cloud/data-storage.html>

Dashboard

Dashboard

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qPCR
- SA** hPSC Scorecard Analysis
qPCR
- PK** Presence Absence Analysis
qPCR
- RQ** Relative Quantification
qPCR
- SC** Standard Curve
qPCR
- NGC** Next-generation Confirmation
Sanger
- QC** Quality Check
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






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-  Proflex890
Ready
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Ready

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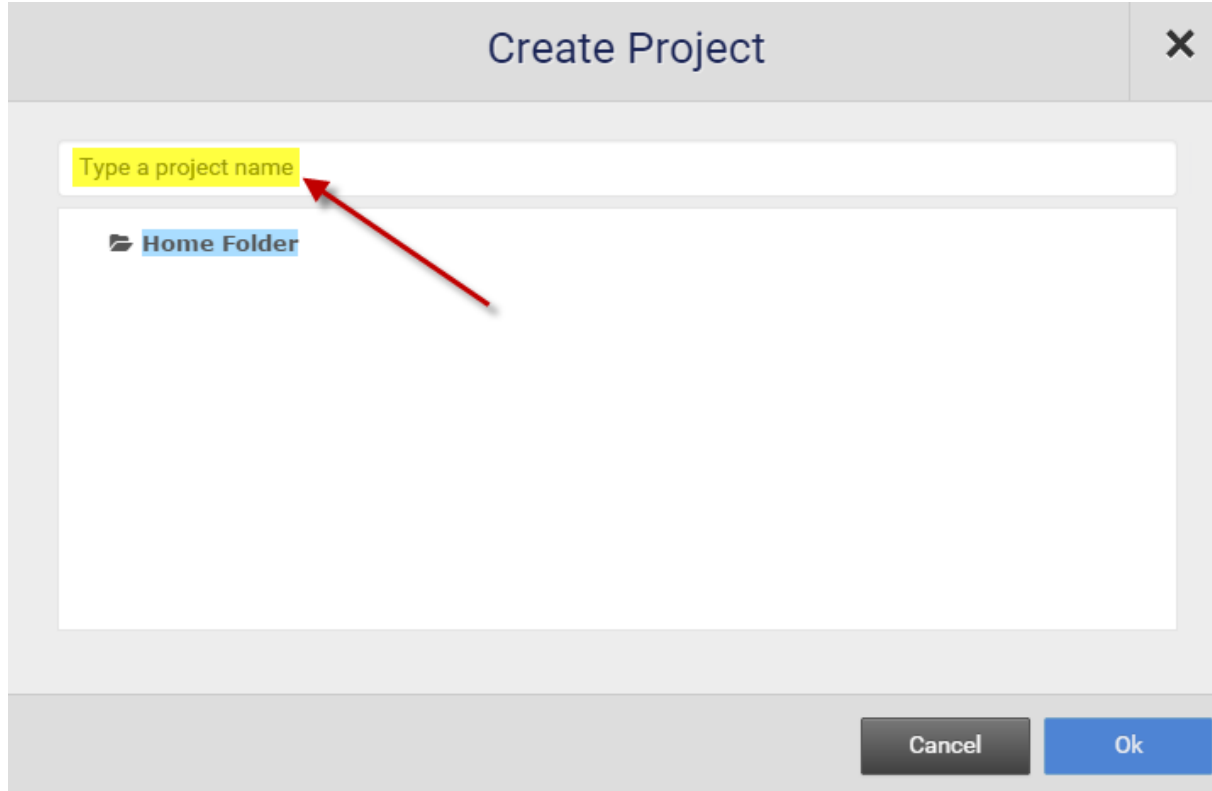
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qPCR
-  Standard Curve
qPCR
-  Next-generation Confirmation
Sanger
-  Quality Check
Sanger
-  Variant Analysis
Sanger

**Upload files
and create
new projects**

Getting Started



In a Project

- Upload eds/sds files either from data manager or local drive
- Select Analysis Module of choice: AQ, RQ or GT

2. Select Analysis Modules

DATA

SC

RQ

Manage Data

1. Upload Files

Files in the project

Import from local

Import from Cloud

Actions

Name	Instrument	Size	Run Date
QS6_384-Well_Comparative_Ct_Example_1.eds	QuantStudio 6 Real-Time PCR System	5190708	10/1/2010 5:18:50 PM
QS6_384-Well_Comparative_Ct_Example_2.eds	QuantStudio 6 Real-Time PCR System	5194201	10/4/2010 1:09:45 PM
QS7_384-Well_Comparative_Ct_Example_1.eds	QuantStudio 7 Real-Time PCR System	5190699	10/1/2010 5:18:50 PM
QS7_384-Well_Comparative_Ct_Example_2.eds	QuantStudio 7 Real-Time PCR System	5194196	10/4/2010 1:09:45 PM
ViiA7_384-Well_Comparative_Ct_Example_1.eds	ViiA 7 Real-Time PCR System	5187789	10/1/2010 5:18:50 PM
ViiA7_384-Well_Comparative_Ct_Example_2.eds	ViiA 7 Real-Time PCR System	5191295	10/4/2010 1:09:45 PM

Relative Quantification Analysis Module

Project Overview

Basic navigation across the top

Analyze Button



RQ Overview Plate Setup Data Review Analysis Export Default Analysis Group Settings Analyze

Experiments 6 Actions

Experiment Name	Block Type	Instrument
QS6_384-Well_Comparative_Ct_Example_1.eds	384-Well	QuantStudio 6 Real-Time PCR System
QS6_384-Well_Comparative_Ct_Example_2.eds	384-Well	QuantStudio 6 Real-Time PCR System
QS7_384-Well_Comparative_Ct_Example_1.eds	384-Well	QuantStudio 7 Real-Time PCR System
QS7_384-Well_Comparative_Ct_Example_2.eds	384-Well	QuantStudio 7 Real-Time PCR System
ViiA7_384-Well_Comparative_Ct_Example_1.eds	384-Well	ViiA 7 Real-Time PCR System
ViiA7_384-Well_Comparative_Ct_Example_2.eds	384-Well	ViiA 7 Real-Time PCR System

Targets 5 Actions

Name	Reporter	Comments
ACTB	FAM	
GAPDH	FAM	
GH1	FAM	
LIPC	FAM	
LPIN1	FAM	

Samples 4 Actions

Name	BioGroup
Brain	
Heart	
Liver	
Lung	

Biogroups 0 Actions

Name	Color	Comments
------	-------	----------

Analysis Groups 2 Actions

Name	Analysis Status
Default Analysis Group	Mixed instrument types
Media v Antigen	Mixed instrument types

Analysis Settings: Endogenous Controls

Endogenous Controls | RQ Settings | Efficiency | Cq Settings | Flag Settings | IC Settings | SC Settings

Use specific endogenous controls Use global normalization (Explanation)

Target	Sample Ct	Endogenous Control	Score
ACTB	min: 17.27 max: 19.20 range: 1.93 	<input checked="" type="checkbox"/>	N/A
GAPDH	min: 17.32 max: 21.03 range: 3.71 	<input type="checkbox"/>	N/A
GH1		<input type="checkbox"/>	N/A
LIPC		<input type="checkbox"/>	N/A

Select to view stability

Target	Sample Ct	Endogenous Control	Score
ACTB	min: 17.27 max: 19.20 range: 1.93 	<input checked="" type="checkbox"/>	1.342
GAPDH	min: 17.32 max: 21.03 range: 3.71 	<input checked="" type="checkbox"/>	1.743
GH1	min: 31.09 max: 35.45 range: 4.36 	<input checked="" type="checkbox"/>	1.536

Stability score need to select at least 3 controls

Analysis Settings: Inter-Plate Calibrator Setting

- No need for endogenous control on every plate
- Required to have same sample and target combination in a particular well for each plate in a project
- Select “Allow calculation as dCT” allows all plates to be analyzed together as one plate
- If checkbox is NOT selected, and no inter-plate calibrator is present, this will result in typical endogenous control calculation

Begin typing and select Target/ Sample from drop down

Analysis Group : Analysis Settings

IC Settings

Allow calculation of ΔCq across all Plates in the Analysis Group

Target

Sample

A

ACTB

Brain

Click box to analyze entire project as a single plate (d CT)

Plate Set Up: Change sample/ target layout here!

No need to go back to instrument software!!!

Overview **Plate Setup** Data Review Analysis Export

Plates

QS6_384-Well_Comparative_Ct... QS6_384-Well_Comparative_Ct... QS7_384-Well_Comparative_Ct... QS7_384-Well_Comparative_Ct... ViiA7_384-Well_Comparative_C... ViiA7_384-Well_Comparative_C...

60 of 384 wells defined 60 of 384 wells defined 60 of 384 wells defined 60 of 384 wells defined 60 of 384 wells defined 60 of 384 wells defined

Overview Plate Setup Data Review Analysis Export

Edit Plate

Apply Template View Options Actions

Well Attributes

Sample Liver

Target LPIN1

Task Unknown

Comments

Plate Attributes

Passive Reference Dye ROX

PCR Stage/Step Stage 2, Step 2

Clear Well Setup

Apply Template

Download Template

Define Standards

Save Plate Image

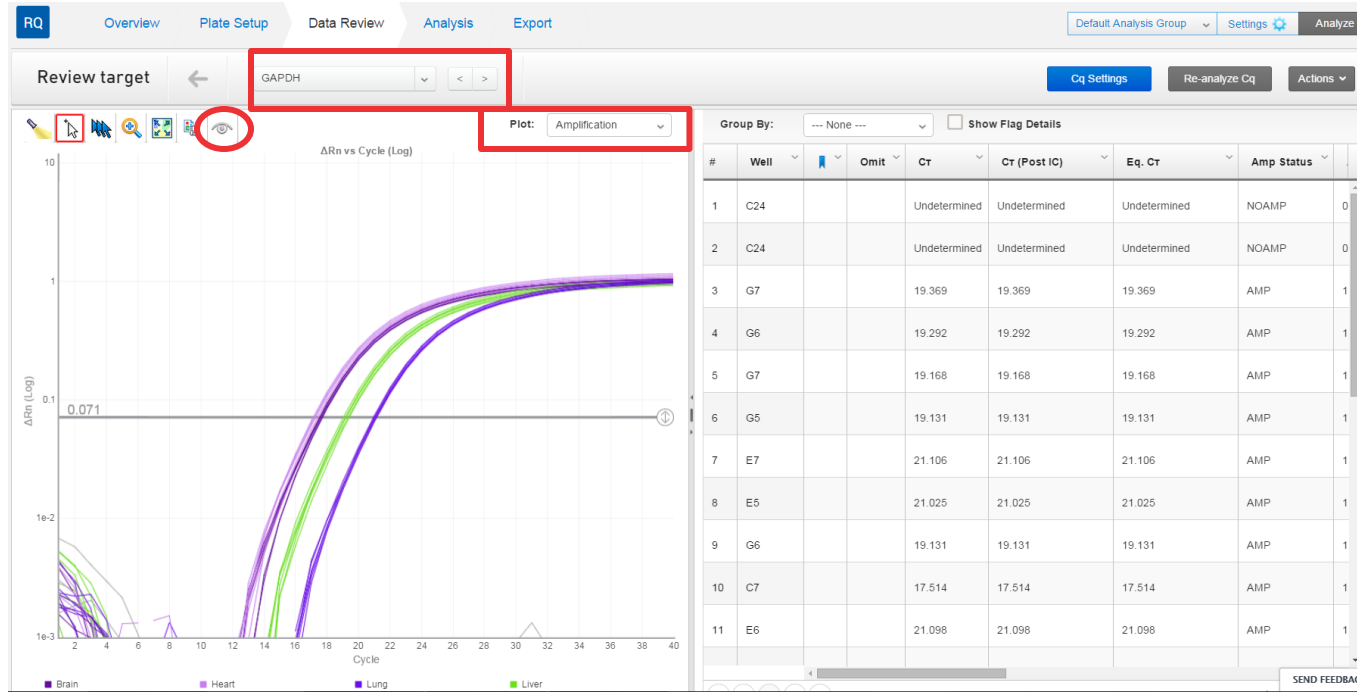
Data Review: Quick View of Samples, Targets, Plates

Change view between samples, targets, plates

The screenshot displays the QIAstudio software interface. At the top, there are navigation tabs: Overview, Plate Setup, Data Review (selected), Analysis, and Export. On the left side, there is a vertical sidebar with icons for Home, DATA, SC, and RQ. The main area is titled 'Targets' and contains a grid of five target thumbnails: ACTB, GAPDH, LIPC, and LPIN1. Each thumbnail shows a graph of fluorescence intensity over time. Below each graph, there is a status indicator: 'No Flag' for ACTB, GAPDH, LIPC, and LPIN1, and 'Flags count : 3' for LIPC. A red arrow points from the 'Data Review' tab to a dropdown menu that is open, showing options: 'Targets' (selected), 'Samples', and 'Plates'. Another red arrow points from the GAPDH thumbnail to a larger view of the GAPDH graph.

Select a thumbnail and drill down

Data Review: Detailed Amplification Curves



Plot Type: ΔRn vs Cycle

Graph Type: Log

Color Type: Sample

Hide Unselected Curves

X-Axis Min: 1 **X-Axis Max:** 40

Y-Axis Min: 0.001 **Y-Axis Max:** 12

Transparency:

Plot: Amplification

- Amplification
- Multicomponent
- Standard Curve






GAPDH

- ACTB
- GAPDH
- GH1
- LIPC
- LPIN1

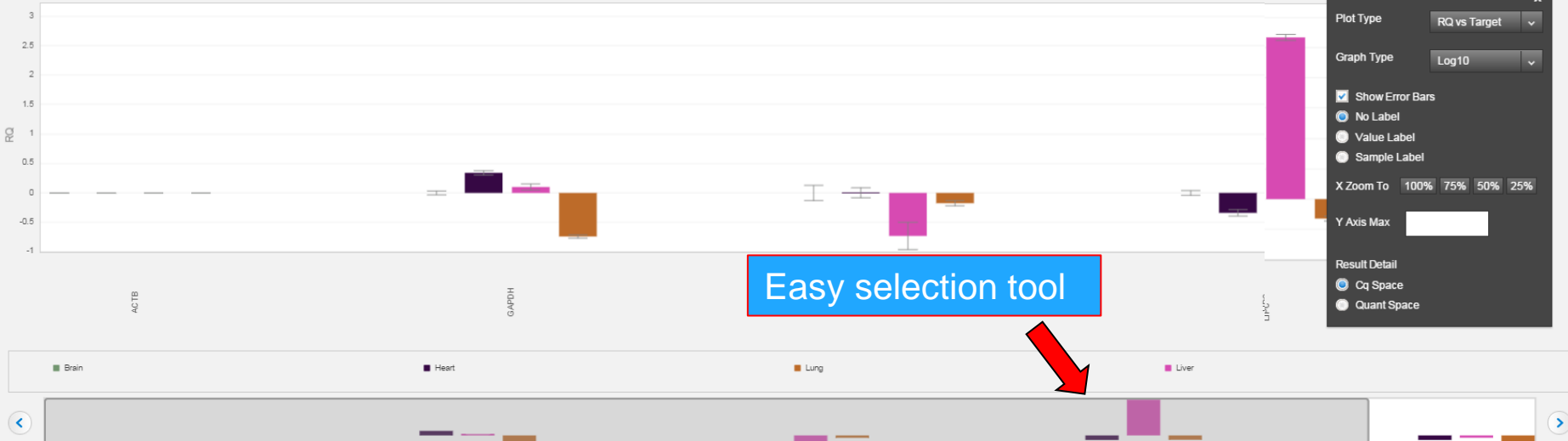
Gene Expression Plots in Analysis Section

RQ Overview Plate Setup Data Review Analysis Export

Default Analysis Group Settings Analyze

RQ Plot      Biogroups Sample

View QC View Options Actions



Easy selection tool

Plot Type: RQ vs Target
Graph Type: Log10
 Show Error Bars
 No Label
 Value Label
 Sample Label
X Zoom To: 100% 75% 50% 25%
Y Axis Max:
Result Detail:
 Cq Space
 Quant Space

Results Details (using equivalent C_t values where the original C_t values are projected to 100% target efficiency) [Clear filter](#)

Target	Sample	Biological Group	Max Ct	Ct Mean	Adjusted Ct Mean	Ct SE	ΔCt Mean	ΔCt SE	F-Factor	ΔΔCt	ΔΔCt - Fσ	ΔΔCt + Fσ	RQ	RQ Min	RQ Max
ACTB	Brain		40.000	17.265	17.265	0.038	-	-	-	-	-	-	-	-	-
ACTB	Heart		40.000	18.136	18.136										
ACTB	Liver		40.000	19.202	19.202										
ACTB	Lung		40.000	18.254	18.254	0.055	-	-	-	-	-	-	-	-	-

New Fields in RQ table explained in HELP

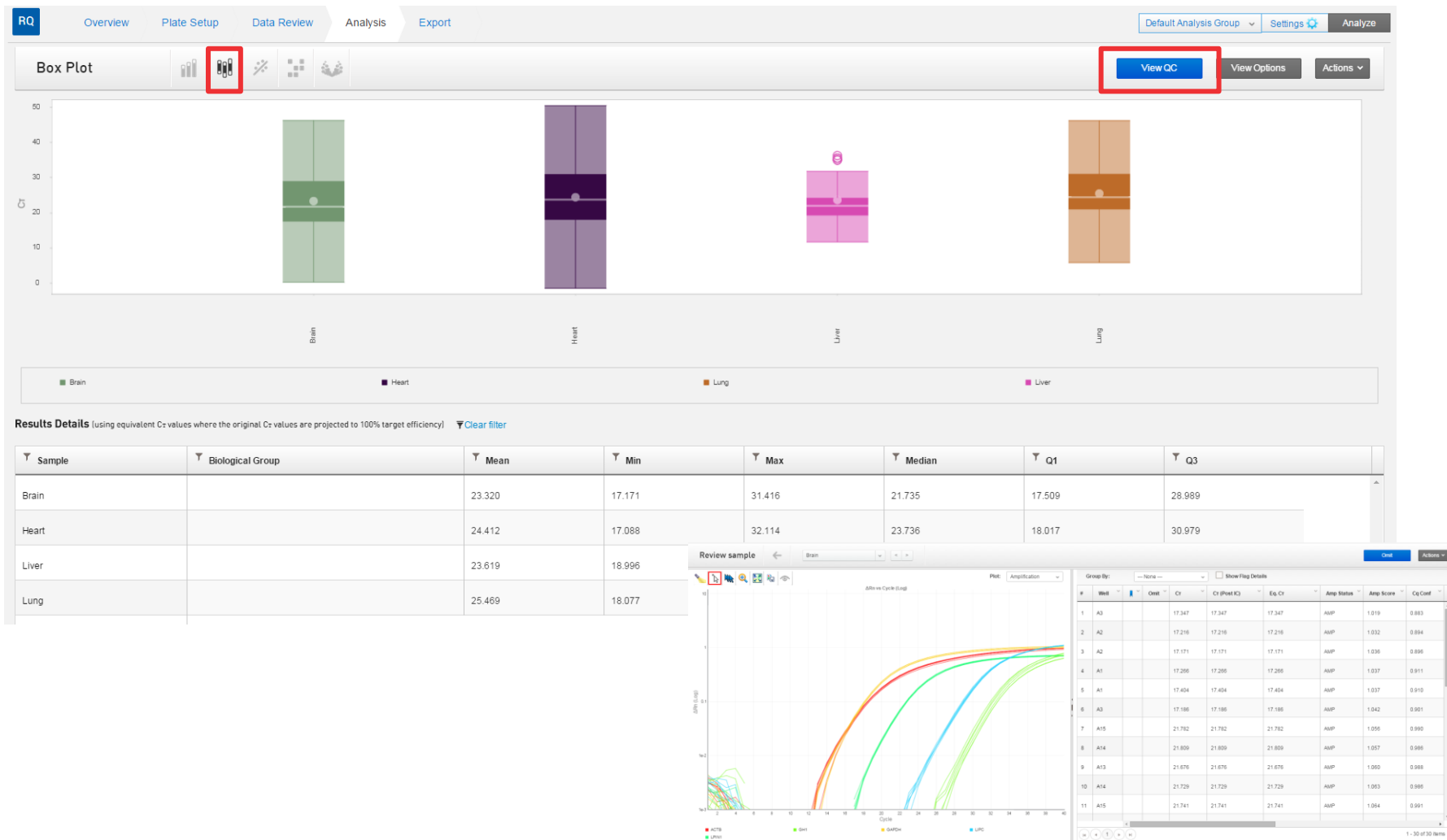
Help Topics

- About Software Interface
- About Analysis Results
- About Quality Metrics
- Save Plot as Image
- View Analysis Settings
- View Box Plot
- View Correlation Plot
- View Gene Expression Plot
- View Heatmap
- View Volcano Plot

40

Box Plots – Quality Checks

- Assess CT distribution among biological replicates, samples, and targets



Correlation Plots – Quality Checks

- Select Correlation Plot of interest, then examine outliers

Brain to Lung
Score: 0.989

View Detail Plot

2) View Amp plot of desired outlier target

1) Select Correlation Plot of interest

Brain to Lung (Score: 0.98890334)
Using equivalent Ct values where the original Ct values are projected to 100% target efficiency

To select point(s) from the plot:

- Click and drag a region
- Click on a single point
- Control-click multiple points

Selected targets	
●	GAPDH
●	LIPC
●	LPIN1

Review Target

3) View Amp plot and well table information

Heat Map

View Options to change settings



Adjustable slider

View Options

- Distance measure: Pearson's Corr...
- Clustering method: Average Linkage
- Map Type: Target-Centric
- Color Scheme: Red-Blue, Red-Green, Red-Blue, Green-Orange

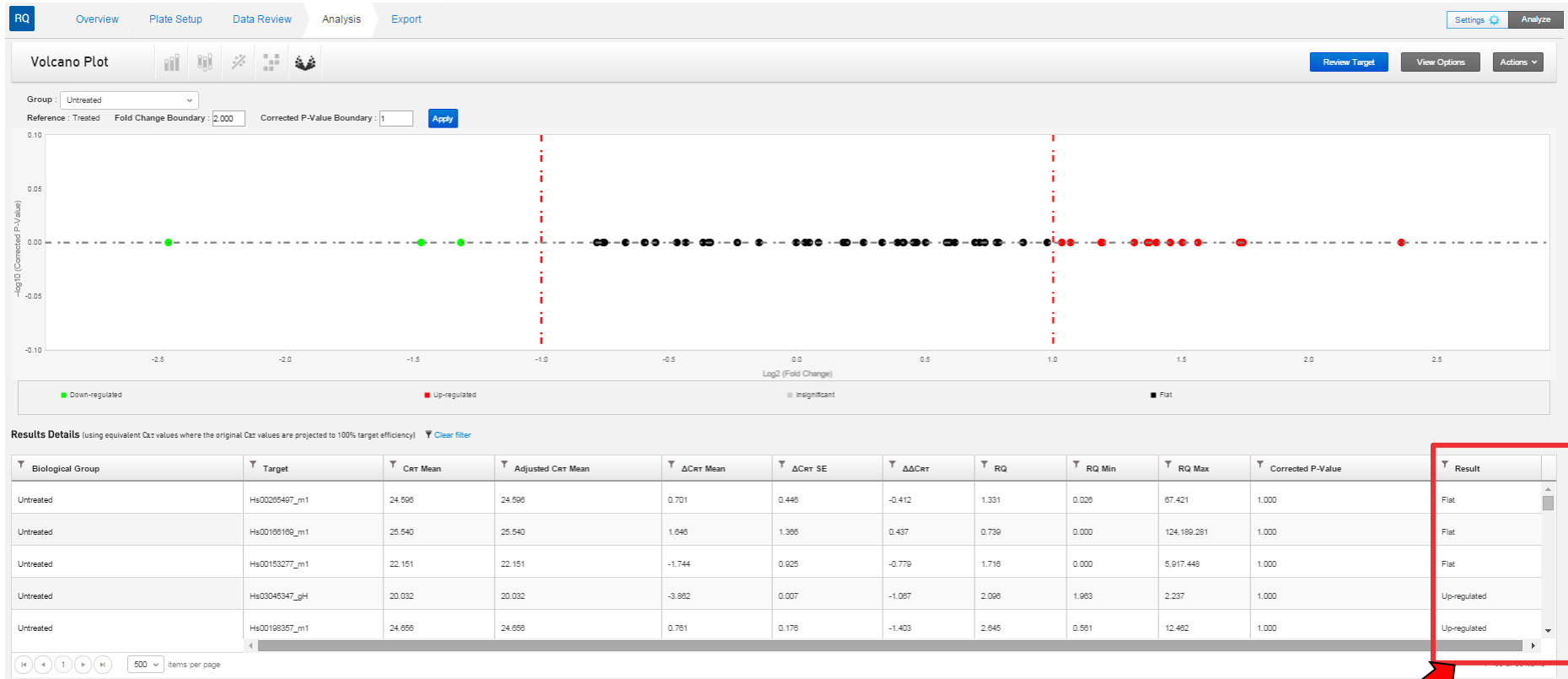
Results Details (using equivalent C₂₇ values where the original C₂₇ values are projected to 100% target efficiency) [Clear filter](#)

Sample	Biological Group	Target	C ₂₇ Mean	Adjusted C ₂₇ Mean	ΔC ₂₇ Mean	ΔC ₂₇ SE	ΔC ₂₇ + Control Median
Brain1-A	Untreated	RNU44_001004	12.763	12.763	-6.426	-	12.400
Brain1-A	Untreated	RNU48_001006	10.100	10.100	-9.091	-	9.737
Brain1-A	Untreated	U6_rRNA_001073	7.287	7.287	-11.905	-	6.923
Brain1-A	Untreated	ath-miR150a_000338	11.946	11.946	-7.245	-	11.583

Human miRNA Starter Kit
*Slider only present with very large data sets

Volcano Plots: New Functionality

- Well table information with gene expression states



Gene expression states: flat, insignificant, upregulated, downregulated

Exporting Data

Export [File Icon] [Preview]

Name :

File type :

Comment :

Biological Group Results
 Sample Results
 Well Results Bookmarks
 Amplification Data
 Volcano Plot Data
 Target/Sample/Plate QC

Must name file first

.txt or .csv formats

Customize your export

Group 1 [Settings] [Analyze]

Export [File Icon] [Start Export]

Experiment Name	Barcode	Well	Biological Group	Sample Name	Target Name
QS0_384-Well_Comparative_Ct...		A2	Untreated	Brain	ACTB
QS0_384-Well_Comparative_Ct...		A3	Untreated	Brain	ACTB
QS0_384-Well_Comparative_Ct...		A11	Untreated	Brain	GH1
QS0_384-Well_Comparative_Ct...		C5	Untreated	Heart	GAPDH
QS0_384-Well_Comparative_Ct...		C15	Untreated	Heart	LPIN1
QS0_384-Well_Comparative_Ct...		E14	Treated	Lung	LPIN1
QS0_384-Well_Comparative_Ct...		E19	Treated	Lung	LIPC
QS0_384-Well_Comparative_Ct...		E24		No Sample	GH1

Select: All | None

Select Contents

- Experiment Name
- Barcode
- Well
- Biological Group Name
- Sample Name
- Target Name
- Task
- Amp Score
- Amp Status
- Cq Conf
- Target Efficiency
- Ct
- Ct (Post IC)
- Eq. Ct
- Quantity

Previewing first 15 rows of data.

Standard Curve Analysis Module

Standard Curve Analysis Module

- No Analysis Groups because this is a single plate analysis module

appliedbiosystems | Standard Curve Example

ThermoFisherCloud

SC Overview Plate Setup Quality Control & Results Export Analyze

Samples 2

Name	Color	Comments
10K		
5K		

Targets 1


Target	Color	Dye	Quencher	...
RNase P		FAM	NFQ-MGB	

Standard Curve Features

SC Overview Plate Setup Quality Control & Results Export

Select an Experiment

Q55_384-Well_Standard_Curve...



No Flag

SC Overview Plate Setup Quality Control & Results Export

Review Result

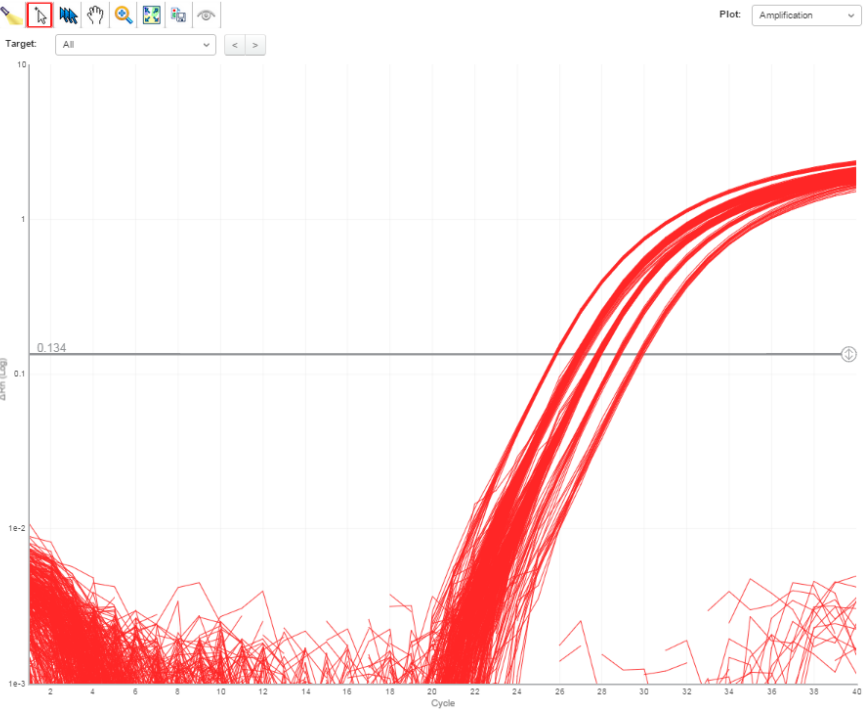
Q55_384-Well_Standard_Curve_Example.edx

Analysis Setting

Actions

Plot: Amplification

Target: All



Well Table

#	Well	Omit	Sample	AMP Status	Amp Score	Cq Conf	Task	Quantity	Quantity Mean	Quantity
1	A1		RNAse P No Sample	NOAMP	0.000	0.000	NTC	-	-	-
2	A2		RNAse P No Sample	AMP	1.253	0.990	STANDARD	1250.000	-	-
3	A3		RNAse P No Sample	AMP	1.257	0.988	STANDARD	2500.000	-	-
4	A4		RNAse P No Sample	AMP	1.259	0.992	STANDARD	5000.000	-	-
5	A5		RNAse P No Sample	AMP	1.255	0.989	STANDARD	10000.000	-	-
6	A6		RNAse P No Sample	AMP	1.268	0.993	STANDARD	20000.000	-	-
7	A7		RNAse P 5K	AMP	1.280	0.989	UNKNOWN	4803.257	4689.373	229.799
8	A8		RNAse P 5K	AMP	1.261	0.989	UNKNOWN	5056.929	4689.373	229.799
9	A9		RNAse P 5K	AMP	1.256	0.985	UNKNOWN	4708.352	4689.373	229.799
10	A10		RNAse P 5K	AMP	1.282	0.988	UNKNOWN	4823.053	4689.373	229.799
11	A11		RNAse P 5K	AMP	1.259	0.993	UNKNOWN	4634.604	4689.373	229.799
12	A12		RNAse P 5K	AMP	1.269	0.988	UNKNOWN	5433.725	4689.373	229.799
13	A13		RNAse P 5K	AMP	1.284	0.992	UNKNOWN	4999.525	4689.373	229.799
14	A14		RNAse P 5K	AMP	1.2570906	0.988	UNKNOWN	4709.555	4689.373	229.799
15	A15		RNAse P 5K	AMP	1.257	0.989	UNKNOWN	4757.323	4689.373	229.799
16	A16		RNAse P 10K	AMP	1.283	0.989	UNKNOWN	9550.178	9533.951	524.797
17	A17		RNAse P 10K	AMP	1.285	0.985	UNKNOWN	9523.232	9533.951	524.797

1 - 384 of 384 items

Importing feature in Analysis Settings

Improved Feature: Importing Standard Curves

1) Select Standard Curve Settings in top navigation pane

The screenshot shows the 'Edit Analysis Setting' dialog box with the 'Standard Curve Settings' tab selected in the top navigation pane. The 'On Plate Standard Curves' radio button is selected, and the 'Export' button is highlighted.

3) Select export

2) Select On Plate Standard Curve

The screenshot shows the software's top navigation pane with 'Standard Curve Settings' selected. Below it, the 'Edit' menu is open, showing options like 'Fill', 'Calibri (Body)', and '12'.

F25

Reporter	Target	Slope	Y-Intercept	R2	Efficiency (%)	Quantities
FAM	RNaseP	-3.2258	38.9715	0.9969	104.174086	1250.0, 2500.0, 5000.0, 10000.0, 20000.0



4) Edit Standard curve details

The screenshot shows the 'Edit Analysis Setting' dialog box with the 'External Standard Curves' radio button selected. The 'Import' button is highlighted. A red arrow points to the 'Standard Curve Settings' tab in the top navigation pane.

5) Import curve details

Exporting Data

SC Overview Plate Setup Quality Control & Results Export Analyze

Export   Preview



Name :

File type :

Comments :

Included in :

- Results Data
- Amplification Data

Export   Back Start Export

Results | Amplification Data

Select: All | None

Select Contents

- Well Number
- Sample Name
- Target Name
- Amplification Score
- Task
- Ct
- Ct Mean
- Ct Standard Deviation
- Quantity
- Quantity Mean
- Quantity Standard Deviation
- Auto Threshold
- Threshold
- Auto Baseline
- Baseline Start

Well Number	Sample Name	Target Name	Amplification Sc...	Task	Ct
P17	10K	RNAse P	1.259	UNKNOWN	26.919
J24	10K	RNAse P	1.261	UNKNOWN	26.988
L19	10K	RNAse P	1.259	UNKNOWN	26.891
E21	10K	RNAse P	1.272	UNKNOWN	26.988
N21	10K	RNAse P	1.256	UNKNOWN	26.945
O16	10K	RNAse P	1.260	UNKNOWN	26.927
M18	10K	RNAse P	1.279	UNKNOWN	26.913
P22	10K	RNAse P	1.270	UNKNOWN	26.863

Previewing first 15 rows of data.

Genotyping Analysis Module

Basic navigation across the top

The screenshot displays the Genotyping Analysis Module interface. At the top, a navigation bar includes a 'GT' logo and tabs for 'Overview', 'Plate Setup', 'Analysis', and 'Export'. The 'Overview' tab is selected and highlighted with a red box. To the right of the navigation bar are 'Settings' and 'Analyze' buttons.

Below the navigation bar, the interface is divided into five main sections:

- Experiments 1:** A table with columns for Experiment Name, Block Type, and Instrument. The data row shows 'Genotyping Starter Kit Example.eds', 'OpenArray', and 'QuantStudio 12K Flex Real-Time PCR System'.
- Assays 63:** A table with columns for Assay Name, Assay ID, Color, and # of wells. It lists six assays, all with a green color and 48 wells.
- Samples 21:** A table with columns for Sample, Color, and # of well. It lists six samples with various colors and 128 wells each.
- References 0:** A table with columns for File Name, Analysis Group, and # of Samples. It is currently empty.
- Analysis Groups 1:** A table with columns for Name and Status. It shows one group named 'Default Analysis Group' with a status of 'Completed'.

General View of Assay, Samples, References

Analysis Groups and Settings



Default Analysis Group : Analysis Setting

Call Setting | Flag Setting | Reference Panels

Analyze Data: Real-time Rn Data Post-PCR Read Pre-PCR and Post-PCR Read Real-time Rn Data

Call Method : Autocalling Classification Scheme

Multiplate Assay Protect Markers Use Reference Panels for Autocalling

Use Hardy-Weinberg for Analysis Use Positive Controls for Analysis

Heterozygote: Allow

Real-time Data Settings

Baseline from 5 to 15

End-point Cycle #

Assay Name	Assay ID	Call Method	Protect	PC	Ref	H-W	Heterozygotes	BL	BL Start	BL End
C_10008862_10	C_1000886...	Autocalling	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Allow	<input checked="" type="checkbox"/>	5	15
C_10024791_10	C_1002479...	Autocalling	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Allow	<input checked="" type="checkbox"/>	5	15
C_10048053_10	C_1004805...	Autocalling	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Allow	<input checked="" type="checkbox"/>	5	15

Back Finish

Real Time Traces to Optimize Cycling Conditions

View Amp plots

Use scroll bar to view clustering trajectory and re-analyze

GT Allele Discrimination C__10024791_10

Settings Analyze

Analysis Setting Call Genotypes Actions

View: Results Group By: --- None --- Show Flag Details

#	Sample ID	Call	Manual	Task	Allele 1	Allele 2	ROX	Quality
1	NA04671	Allele 1/Allele 1		Unknown	2488.135	490.756	717.529	0.984
2	NA04671	Allele 1/Allele 1		Unknown	2450.372	499.233	709.941	0.984
3	NA17004	Allele 1/Allele 1		Unknown	2455.007	463.317	718.294	0.984
4	NA17004	Allele 1/Allele 1		Unknown	2335.937	472.934	714.500	0.984

C__10024791_10 (Call Rate = 100.000%)

Allele 2 (Allele 2)

Assay: C__10024791_10

NTC (8) Control (8) Allele 2/Allele 2 (4)

Plot: Amplification ARn Vs Cycle Number

Allele 1 Allele 2

C__10024791_10 (Call Rate = 100.000%)

Allele 2 (Allele 2)

Allele 1 (Allele 1)

NTC (8) Allele 1/Allele 1 (26) Allele 1/Allele 2 (6) Allele 2/Allele 2 (4) Control (8)

Exporting Genotyping Data

- New Export Files: Sample Call Rates

GT Overview Plate Setup Analysis **Export** Settings Analyze

Export Preview

Export Results

Name :

File type :

Comments :

Included in :

- Analysis Results
- Basic Advanced (Include omitted wells Bookmarked wells only)
- Genotype Matrix (No preview) QC by Samples
- Analysis Settings QC by Assays
- Populations QC by Plates

Call **Exported**

Undetermined

No Amplification

Possible Rare Allele

Invalid

Export

[Advanced Analysis Results](#) | [Analysis Settings](#) **QC by Samples**

Sample ...	Sample Call Rate	Sample Call Rate Low
NA1700	97.62 %	Passed
NA1700	96.83 %	Passed
NA1703	97.62 %	Passed
NA1705	96.83 %	Passed
NA1705	100.00 %	Passed
NA1705	98.41 %	Passed
NA1705	96.83 %	Passed
NA1705	98.41 %	Passed

Previewing first 15 rows of data.

Thank You!

技術服務E-mail: Support.TW@lifetech.com

訂貨及維修服務專線: 0800-251-326