

Gaining Advantage

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Applied Biosystem QuantStudio™ 3 Real-Time PCR System之原理與應用介紹

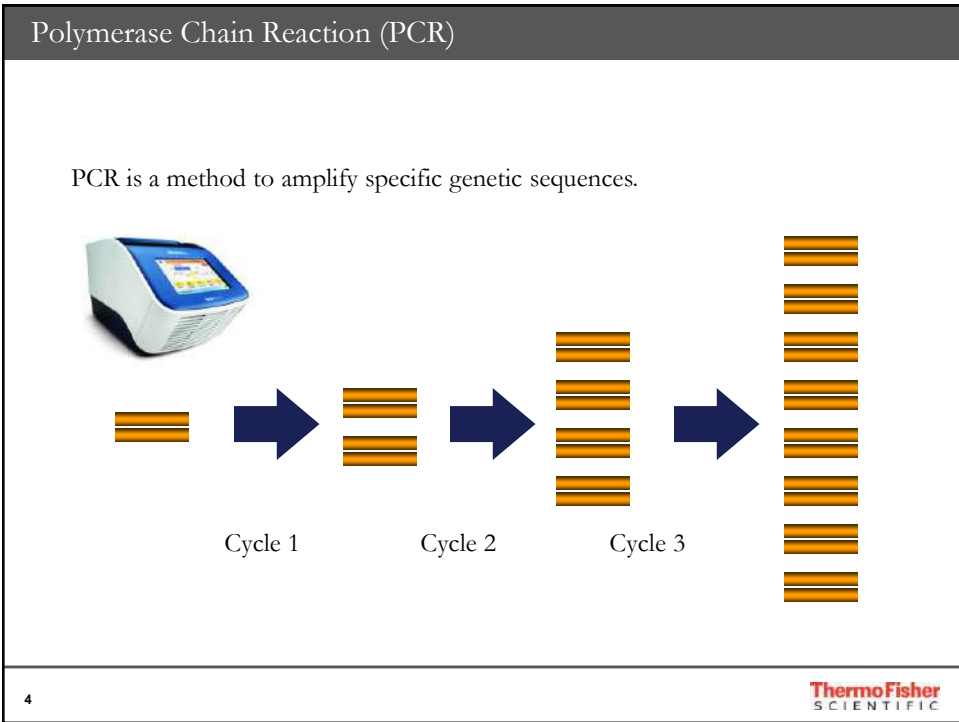
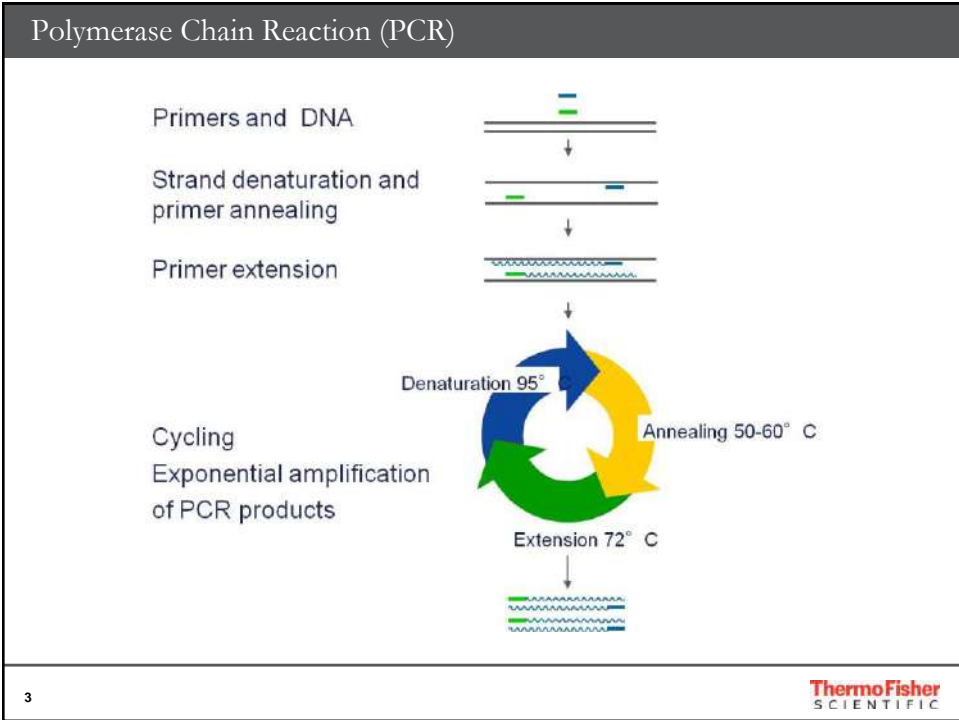
陳立德 (Erwin Chen)
Field Application Scientist

Proprietary & Confidential

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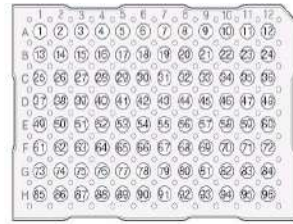
Agenda

- **Principle of Real-time PCR**
 - TaqMan® V.S. SYBR® Green
 - **Real-time PCR Application**
 - Reverse Transcription and Real-time PCR Reaction
- **Assay design**
 - Predesigned Assay Search
 - Primer Express
- **QuantStudio™ 3 Real-Time PCR System**
- **Thermo Fisher Cloud**



Traditional PCR

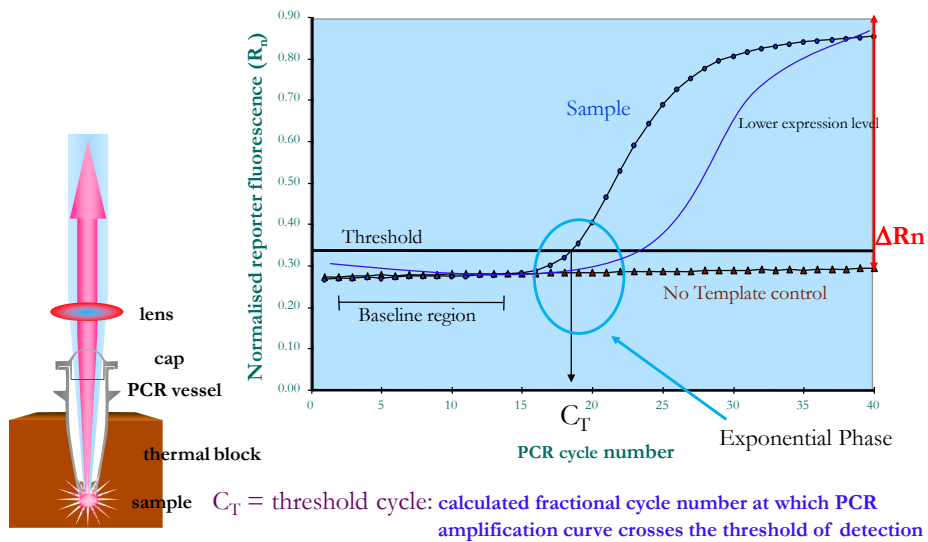
In traditional PCR, the product is detected only after amplification is completed, using methods such as gel electrophoresis



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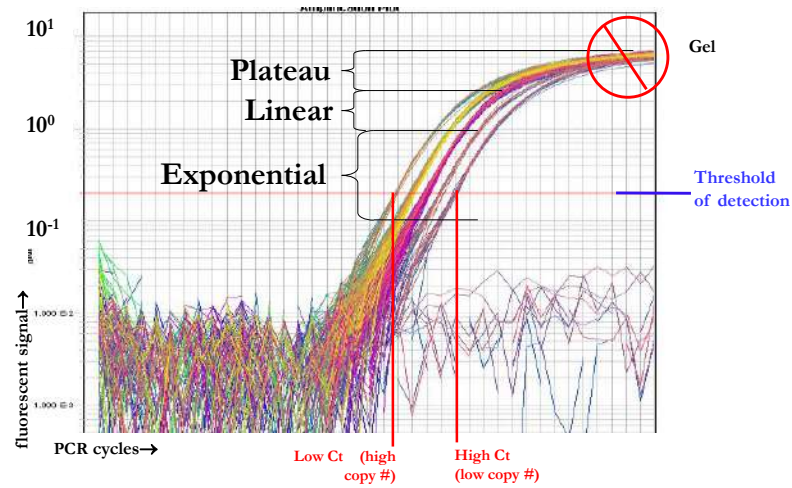
Principle of Real-time PCR



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Real-time PCR Signal Detection: Exponential Phase



$Y = N_0 \cdot 2^n$, C_T 與起始濃度之對數值成反比

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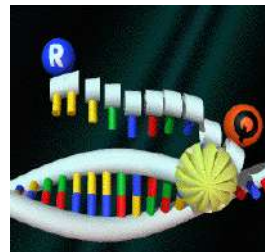
Real-time PCR Chemistries

SYBR® Green I dye



Binds Double-stranded DNA

TaqMan® and TaqMan® MGB



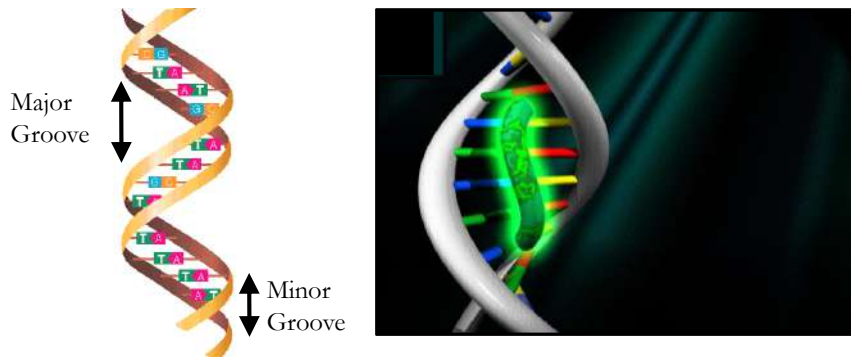
Fluorogenic 5' Nuclease Assay

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Real-time PCR Chemistries: SYBR® Green I Dye

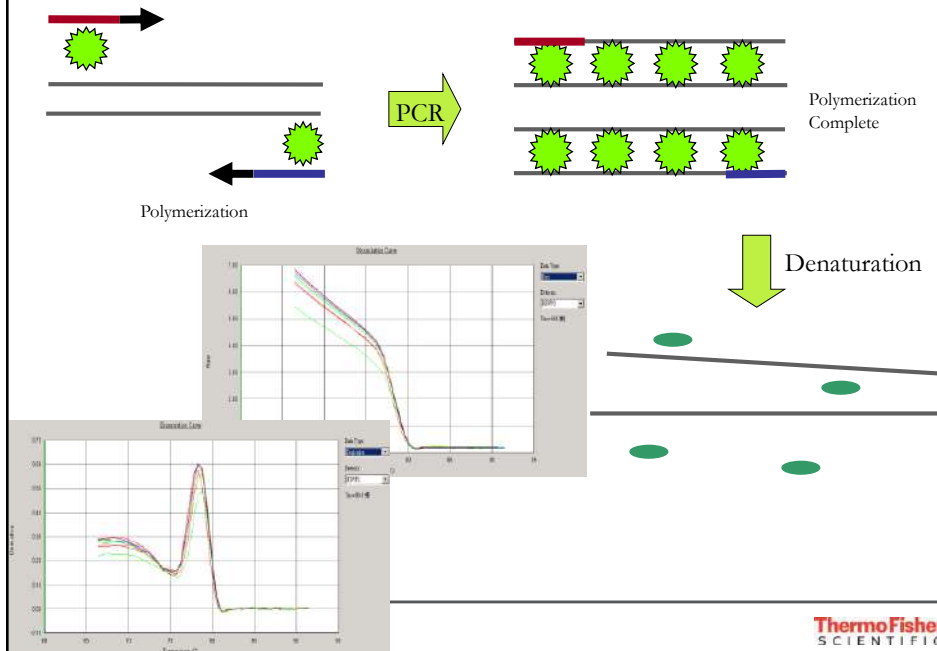
- A 'minor groove'-binding molecule specific to the minor groove of double-stranded DNA
- Fluoresces at an increased intensity when bound



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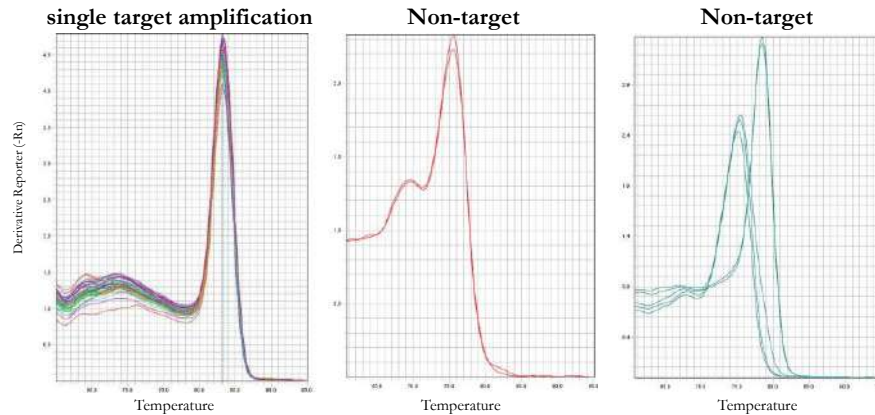
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SYBR® Green I Dye: Melting Curve Analysis



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SYBR® Green I Dye: Melting Curve Analysis



Use **NTC** to check whether non-specific product is primer dimer

- If the non-specific product is primer-dimer:

- Optimize primer concentration

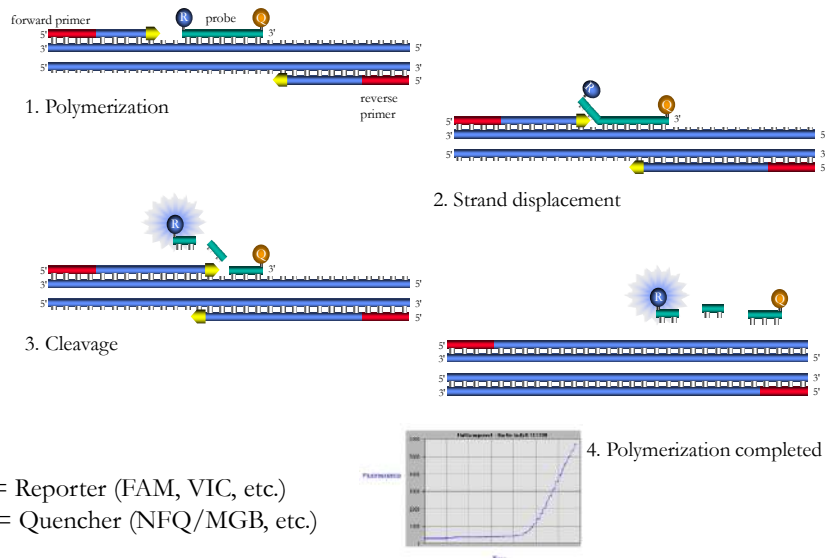
- Re-design primer pair

(NTC: No Template Control)

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TaqMan® Assay: Fluorogenic 5'-nuclease Assay

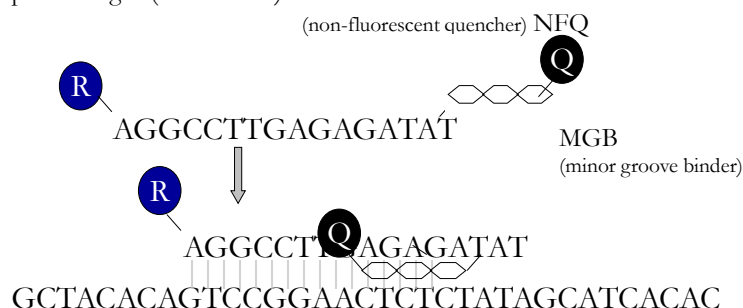


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TaqMan® Probe: TaqMan® MGB/NFQ Probes

- Minor Groove Binder (MGB)
 - Small molecule that fits snugly into minor groove of duplex DNA
 - Stabilizes probe annealing
- Non-fluorescent Quencher (NFQ)
 - “Dark” quencher acts as energy transfer acceptor that doesn’t emit a detectable fluorescent signal
 - MGB probe design uses a special algorithm in Primer Express® Software
- Shorter probe length (13-25-mers)



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Real-time PCR Chemistries

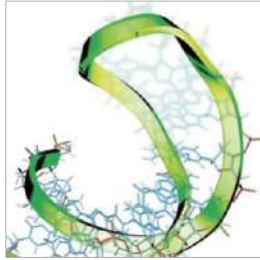
	TaqMan® Assay	SYBR® Green I Dye
Specificity	More specific	Less specific
	Probe hybridization	
Sensitivity	Very high	Very high
Flexibility	Multiplex PCR	No probe required
	SNP detection	Screening tool
	+/- application	
Optimization	Ready to use 20x primer/probe mix - no need to optimize	Need to optimize PCR program
	Gold standard for MAQC	Need to check primer-dimer info
	PCR efficiency 100±10%	Need to check PCR efficiency

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Real-time PCR Application

- Absolute Quantitation vs. Relative Quantitation

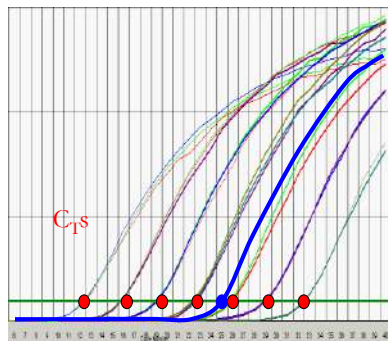
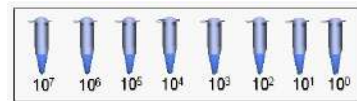


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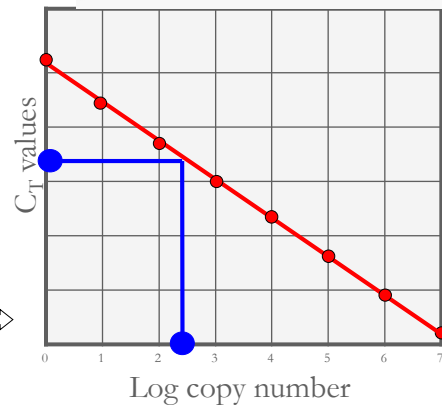
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絕對定量 (Absolute Quantitation)

- 主要應用於病毒量及病原菌偵測
- To determine the actual number of copies of a target nucleic acid within a sample with statistical confidence.



C_T is directly proportional to log of amount of input template



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相對定量 (Relative Quantitation)

- To determine fold differences of a target nucleic acid in a starting material with statistical confidence.
 - $\Delta\Delta C_t$ analysis (most common)
 - Relative standard curve
- Need endogenous gene normalizes the amount of sample added
 - Endogenous control (e.g. *GAPDH*, *β -actin*, etc.)
- Most powerful and widely used method
- Check primer PCR efficiency if using SYBR Green Dye

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Reverse Transcription and Real-time PCR
Reaction

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One-step vs Two-step Workflows

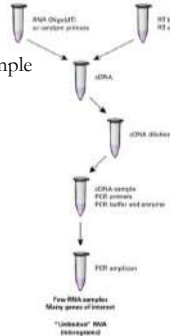
• One-step Technology

- RT and PCR are performed in single buffer system
- ✓ One tube, one step
- ✓ Reduce chance of cross-contamination
- ✓ Easy for high throughput workflow
- ✓ Cost effective when few targets/sample analyzed
- ✓ Uses gene-specific primers
- X cDNA cannot be stored



• Two-step Technology

- RT and PCR are performed in two separate reactions
- ✓ Cost advantaged when interrogating multiple targets
- ✓ cDNA can be stored and used for further experiments
- ✓ Best choice if RNA is limiting
- X Multiple steps, longer time to result



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One-step Workflow: Real-time PCR Reactions

Component	Volume for one reaction	Notes
4X TaqMan® Fast Virus 1-Step Master Mix	5 µL	—
TaqMan® Gene Expression Assay (20X)	1 µL	If you are not using pre-formulated TaqMan® Gene Expression Assays, Applied Biosystems recommends primer concentrations of 400 to 900 nM and a probe concentration of 100 to 250 nM.
Sample	Variable	Use as much sample as needed, up to the maximum allowed by the reaction volume.
RT-PCR Grade Water	Variable	Fill to the total reaction volume.
Total volume per reaction	20 µL	—



For sample volumes ≤30 µL

Run mode	Default†	Step	Stage	No. of cycles	Temperature	Time
Thermal cycling conditions		Reverse transcription	1	1	50 °C‡	5 minutes
		RT inactivation/initial denaturation	2	1	95 °C	20 seconds
		Amplification	3	40	95 °C	3 seconds
					60 °C	30 seconds

† Use the default run mode for your system and sample block module (that is, Fast mode on Fast instruments and standard mode on standard instruments).

‡ Reverse transcription works best between 48 °C and 55 °C.

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One-step Workflow: Master Mixes

- TaqMan® Fast Virus 1-Step Master Mix (PN 4444434)
 - 4X master mix to amplify both RNA and DNA
 - Formulated to handle common RT-PCR inhibitors found in blood, stool, and other difficult samples
 - Up to triplex (ROX as passive reference)
- TaqPath™ 1-Step Multiplex Master Mix (PN A28526)
 - 4X master mix to amplify both RNA and DNA
 - Tolerant to common RT-PCR inhibitors
 - Manufactured in an ISO 13485 certified facility
 - Up to quadruplex (Mustang Purple as passive reference): Only in QS5

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Two-step Workflow: Real-time PCR Reactions

Reverse Transcription : SuperScript IV VILO Kit (Only 25 min)

Step	Notes	Component	Amount
1	Prepare gDNA reaction (see table)	gDNA reaction mix	10 µl
2	Digest gDNA (see table)	gDNA reaction mix	10 µl
3	Prepare RT and No RT Control reaction mixes (see table)	RT reaction mix	10 µl
		No RT Control reaction mix	10 µl



4	Anneal primers	Early mix and incubate at 25°C for 10 minutes.
5	Reverse transcribe RNA	Incubate at 50°C for 10 minutes.
6	Inactivate enzyme	Incubate at 85°C for 5 minutes.
7	qPCR amplification	Use the diluted or undiluted cDNA for qPCR or store at -20°C for up to one week, or -80°C for long-term storage. Click to see guidelines for optimizing qPCR amplification.

Real-time PCR:

TaqMan Chemistry

2x TaqMan Master Mix	1x	10 µl
20x Probe/primer Assay Mix	1x	1 µl
Water		NA
cDNA		5 µl

SYBR Chemistry

2x Power SYBR Master Mix	1x	10 µl
F Primer	optimized	NA
R Primer	optimized	NA
Water		NA
cDNA		5 µl

Standard mode

PCR condition:
50°C, 2min
95°C, 10 min
95°C, 15 sec
60°C, 1min } 40 cycles

Fast mode

PCR condition:
95°C, 20 sec
95°C, 1 sec
60°C, 20 sec } 40 cycles

SYBR Green:

- Check Primer Concentration
- Add Melt Curve Program

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Two-step Workflow: Master Mixes

Standard & Fast Mode

- TaqMan® Chemistry
 - TaqMan® Fast Advanced Master Mix (PN 4444557)
- SYBR® Green Chemistry
 - PowerUp™ SYBR® Green Master Mix (PN A25742)

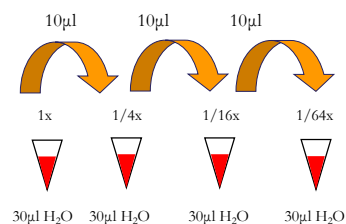


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相對定量 (Relative Quantitation): PCR Efficiency Validation

- 2µg RNA in 20µl RT
- Gene name: *C-Myc* and *GAPDH*
- cDNA 4-fold serial dilution: 10µl cDNA + 30µl H₂O
 - 1. 1x
 - 2. 1/4 x
 - 3. 1/16 x
 - 4. 1/64 x
 - 5. NTC (duplicate for each sample)
- 每個濃度點各做二重複

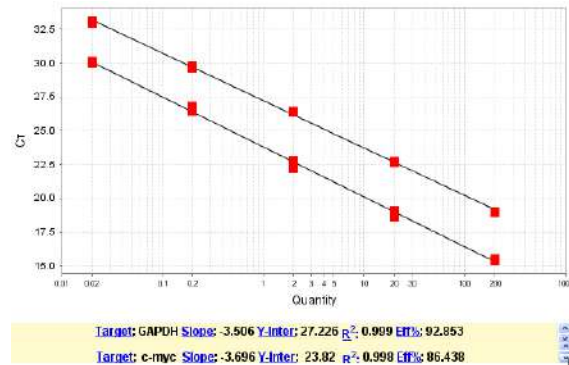


- Prepare a Premix for each gene
- Aliquot 15µl of Premix to each well
- Add 5µl of RT product to the well
- Real-time PCR reaction

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相對定量 (Relative Quantitation): PCR Efficiency Validation



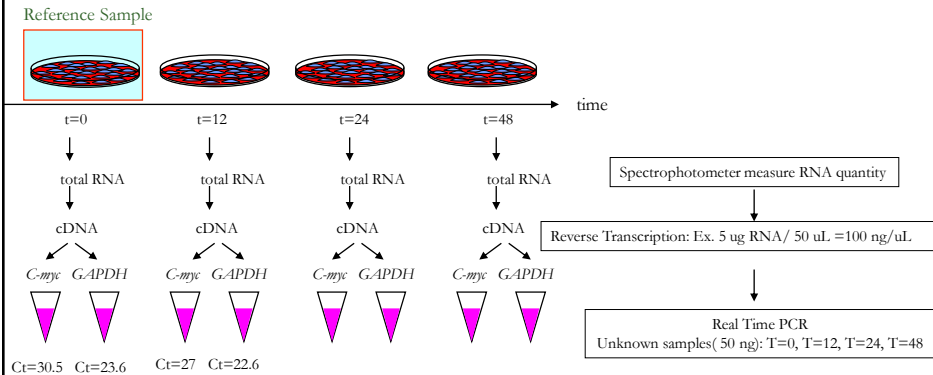
$90 \leq \text{Eff\%} \leq 110 \rightarrow \Delta\Delta\text{Ct}$
 $\text{Eff\%} < 90 \rightarrow \text{Relative standard curve}$

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相對定量 (Relative Quantitation): Comparative Ct Method ($\Delta\Delta\text{Ct}$)

Comparison of the c-myc expression level
 in T=0, T=12, T=24, T=48 time course study



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相對定量 (Relative Quantitation): Comparative Ct Method ($\Delta\Delta C_t$)

step 1: *Normalization to endogenous control*

Sample: Ct c-Myc – Ct GAPDH = ΔC_t sample

Reference: Ct c-Myc – Ct GAPDH = ΔC_t reference sample

step 2: *Normalization to reference sample*

ΔC_t sample – ΔC_t reference sample = $\Delta\Delta C_t$

step 3: *use the formula*

$$2^{-\Delta\Delta C_t}$$

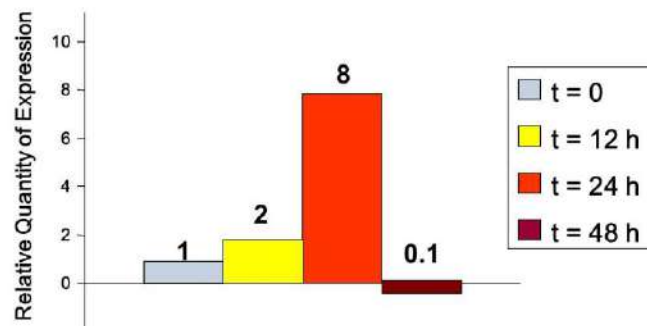
A reference sample is a sample to which unknown samples are compared (*e.g.* untreated sample or control).

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相對定量 (Relative Quantitation): Comparative Ct Method ($\Delta\Delta C_t$)

	c-Myc	GAPDH	ΔC_t	$\Delta\Delta C_t$	$2^{-\Delta\Delta C_t}$
T=0 (Reference)	25	10			
T=12hr	24	10			
T=24hr	23	11			
T=48hr	28	10			

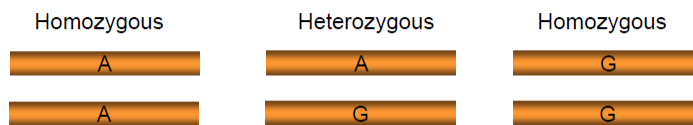


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Single Nucleotide Polymorphisms (SNPs)

- SNPs are single base changes in genomic DNA
- SNPs may cause diseases, alter metabolisms or act as markers
- SNPs in diploid loci commonly have 3 genotypes..

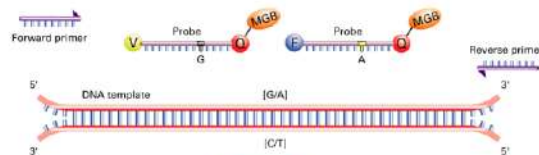


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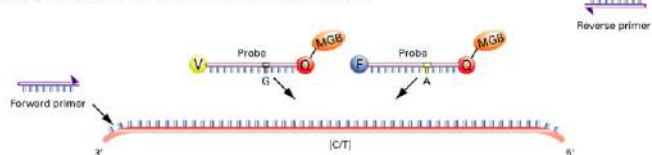
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TaqMan® SNP Genotyping Assay Overview

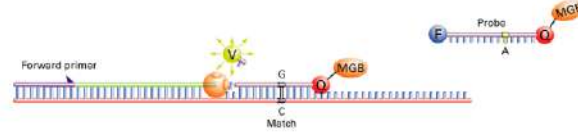
1. Assay Components and DNA Template



2. Denatured Template and Annealed Assay Components



3. Signal Generation

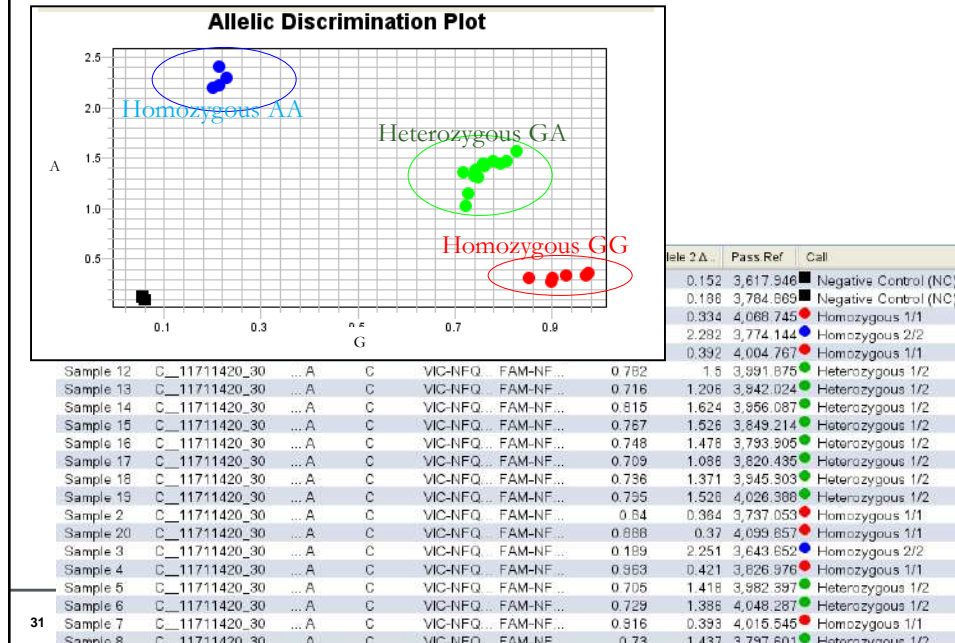


LEGEND

- V VIC[®] dye
- F FAM[®] dye
- Q Quencher
- MGB Minor Groove Binder
- A AmpliTaq Gold[®] DNA Polymerase
- Probe
- Primer
- Template
- Extended Primer

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Allelic Discrimination (SNP) Data

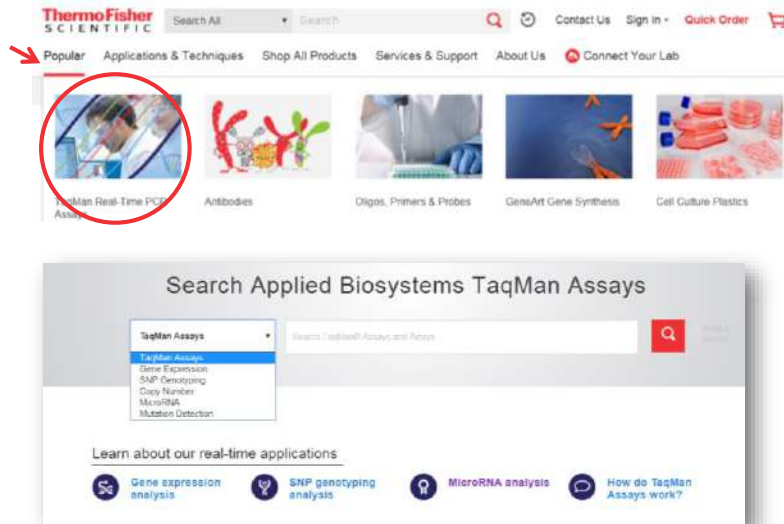


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Assay Design

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Pre-Designed TaqMan® Assay

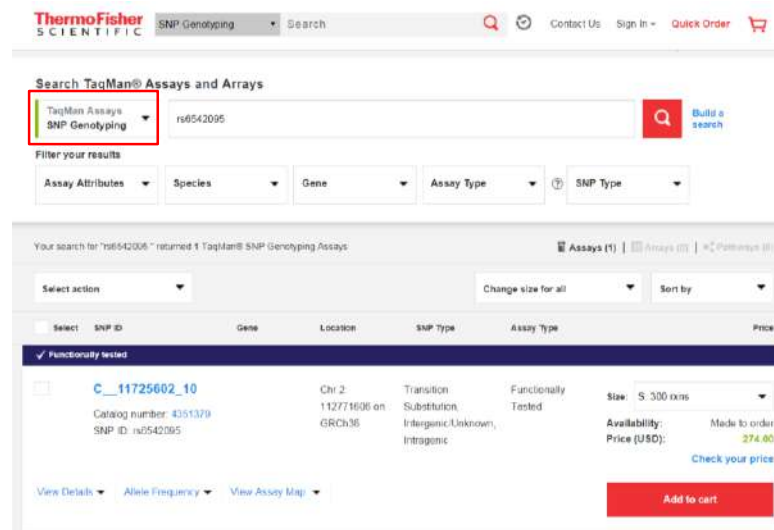


<http://www.thermofisher.com/tw/en/home/life-science/pcr/real-time-pcr/real-time-pcr-assays.html>

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Pre-Designed TaqMan® Assay - SNP



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Pre-Designed TaqMan® Assay – Gene expression

Search TaqMan® Assays and Arrays

TaqMan Assays
Gene Expression

gapdh

Build a search

Filter your results

Assay Attributes Species Gene GAPDH Assay Design Cross Reactivity

Select action Select product(s) Change dye for all Change size for all Sort by

Select	Assay ID	Gene	Transcripts	Assay Design	Amplicon Length	Price
<input type="checkbox"/>	Hs02786624_g1 Catalog number: 4331182 Target species: Human Important information	GAPDH	4 RefSeq (NM)	Both primers and probe map within a single exon	157	Dye: FAM-MGB Size: 5, 250 rxns Availability: Price (USD): Contact Us

View Details Related Reagents Related Controls View Assay Map

99 / 100 Bioe Stars 50 Citations: Erythropoietin Receptor Expression L. (PLoS One 2013 Oct 1) GAPDH: Hs02786624_g1 (Applied) (More...)

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Primer Design: Bioinformatics Evaluations

- NCBI (<http://www.ncbi.nlm.nih.gov/>)
 - BLAST: Sequence specificity
 - SNPs within sequence (N masked SNPs)
- Repeat Masker (<http://www.repeatmasker.org>)
 - N masked repeat sequence
- Custom TaqMan® Assay Design Tool
(<https://www.thermofisher.com/order/custom-genomic-products/tools/cadt/>)
- Primer Express® 3.0.1

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Custom TaqMan® Assay Design Tool - TaqMan® Gene Expression Assays

Custom TaqMan® Assay Design Tool

Home Enter Sequences Select Assays Additional Products Review & Order

Indicate Bioinformatics Preferences

Tell us which species your sequences are associated with: Human (Human)

Select a bioinformatic analysis preference:

[More Info](#)

☐ No bioinformatic analysis on my sequences

☒ Yes, please perform bioinformatic analysis on my sequences (Customs Plus)

Enter or Search for Sequence Information

Please enter your sequence in the 5' to 3' direction. Sequences must be between 61 and 5000 nucleotides in length and composed solely of the nucleotides A, C, G, T, or N. Ensure [successful design and performance](#) by reading our [Design and Ordering Guide](#).

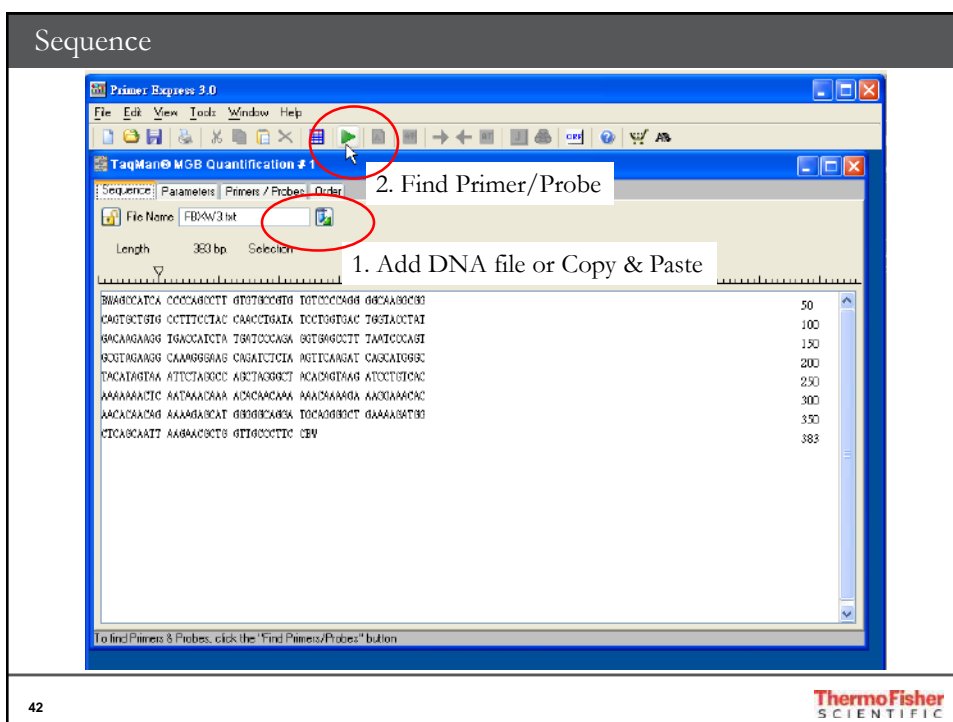
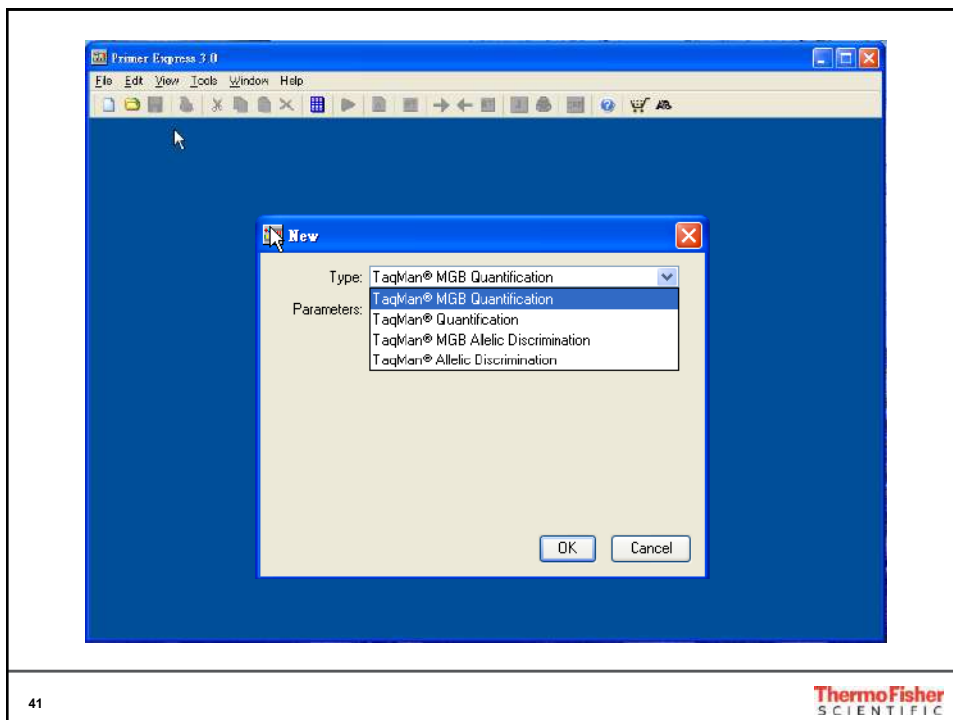
[Search for Sequences by Keyword or Location](#)

☐ Keep all sequences confidential

Status	Name	Sequence	Target Position
		<p>e.g. CAGTTCGACAGGCACTCTGACCTA GAGGTCAGACAGGCACTCTGACCTA</p> <p>Do you wish to permanently hide your assay primer/probe sequences in all documents? More Info</p> <p><input checked="" type="radio"/> No <input type="radio"/> Yes</p>	<p><input checked="" type="button" value="Remove All"/> <input checked="" type="button" value="Refresh"/></p> <p>After entering sequence, click 'Check Format' below</p>

[+ Enter More Sequences](#)

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Results

TagMan® MGB Quantification # 1

Sequence | Parameters | Primers / Probes | Order

☐ Candidate Primers & Probes

#	Find Start	Find Len.	Find Tm	Find %GC	Rev Start	Rev Len.	Rev Tm	Rev %GC	Probe Start	Probe Len.	Probe Tm	Probe %GC	Amp Tm	Amp %GC	Amp Tm	Amp Len
1	46	18	50	61	112	26	53	46	57	17	53	47	51	52	50	65
2	46	18	50	61	112	26	53	46	57	18	53	44	51	52	50	65
3	46	18	50	61	112	26	53	46	58	18	70	44	51	52	50	65
4	46	18	50	61	112	26	53	46	70	16	53	50	51	52	50	65
5	122	22	56	59	167	26	53	38	145	15	56	60	73	48	58	66
6	51	21	55	52	115	26	56	44	75	19	56	53	50	49	58	67
7	56	25	58	44	151	22	55	50	121	17	55	59	50	49	58	67
8	56	25	58	44	151	22	55	50	123	16	58	53	50	49	58	67
9	121	21	53	52	167	26	53	38	143	17	70	53	73	48	58	67
10	121	21	50	52	157	26	53	38	144	16	53	56	73	48	58	67
11	56	26	56	42	151	22	55	50	123	16	58	53	50	49	58	67
12	121	22	50	50	167	26	53	38	144	16	53	56	73	48	58	67
13	122	22	56	50	168	27	50	41	145	15	58	60	50	49	58	67
14	46	18	50	61	115	26	53	48	57	17	53	47	51	52	50	68
15	46	18	50	61	115	26	53	48	57	18	53	44	51	52	50	68
16	46	18	50	61	115	25	53	48	58	18	70	44	51	52	50	68

☐ Location

☐ Secondary Structure

Oligo Length: 18
Reverse Primer Length: 26
Probe Length: 17

Forward Primer: CCGCAGTGTGTGCTTT
Reverse Primer: GACTTCTTTGATAGTACCATCA
Probe: CTACCACTGATATCC

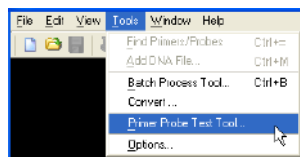
Most Stable Structure Found

5'-TGAAGG-3'
3'-CTGAGTTT-3'

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Check Tm of Primers



Primer Probe Test Tool

Parameters

Document Type: TagMan® MGB Quantification Parameter: Default Browse

Primers and Probes

Find Primer: ACTGATCGATCAGCTACCGATC Tm: 56.1 %GC: 50 Length: 22

Rev Primer: TCGATCGATCGATCGATGC Tm: 59.2 %GC: 53 Length: 19

Probe 1: Tm: 0.0 %GC: 0 Length: 0

Probe 2: Tm: 0.0 %GC: 0 Length: 0

Tab

life technologies™

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SYBR Green Experiment Notes

1. Primer Concentration Optimization

- Primer final concentration
- No primer dimer or non-specific product involved

2. PCR Primer Efficiency Validation

- Serially-diluted sample to generate standard curve for target gene and endogenous control gene

3. Test with samples that are comparable to real experiment for each gene

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Applied Biosystems 提供 Primers/Probe 設計的全方位解決方案

<input type="checkbox"/> H. sapiens	<input type="checkbox"/> A. thaliana
<input type="checkbox"/> R. norvegicus	<input type="checkbox"/> D. melanogaster
<input type="checkbox"/> M. musculus	<input type="checkbox"/> C. elegans
<input type="checkbox"/> M. mulatta (Rhesus)	<input type="checkbox"/> C. familiaris (Canine)
<input type="checkbox"/> D. rerio (Zebrafish)	<input type="checkbox"/> B. taurus (Cow)
<input type="checkbox"/> G. gallus (Chicken)	<input type="checkbox"/> O. cuniculus (Rabbit)
<input type="checkbox"/> S. scrofa (Pig)	<input type="checkbox"/> E. caballus (Horse)
<input type="checkbox"/> O. sativa (Rice)	<input type="checkbox"/> Pathogens



- *TaqMan Gene Expression Assays*
 - > 1,300,000 個已設計及測試過的基因定量試劑組
 - 提供所有相關生物資訊 (23 species)
- *TaqMan microRNA and primary microRNA Assays*
- *TaqMan SNP Genotyping Assays*
- *TaqMan Copy Number Assays*
- *TaqMan Mutation Detection Assays*

- **Custom** TaqMan Assays
 - All-in One tube TaqMan-based Assay

- Primer Express Software
- 上機條件皆相同~~不用再花時間測試primer溫度了



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Applied Biosystems QuantStudio™ 3 Real-Time PCR System

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QuantStudio™ 3 Real-Time PCR Systems: The Basics

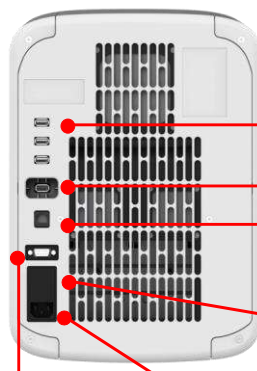
Touchscreen (stand-alone capabilities, PIN-protected user accounts, and dye calibration/RNaseP functionality)

USB port for template upload and data download

Motorized block drawer (controlled by touchscreen)



RS232 port (Service only)



USB ports

WiFi adapter port (optional use)

Ethernet port : RJ45 (10/100Mbps)

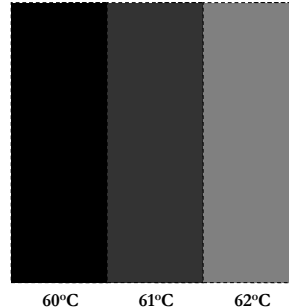
Fuse cover

Power port: 100/240 VAC

QuantStudio™ 3 Real-Time PCR System: The Basics

- **VeriFlex™ Block with 3 programmable zones**

- Independent temperature control in each zone (more precise than gradient)
- Can program at will, including multiple zones with same temp
- Great for optimization and also running multiple assays at the same time



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Multiplexing Capabilities

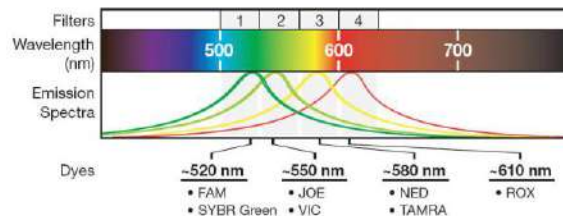
- **OptiFlex™ System with Bright White LED**

- **4-color instrument**

- FAM™/SYBR® Green dyes
- VIC®/JOE™ dyes
- ROX™ dye
- NED™/TAMRA™ dye

- **Factory calibrated**

Peak channel	Color	Filter wavelength (nm) ^[1]		Pre-calibrated dyes	Example custom dyes
		Excitation	Emission		
x1-m1	Blue	470 ± 15	520 ± 15	FAM™ and SYBR® Green	SYT09
x2-m2	Green	520 ± 10	558 ± 12	VIC®	HEX™, TET™, and JOE™ ^[2]
x3-m3	Yellow	550 ± 10	587 ± 10	ABY®, NED™, and TAMRA™	Cy®3
x4-m4	Orange	580 ± 10	623 ± 14	JUN® and ROX™	Texas Red®



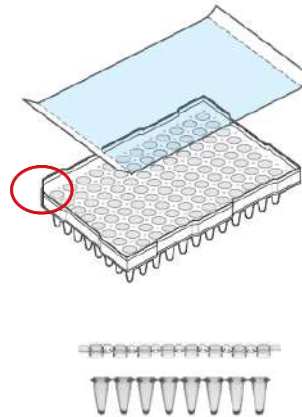
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QuantStudio™ 3 Real-Time PCR System: Consumables

For 96-well Fast Block:

- 样品量多時
 - MicroAmp® **Fast** 96-Well Reaction Plate (0.1ml) - 10 plates (P/N 4346907)
 - MicroAmp® Optical Adhesive Film - 25 films (P/N 4360954)
- 样品量少時
 - MicroAmp® **Fast** 8-Tube Strip (0.1ml) - 125 strips (P/N 4358293)
 - MicroAmp® Optical 8-Cap Strip - 300 strips (P/N 4323032)



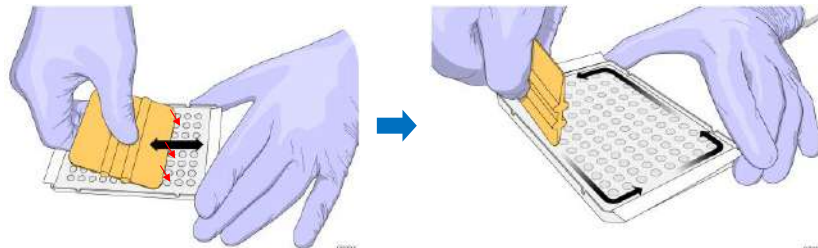
★ Load at least 16 tubes with tray

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Sealing the Plate

1. The **flat edge of an applicator** is rubbed back-and-forth along the **length** of the plate with a significant downward pressure to form a complete seal on top the wells
2. The **end of an applicator** is rubbed around all the **outside edges** of the plate with a significant downward pressure to form a complete seal around the outside wells



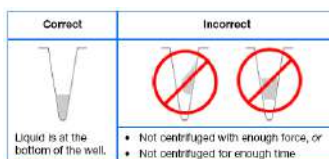
Note: **Pressure** is required to activate the adhesive on the optical cover

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QuantStudio™ 3 Real-Time PCR System: Operation Notes

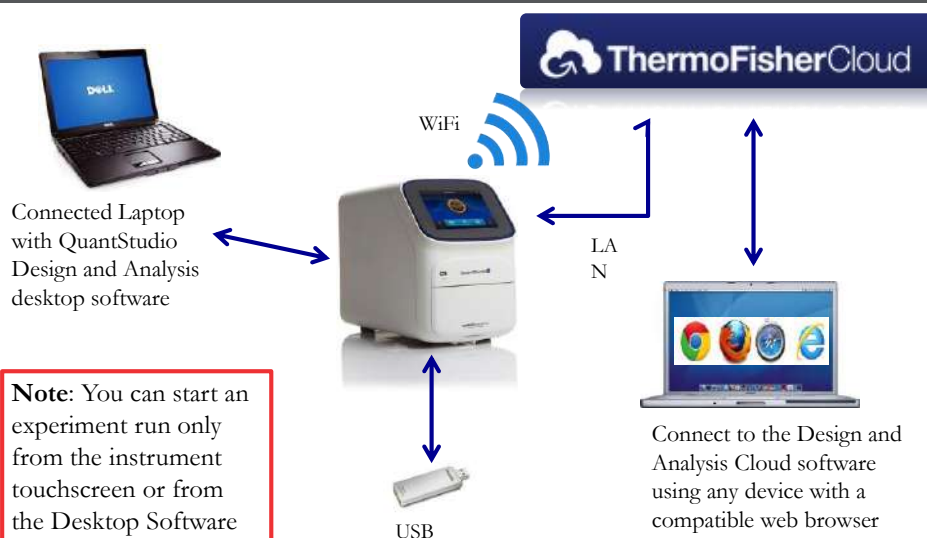
- Use a **tray** for 8-tube strips
- Do not label on the consumables
 - This may increase the background signal
- Avoid bubbles when pipetting into each well
 - Centrifuge samples



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Stand-alone, Desktop, or Online



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QuantStudio™ 3 Real-Time PCR System: Touch Screen




QuantStudio™ 3 Real-Time PCR System: Edit Run Protocol




Full method editing capabilities on the touch screen, including VeriFlex, Pause, and Melt

Monitor Progress During the Run


Time Remaining





Thermal Protocol Status




Live Amplification Curves






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Options to Upload Data



1. Cloud = Data saved to user's online account
2. USB = Data saved to attached USB drive
3. Desktop = Data automatically saves back to desktop if run started from desktop

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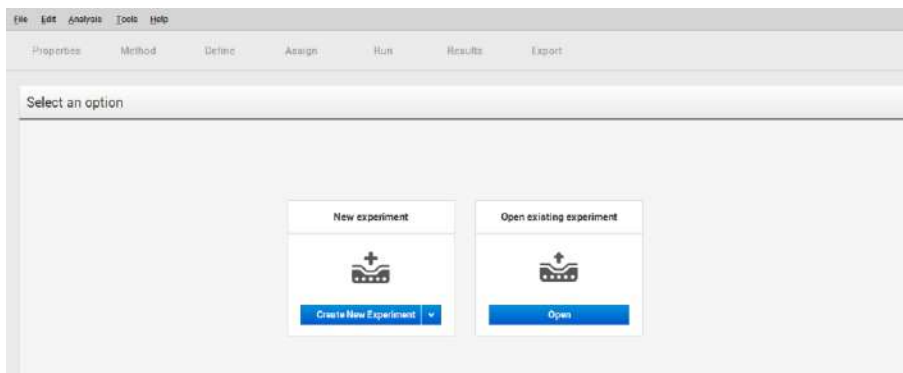
QuantStudio™ Design and Analysis Software

- QuantStudio™ Design and Analysis Software supports a variety of analysis methods, including:
 - Absolute Quantitation
 - Standard Curve
 - Relative Quantitation
 - Relative Standard Curve
 - Comparative CT ($\Delta\Delta$ CT)
 - Melt curve analysis
 - Genotyping (including real-time amplification)
- Multiplate GEx analysis available online on the QuantStudio Design and Analysis **Cloud** Software (<https://www.thermofisher.com/tw/en/home/cloud.html>)

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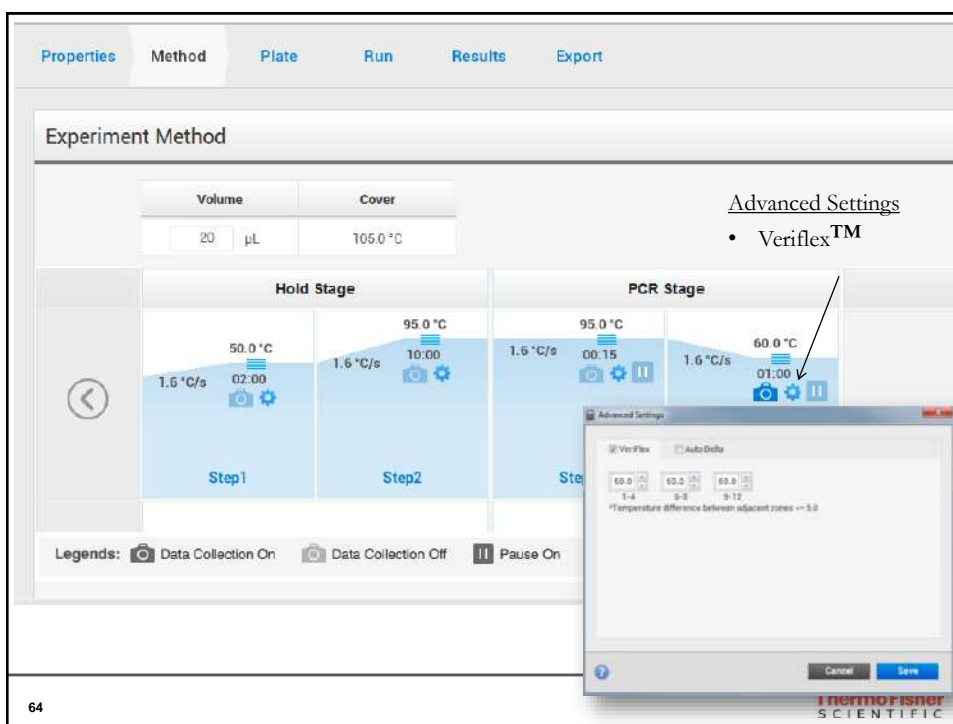
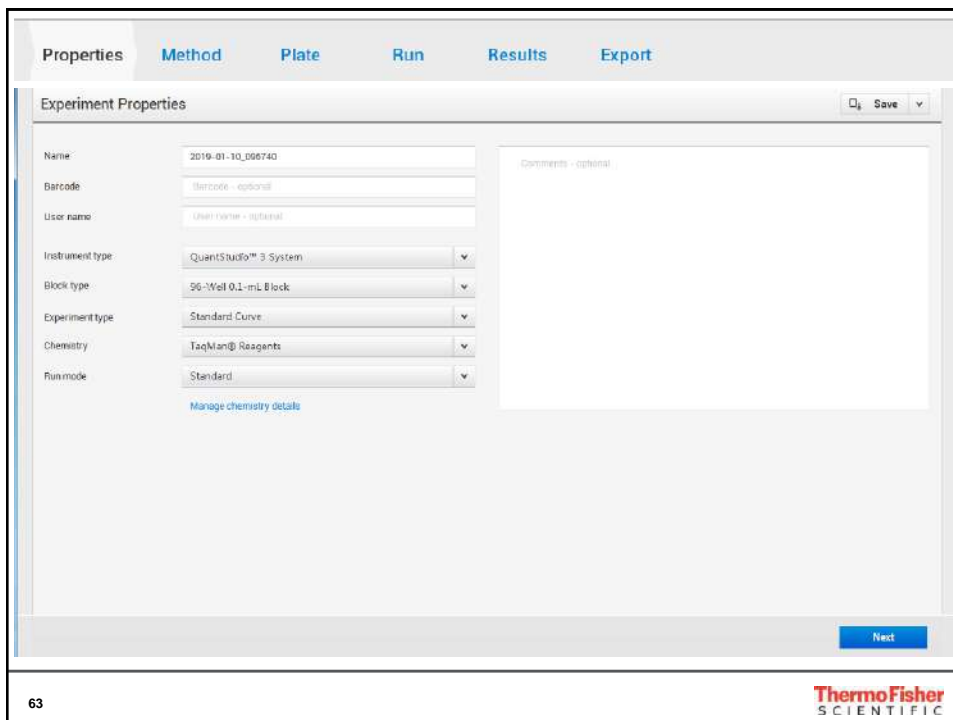
QuantStudio™ Design and Analysis Software



- Similar look and feel as online software
- <http://www.thermofisher.com/tw/en/home/technical-resources/software-downloads/ab-quantstudio-3-and-5-real-time-pcr-system.html>

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Properties

Method

Plate

Run

Results

Export

Assign Targets and Samples

Quick Setup

Advanced Setup

Well Attributes

Sample

New Sample

Target

New Target

Well Comments

Well Comments

Plate Attributes

View

1

2

3

A

B

C

D

E

F

G

H

Wells: U 0 S 0 N 0

Select well and type sample names

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Properties

Method

Plate

Run

Results

Export

Assign Targets and Samples

Quick Setup

Advanced Setup

Targets

+

Add

✎

Action

▼

	Name	Reporter	Quencher	Comments	Task	Quantity	
<input type="checkbox"/>	Target 1	FAM	NFQ-MGB		▼		✕

Samples

+

Add

✎

Action

▼

	Sample Name	Comments	+	
<input type="checkbox"/>	Sample 1			✕

View

1

2

3

A

B

C

D

E

F

G

H

Wells: U 0 S 0 N 0

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Define and Set Up Standards

Properties Method **Plate** Run Results Export

Assign Targets and Samples

Quick Setup Advanced Setup

Well Attributes

Sample: New Sample

Target: New Target

Well Comments: Well Comments

Plate Attributes

View

Define and Set Up Standards

Wells: 10 0 0 0 0 96 Empty

Previous Next

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Define and Set Up Standards

Select a target

Model: Singleplex Select the target for this standard curve: Target 1

Define the standard curve

of Points: 5 (5 Recommended)

of Replicates: 3 (3 Recommended)

Starting Quantity: 1.0 (Enter the highest or lowest standard quantity for the standard curve)

Serial Factor: 1.5 (Select a value from 1:10 to 10x)

5 Points X 3 Replicates = 15 Required Wells

Standard Curve Preview

1E0
2E-1
4E-2
8E-3
1.6E-3

Select and arrange wells for the standards

Arrange standards in: Columns Rows

Use Wells: Automatically Select Wells for Me Let Me Select Wells

15 Required Wells / 15 Selected Wells

A1,A2,A3,A4,A5,A6,A7,A8,A9,A10,A11,A12,B1,B2,B3

Apply Reset Close

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Properties Method **Plate** Run Results Export

Assign SNP Assays and Samples

Quick Setup

Advanced Setup

SNPs

+

 Add

📄

 Action ▼

	Name	NCBI...	Cont...	Allel...	Rep...	Qu...	Allel...	Re...	Q...	Task
<input type="checkbox"/>	SNP Assay 1			Alle...	VIC	N...	Alle...	F...	...	▼

Samples

+

 Add

📄

 Action ▼

	Sample Name	Comments
<input type="checkbox"/>	Sample 1	

View ▼

	1	2	3
A			
B			
C			
D			
E			
F			
G			
H			

Wells: U 0 N 0 P 0 2 0

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Properties Method Plate **Run** Results Export

Run Control

Start Run ▼

Save ▼

Reset

Amplification Plot

Plot Run Summary

Amplification Plot

Y-axis: 0.001 to 10

X-axis: Cycles

Plot Run Summary

Y-axis: 1 to 4

X-axis: 1 to 384

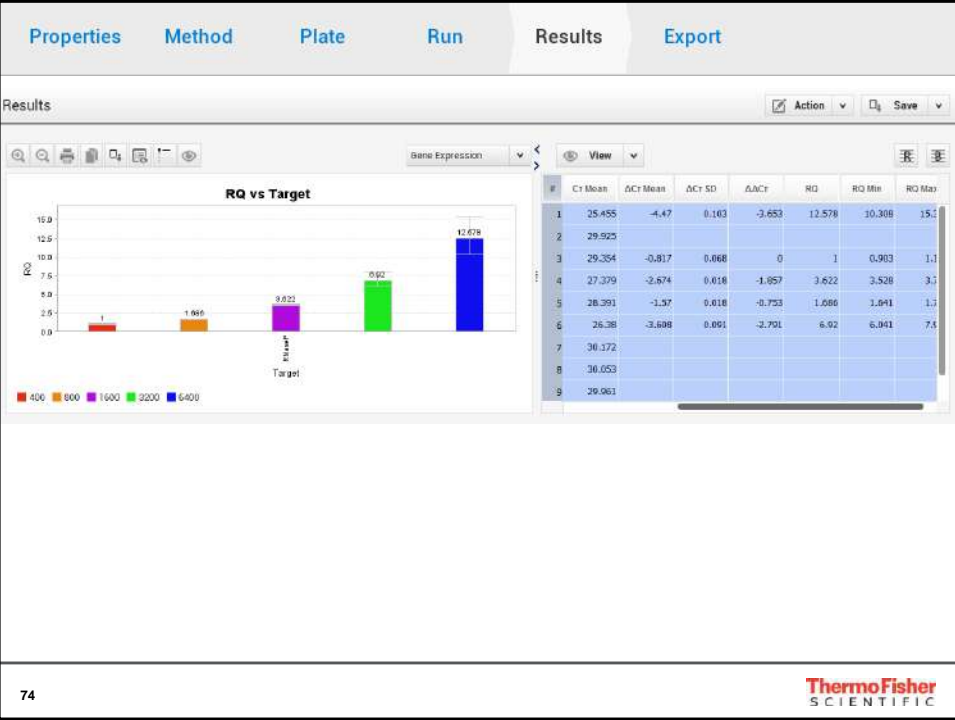
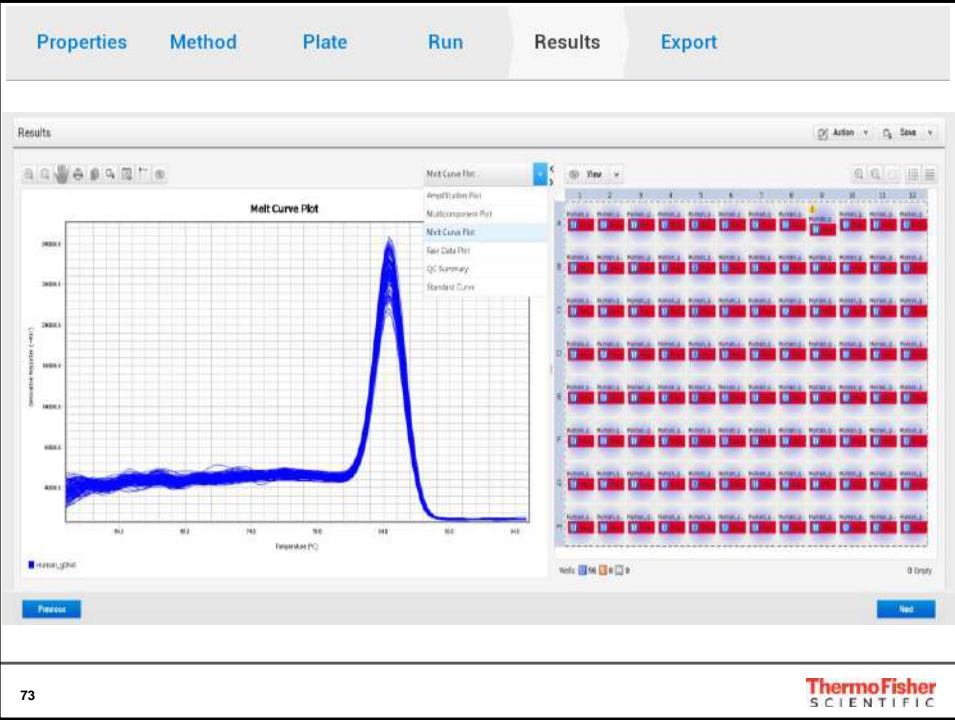
Previous

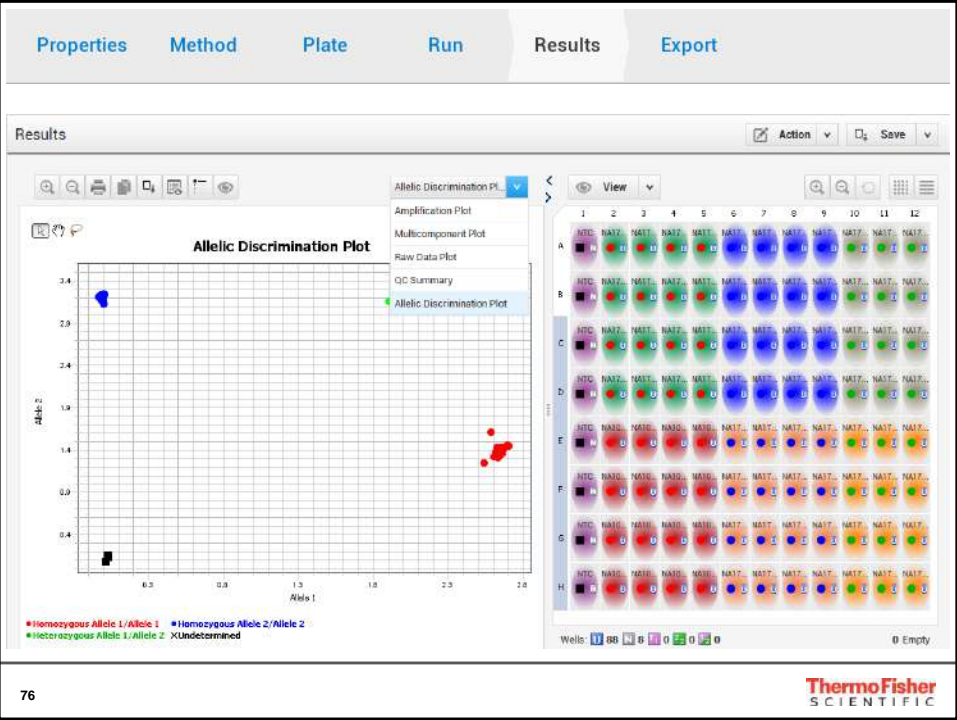
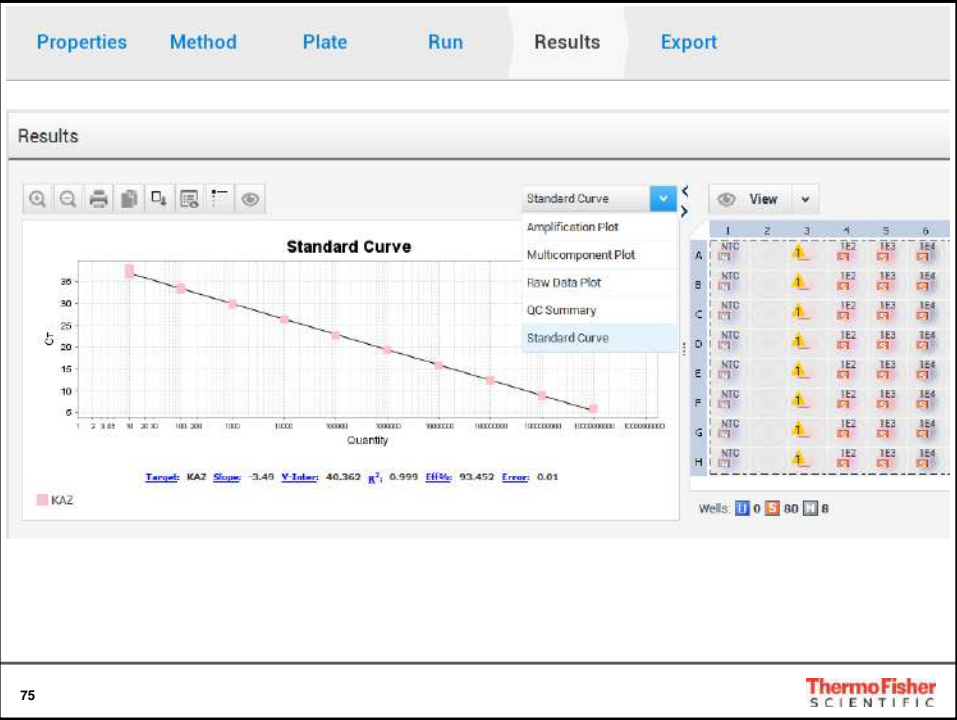
Test

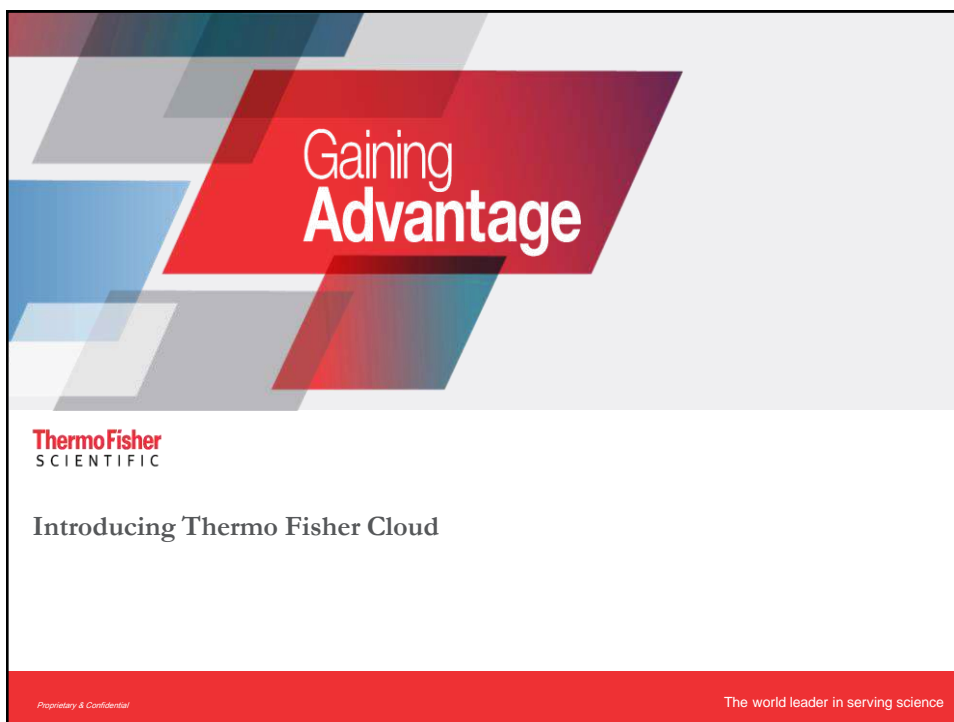
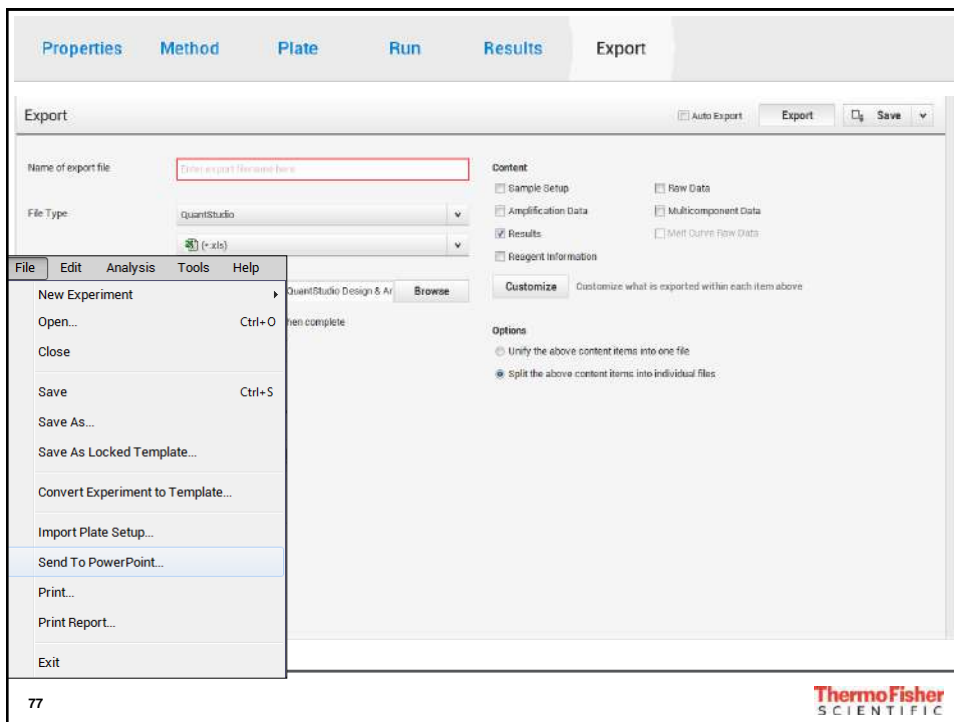
Start Run from touchscreen or desktop

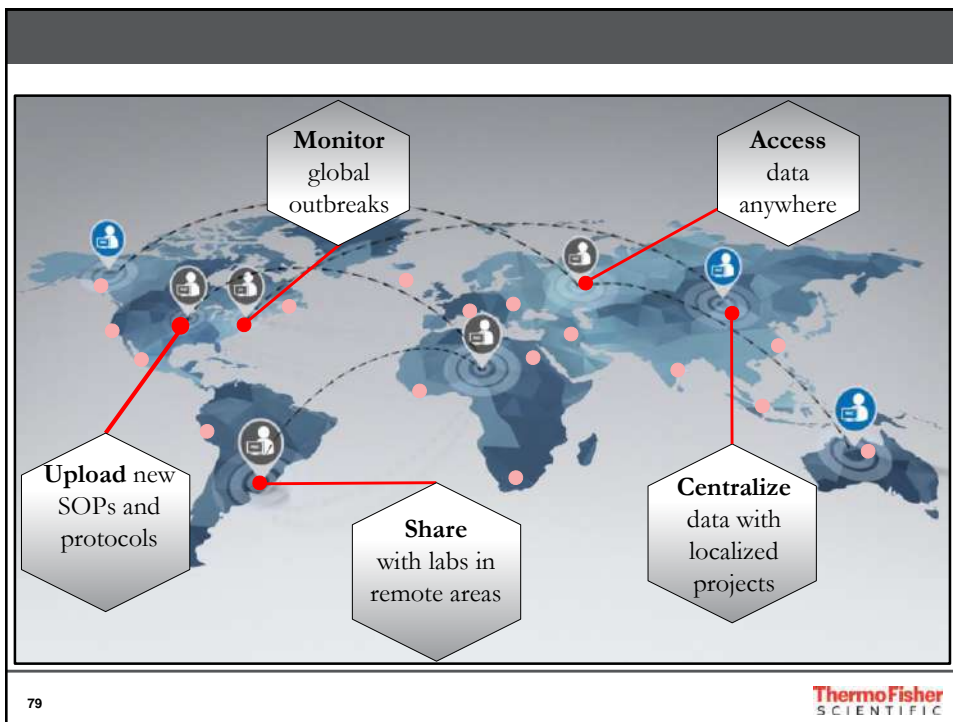
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Thermo Fisher Cloud: Dashboard

The front page of Thermo Fisher

Quick access to Files, Applications, and Instruments

Upload files and create new projects

Check the status of connected instruments

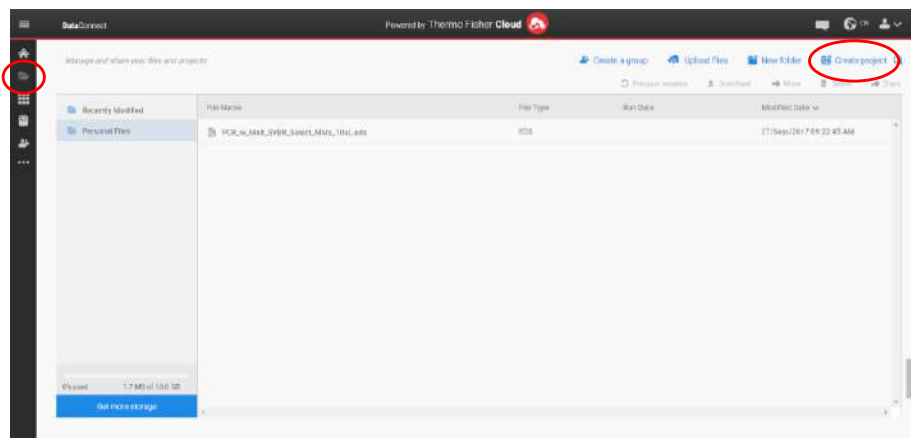
Manage Profile Switch to Region Get Notifications

Connect Your Lab

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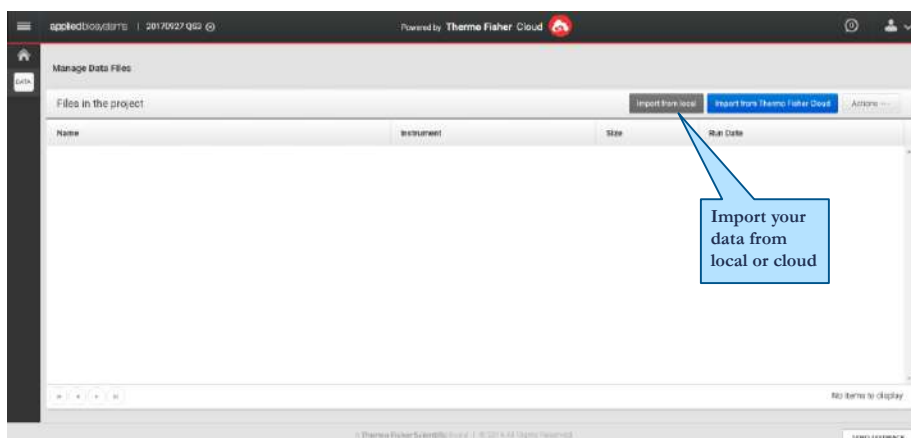
Thermo Fisher Cloud: Files



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In the Project



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Analysis modules

powered by ThermoFisher Cloud

Manage Data Files

Files in the project

Name	Instrument	Size	Run Date
PCR_w_Meth_SyBR_Select_MMx_10ul.xls	QuantStudio 5 System	1,742,810	4/6/2015 9:10:23 AM

Select analysis module

SC: Standard curve
RQ: Relative Quantification

1 - 1 of 1 items

ThermoFisher Scientific Cloud 1 © 2014 All Rights Reserved

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Relative Quantification Analysis Module: Project Overview

Basic navigation across the top

Analyze button

Overview Plate Setup Data Review Analysis Export

Default Analysis Group Settings Analyze

Experiments

Experiment Name	Block Type	Instrument
Q96_164Real_Comparative_2_Example_1.xls	164-Real	QuantStudio 6 Real-Time PCR System
Q96_164Real_Comparative_2_Example_2.xls	164-Real	QuantStudio 6 Real-Time PCR System
Q97_164Real_Comparative_2_Example_1.xls	164-Real	QuantStudio 7 Real-Time PCR System
Q97_164Real_Comparative_2_Example_2.xls	164-Real	QuantStudio 7 Real-Time PCR System
V967_164Real_Comparative_2_Example_1.xls	164-Real	VIA 7 Real-Time PCR System
V967_164Real_Comparative_2_Example_2.xls	164-Real	VIA 7 Real-Time PCR System

Targets

Name	Reporter	Comments
ACTB	Real	
GLP1R	Real	
BDH	Real	
EPIC	Real	
EPHX	Real	

Samples

Name	Biogroup
Brain	
Heart	
Liver	
Lung	

Biogroups

Name	Color	Comments

Analysis Groups

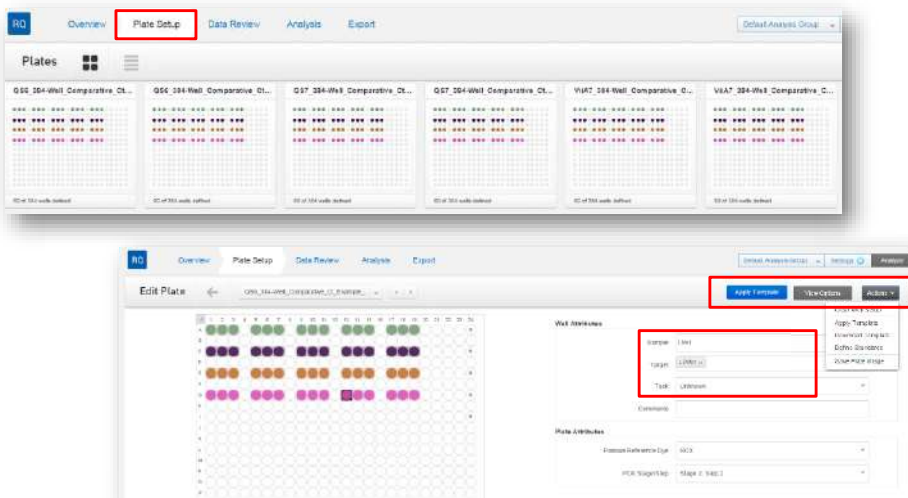
Name	Analysis Status
Default Analysis Group	Selected instrument type
Metabolic Analysis	Selected instrument type

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Plate Set Up: Change sample / target layout

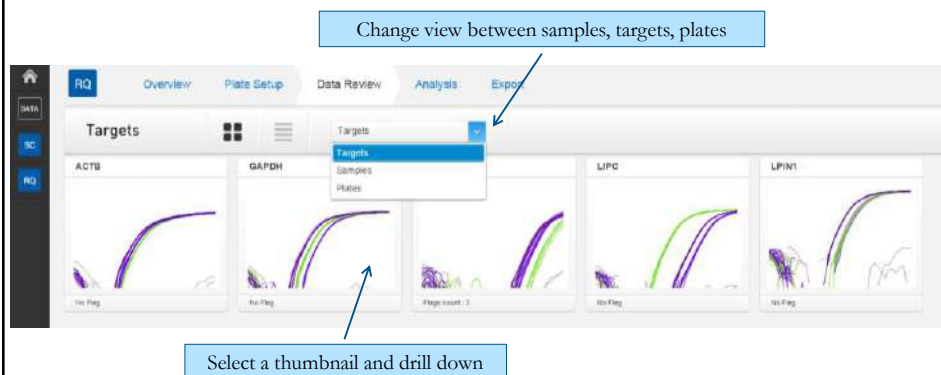
No need to go back to instrument software!!!



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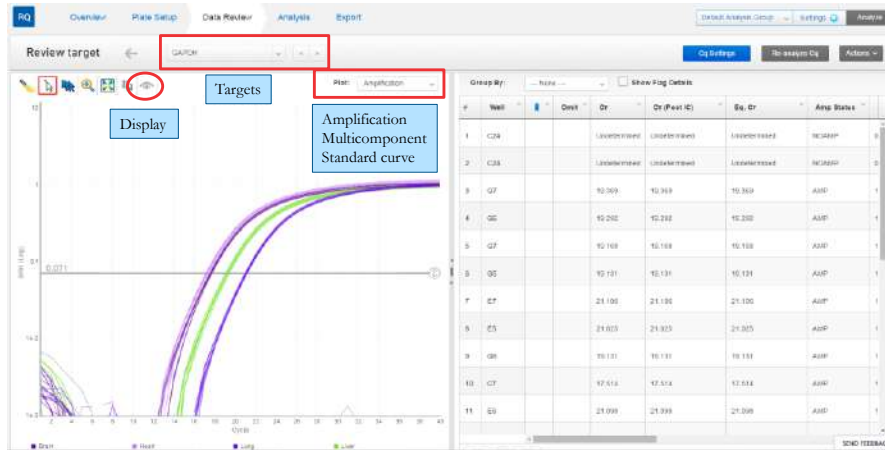
Data Review: Quick View of Samples, Targets, Plates



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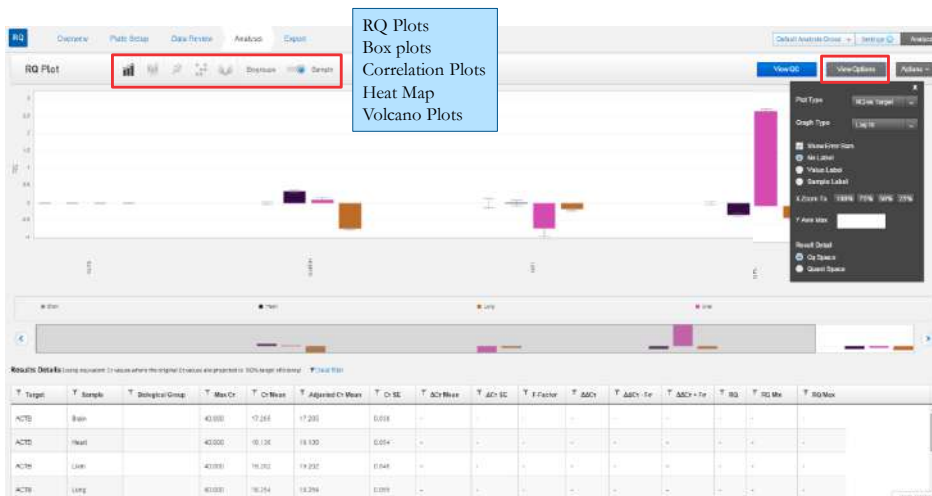
Data Review: Detailed Amplification Curves



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Gene Expression Plots in Analysis Section



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Export

Name:

File type: Text CSV Excel

Comments:

Included in:

- ☒ Biological Group Results
- ☒ Sample Results
- ☒ Well Results (1/10 wells)
- ☐ Amplification Data
- ☐ Volume Plate Data
- ☐ Target Sample Plate QC

Export

Must name file first

.txt or .csv formats

Customize your export

Experiment Name	Barcode	Well	Biological Group	Sample Name	Target Name
Q04_204	WV1_Comparative_Q	A2	Untreated	Shen	ACTB
Q04_204	WV1_Comparative_Q	A3	Untreated	Shen	ACTB
Q04_204	WV1_Comparative_Q	A11	Untreated	Shen	SH4
Q04_204	WV1_Comparative_Q	C5	Untreated	Heal	SNP-Div
Q04_204	WV1_Comparative_Q	C19	Untreated	Heal	LP-B1
Q04_204	WV1_Comparative_Q	E14	Treated	LPB	LP-B1
Q04_204	WV1_Comparative_Q	E16	Treated	LPB	LPB
Q04_204	WV1_Comparative_Q	E20	No Sample		SH4

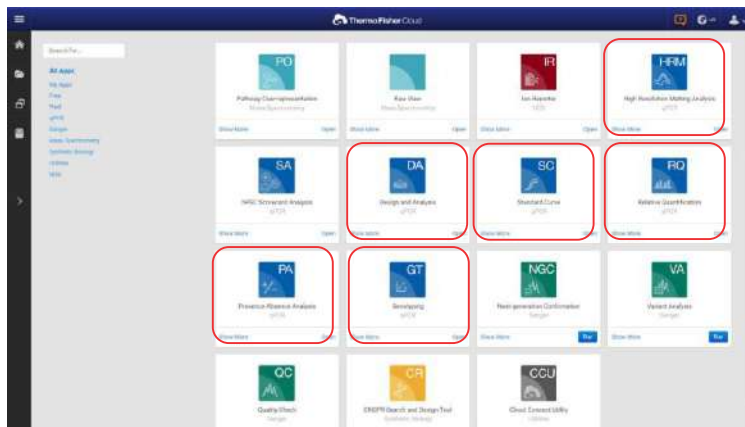
Applied Biosystems qPCR Analysis Modules on Thermo Fisher Cloud

The screenshot displays the ThermoFisher Cloud interface, which is a web-based platform for managing and analyzing qPCR data. The interface features a sidebar on the left with navigation options such as 'All Apps', 'My Apps', 'Files', 'Tools', 'Reports', 'Data', 'Settings', 'Help', 'About', 'Contact Us', 'Feedback', 'Privacy Policy', 'Terms of Service', and 'Help'. The main area shows a grid of application tiles, each representing a different qPCR analysis module. The tiles are arranged in a 4x4 grid, with some tiles highlighted by red boxes. The highlighted tiles are: Pathway Characterization (PO), High Resolution Melting Analysis (HRM), SA (qPCR), DA (Design and Analysis), SC (Standard Curve), RA (Relative Quantification), PA (Precision/Ratio Analysis), GT (Genotyping), NGC (Next-generation Confirmation), and VA (Variant Analysis). Each tile includes a small icon, the module name, and a 'Show More' button. The interface is designed to be user-friendly and accessible, allowing researchers to easily find and use the tools they need for their qPCR analysis.

A suite of qPCR analysis tools for the entire QuantStudio™ Family:
QuantStudio1, 3, 5, 6, 7, 12K including Open Array format

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A suite of qPCR analysis tools for the entire QuantStudio™ Family:
QuantStudio1, 3, 5, 6, 7, 12K including Open Array format

On-Line Support Center

[Support Centers](#)
Real-Time PCR and Digital PCR Instruments Support Center

- 7500 and 7500 Fast Real-Time PCR Systems Support
- 7900HT Fast Real-Time PCR System Support
- QuantStudio™ 3D Digital PCR System Support
- QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Support
- QuantStudio™ 12K Real-Time PCR System Support

One resource for all of your real-time PCR and digital PCR instrument support needs. Navigate through the instrument support categories below to obtain relevant technical information, view tips and tricks when starting an experiment, and find fixes for common instrument and software errors.

QuantStudio™ 12K Flex Real-Time PCR System Support
 Find useful resources and technical FAQs on calibration, operation, and software support across all block types of the QuantStudio™ 12K Flex Real-Time PCR System (96-well, 384-well, TaqMan® array, and OpenArray® plate).

QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Support
 Access in-depth technical information on the QuantStudio™ 6 and 7 Flex Real-Time PCR systems (96-well, 384-well, TaqMan® array). Browse our useful documents and manuals, get tips on using the qPCR instruments, or find help with calibration, operation, and software support across all block types.

QuantStudio™ 3 and 5 Real-Time PCR Systems Support
 Obtain technical documents on calibration, operation, and software support across all block types of the StepOne™ and StepOnePlus™ qPCR instruments (48-well or 96-well, respectively). View our frequently asked questions for setting up your experiment or our recommendations for commonly encountered error messages.

Via™ 7 Real-Time PCR System Support
 Find useful resources and technical FAQs on calibration, operation, and software support across all block types.

7500 and 7500 Fast Real-Time PCR Systems Support
 Learn best practices for calibration, operation, and data analysis when using the 7500 or 7500 Fast qPCR instruments. Browse through useful resources including technical guides and tools.

Protein Electrophoresis and Western Blotting Support
 Obtain support on digital PCR applications and analyzing your data.

How-To and Educational Videos
 How-To and

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<https://www.thermofisher.com/tw/en/home/technical-resources/technical-reference-library/real-time-digital-PCR-instruments-support-center.html>

ThermoFisher Scientific

On-Line Support Center

Real-Time PCR and Digital PCR Applications Support Center

[Support Centers](#)
Real-Time PCR and Digital PCR Applications Support Center

- Pharmacogenomics Support
- Mutation Detection Support
- miRNA and pri-miRNA Support
- High Resolution Melt Support
- Gene Expression Support
- Digital PCR Support
- Copy Number Variation (CNV) Support
- Protein Thermal Shift Support

One resource for all of your Real-Time PCR and Digital PCR support needs. Navigate through the qPCR support categories below to obtain relevant technical information, view tips and tricks when starting an experiment, and find answers to everyday qPCR problems.

Real-Time PCR Basics Support
 Support for each step of your qPCR experiment from reverse transcription and primer/probe design to gene expression analysis.

SNP Genotyping Support
 Learn how to prepare your samples to detect polymorphisms, find the right SNP assay and analyze the results.

Copy Number Variation (CNV) Support
 View information on determining and analyzing copy number changes due to gene deletion or duplication.

High Resolution Melt (HRM) Support
 Access information about analyzing genetic variations.

Gene Expression Support
 Learn how to set up your gene expression assay and find suggestions for common amplification problems.

miRNA and pri-miRNA Support
 Find out how to design and run your pri-miRNA and microRNA assays.

Mutation Detection Support
 Get help with assaying rare allele mutations using castPCR technology to determine mutation status.

Digital PCR Support
 Obtain support on digital PCR applications and analyzing your data.

TaqMan™ Protein Assay Support
 Find information and help about analyzing protein-protein interactions through Taqman™ assays.

Protein Electrophoresis and Western Blotting Support
 Obtain support on digital PCR applications and analyzing your data.

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<https://www.thermofisher.com/tw/en/home/technical-resources/technical-reference-library/real-time-digital-PCR-applications-support-center.html>

her IC

Thank You!

技術服務E-mail: Support.TW@lifetech.com

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