



Gaining Advantage

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Applied Biosystem QuantStudio™ 1 Real-Time PCR System
原理與應用介紹

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Agenda

- **Principle of Real-time PCR**
 - TaqMan® V.S. SYBR® Green
- **Real-time PCR Application**
- **Assay design**
 - Predesigned Assay Search
 - Custom Assay Design tool
- **QuantStudio™ 1 Real-Time PCR System**
- **Thermo Fisher Cloud**

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Polymerase Chain Reaction (PCR)

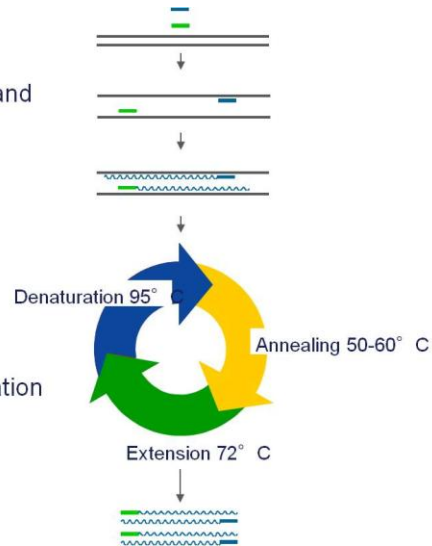
Primers and DNA

Strand denaturation and
primer annealing

Primer extension

Cycling

Exponential amplification
of PCR products



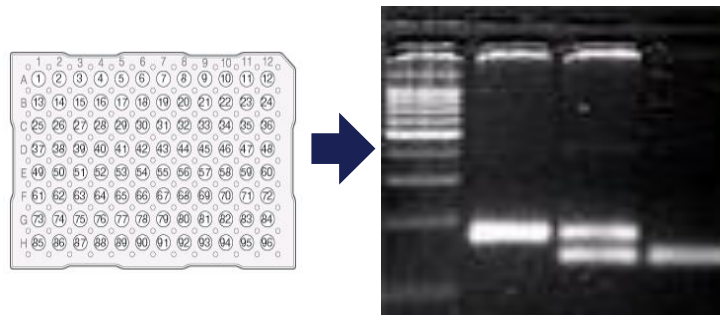
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Traditional PCR

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

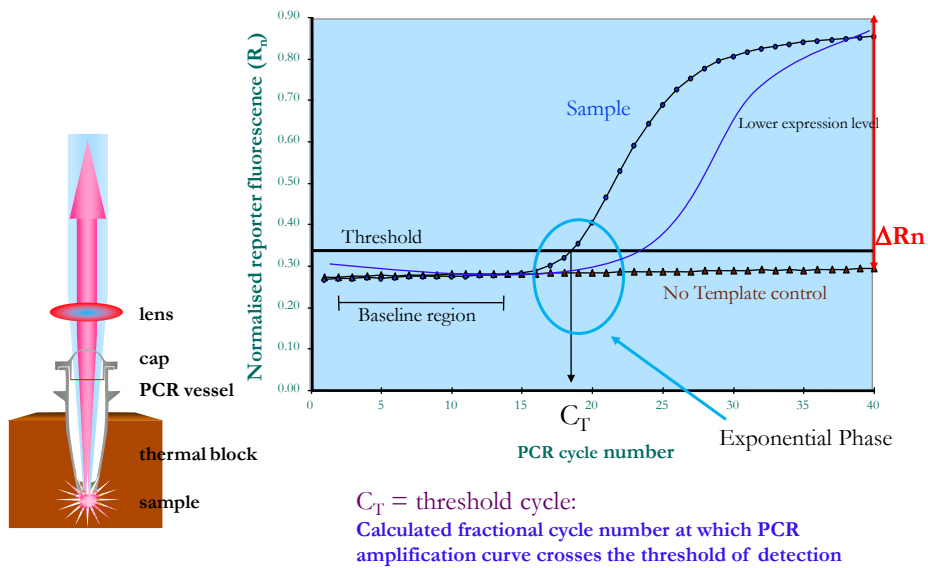


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Principle of Real-time PCR

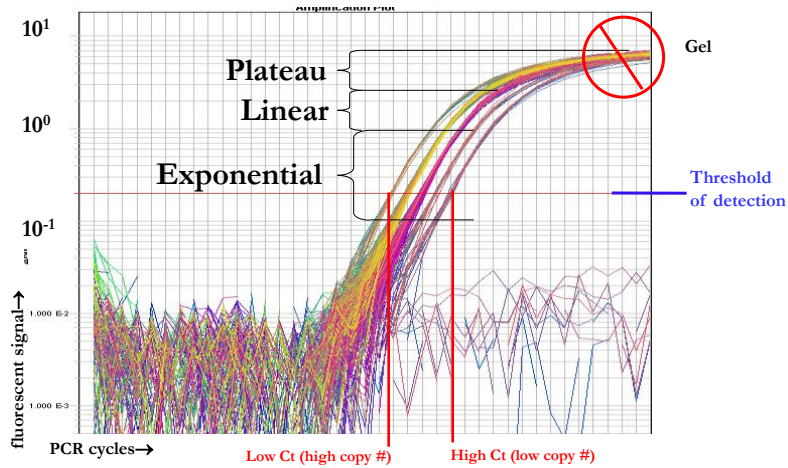


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Real-time PCR Signal Detection: Exponential Phase



$Y = N_0 2^n$, C_T 與起始濃度之對數值成反比

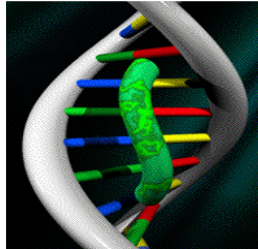
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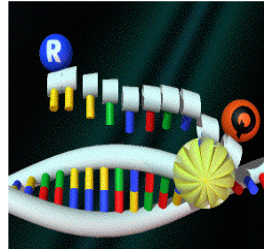
Real-time PCR Chemistries

SYBR® Green I dye



Binds Double-stranded DNA

TaqMan® and TaqMan® MGB



Fluorogenic 5' Nuclease Assay

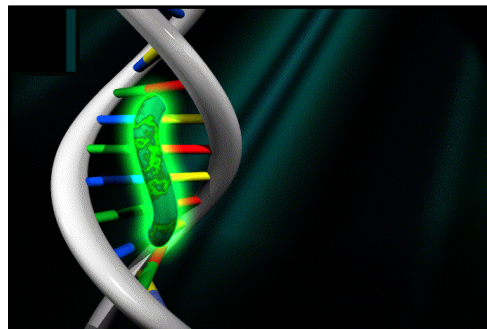
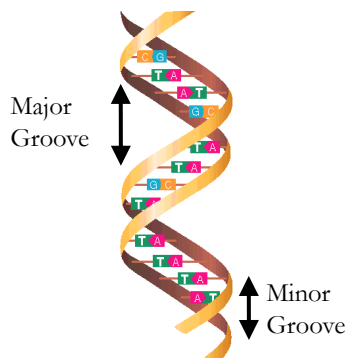
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Real-time PCR Chemistries: SYBR® Green I Dye

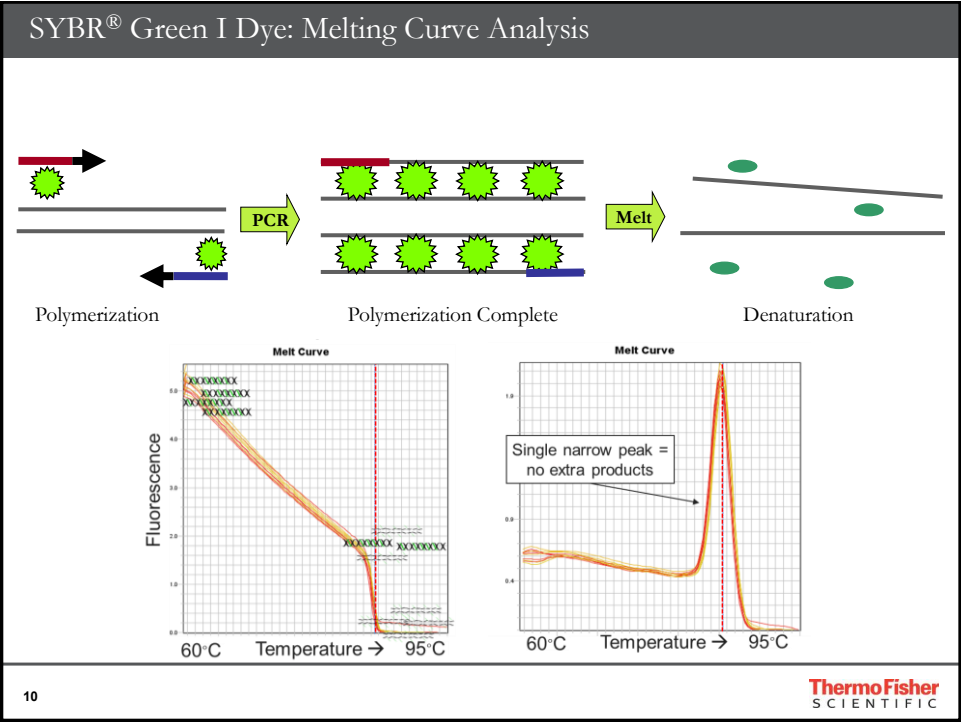
- A 'minor groove'-binding molecule specific to the minor groove of double-stranded DNA
- Fluoresces at an increased intensity when bound



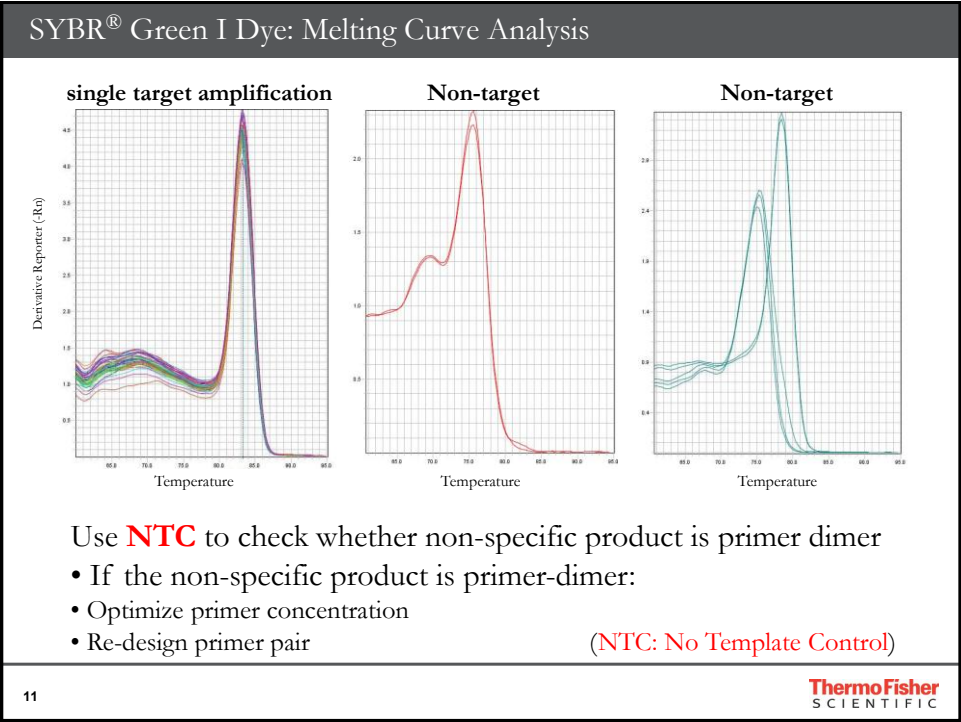
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TaqMan® Assay: Fluorogenic 5'-nuclease Assay

1. Polymerization

2. Strand displacement

3. Cleavage

4. Polymerization completed

R = Reporter (FAM, VIC, etc.)
Q = Quencher (NFQ/MGB, etc.)

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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)

(non-fluorescent quencher) NFQ

R AGGCCTTGAGAGATAT Q

MGB (minor groove binder)

R AGGCCTTGAGAGATAT Q

GCTACACAGTCCGGAAGTCTCTATAGCATCACAC

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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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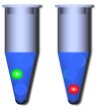
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Singleplex vs Multiplex

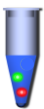
- Singleplex- one gene target detected per reaction

	1	2	3
A	Sample 1 Target 1	Sample 1 Target 1	Sample 1 Target 1
	Target 1	Target 1	Target 1
B	Sample 2 Target 1	Sample 2 Target 1	Sample 2 Target 1
	Target 1	Target 1	Target 1
C	Sample 1 Target 2	Sample 1 Target 2	Sample 1 Target 2
	Target 2	Target 2	Target 2
D	Sample 2 Target 2	Sample 2 Target 2	Sample 2 Target 2
	Target 2	Target 2	Target 2



- Multiplex -more than one gene target detected per reaction
- Advantages of Multiplexing include:
 - ✓ Increased throughput
 - ✓ Reduced reagent consumption
 - ✓ Reduced sample consumption
 - ✓ Improved precision

	1	2	3
A	Sample 1 Target 1 Target 2	Sample 1 Target 1 Target 2	Sample 1 Target 1 Target 2
	Target 1 Target 2	Target 1 Target 2	Target 1 Target 2
B	Sample 2 Target 1 Target 2	Sample 2 Target 1 Target 2	Sample 2 Target 1 Target 2
	Target 1 Target 2	Target 1 Target 2	Target 1 Target 2



- Most Real-Time PCR instruments are capable of 2, 3, 4, or even 5 plex reactions

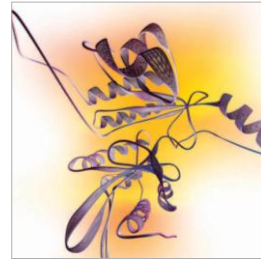
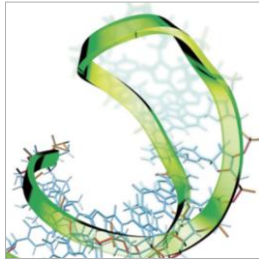
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Real-time PCR Application

- Absolute Quantitation vs. Relative Quantitation



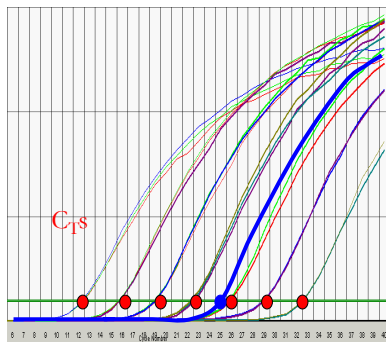
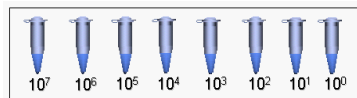
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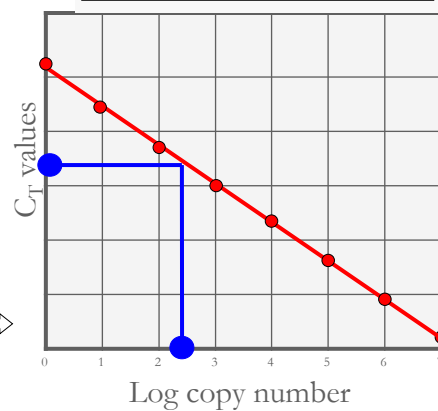
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絕對定量 (Absolute Quantitation)

- 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

RNA

Gene-specific primers

One-step buffer

PCR enzyme

RT enzyme

PCR amplification

Many RNA samples

Few genes of interest

Limited RNA amount (Intergenic and intragenic)

• 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

RNA (Oligo(dT) or random primers)

RT buffer

RT enzyme

cDNA

cDNA dilution

cDNA sample

PCR primers

PCR buffer and enzyme

PCR amplification

Few RNA samples

Many genes of interest

"Universal" RNA (Intergenic)

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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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Two-step Workflow: Real-time PCR Reactions

Reverse Transcription : SuperScript V Kit (Only 11 min)

Step	Action	Procedure Details												
1	Prepare cDNA digestion reaction mix (see table)	For each RT reaction or No-RT Control reaction, prepare a 10 µL cDNA digestion reaction mix in a RNase-free tube as per the following components: <table border="1"> <thead> <tr> <th>Component</th> <th>Volume</th> </tr> </thead> <tbody> <tr> <td>10x cDNAase Buffer</td> <td>1 µL</td> </tr> <tr> <td>cDNAase enzyme</td> <td>1 µL</td> </tr> <tr> <td>Template RNA (1 µg to 2.5 µg total RNA)</td> <td>varies</td> </tr> <tr> <td>Nuclease-free Water</td> <td>to 10 µL</td> </tr> </tbody> </table>	Component	Volume	10x cDNAase Buffer	1 µL	cDNAase enzyme	1 µL	Template RNA (1 µg to 2.5 µg total RNA)	varies	Nuclease-free Water	to 10 µL		
Component	Volume													
10x cDNAase Buffer	1 µL													
cDNAase enzyme	1 µL													
Template RNA (1 µg to 2.5 µg total RNA)	varies													
Nuclease-free Water	to 10 µL													
2	Digest cDNA	Seal mix and incubate at 37°C for 2 minutes. Briefly centrifuge the reaction and place on ice.												
3	Prepare RT and No RT Control reaction mixes (see table)	Add the following components to the tube containing the 10 µL reaction mix from step 2 on ice: <table border="1"> <thead> <tr> <th>Component</th> <th>RT reaction</th> <th>No-RT Control reaction</th> </tr> </thead> <tbody> <tr> <td>SuperScript[®] V RT[™] Master Mix</td> <td>4 µL</td> <td>—</td> </tr> <tr> <td>SuperScript[®] V RT[™] RT Control</td> <td>—</td> <td>4 µL</td> </tr> <tr> <td>Nuclease-free Water</td> <td>2 µL</td> <td>2 µL</td> </tr> </tbody> </table>	Component	RT reaction	No-RT Control reaction	SuperScript [®] V RT [™] Master Mix	4 µL	—	SuperScript [®] V RT [™] RT Control	—	4 µL	Nuclease-free Water	2 µL	2 µL
Component	RT reaction	No-RT Control reaction												
SuperScript [®] V RT [™] Master Mix	4 µL	—												
SuperScript [®] V RT [™] RT Control	—	4 µL												
Nuclease-free Water	2 µL	2 µL												
4	Anneal primers	Seal 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 -70°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 } 40 cycles
 60 °C, 1min }

Fast mode

PCR condition:

95 °C, 20 sec
 95 °C, 1 sec } 40 cycles
 60 °C, 20 sec }

SYBR Green:
 - Check Primer Concentration
 - Add Melt Curve Program

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Real-time PCR master mix

Two-step workflow

- **SYBR[®] Green Chemistry**
 - PowerTrack[™] SYBR Green Master Mix(PN A46012)
- **TaqMan[®] Chemistry**
 - TaqMan[®] Fast Advanced Master Mix(PN 4444557)

One-step workflow

- **SYBR[®] Green Chemistry**
 - Power SYBR[™] Green RNA-to-CT[™] 1-Step Kit (PN# 4391178)
- **TaqMan[®] Chemistry**
 - TaqPath[™] DuraPlex[™] 1-Step RT-qPCR Master Mix (PN# A58666)

<https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/real-time-pcr-reagents.html>

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Real-time PCR master mix selection tool

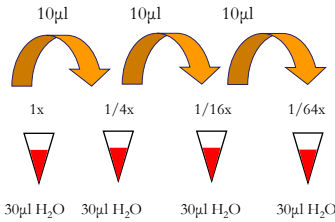
Application	Chemistry	Type
Gene expression	Probe (TaqMan)	qPCR
Genotyping	SYBR™ Green	1-step RT-qPCR
miRNA analysis		
Pre-amplification		
Virus detection		

Application	Detection chemistry	Type/format	Multiplexing	Mode/speed	Lab type	Instruments	Recommended
Gene expression	Probe (TaqMan)	qPCR	No	Fast	Any except clinical	Any	TaqMan Fast Advanced Master Mix
Gene expression	Probe (TaqMan)	qPCR	No	Fast or standard	Clinical	Any	TaqPath qPCR Master Mix, CG
Gene expression	Probe (TaqMan)	qPCR	Yes	Fast or standard	Any except clinical	See instruments list	TaqMan Fast Advanced Master Mix
Gene expression	Probe (TaqMan)		Yes	Fast or standard	Any except clinical	See instruments list	TaqMan Multiplex Master Mix
Gene expression	SYBR™ Green	qPCR	No	Fast or standard	Any	Any	PowerUp SYBR Green Master Mix
Gene expression	Probe (TaqMan)	1-step RT-qPCR	No	Fast or standard	Any except clinical	Any	TaqMan Fast Virus 1-Step Master Mixes

<https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/real-time-pcr-reagents.html>

相對定量 (Relative Quantitation): PCR Efficiency Validation

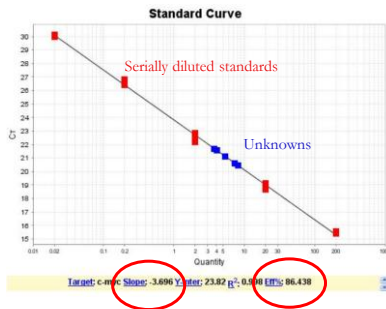
- 2µg RNA in 20µl RT
- Gene name: *C-Myc* and *GAPDH*
- cDNA 4-fold serial dilution: 10µl cDNA + 30µl H₂O
 - 1. 1x
 - 2. 1/4 x
 - 3. 1/16 x
 - 4. 1/64 x
 - 5. NTC (duplicate for each sample)
- 每個濃度點各做二重複



- 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

Assay Efficiency (E) – determined by serial dilution

- A serial dilution can generate a standard curve similar to below
 - Software will determine the slope of the standard curve using the following equation: $Y = mx + b$
- Where Y = Quantity, m = slope, x = concentration and b = y-intercept



The slope of a standard curve can be used to calculate reaction efficiency using the general equation:

$$(E) \text{ Efficiency} = 10^{(-1/m)} - 1$$

The slope of this standard curve (m) is -3.696

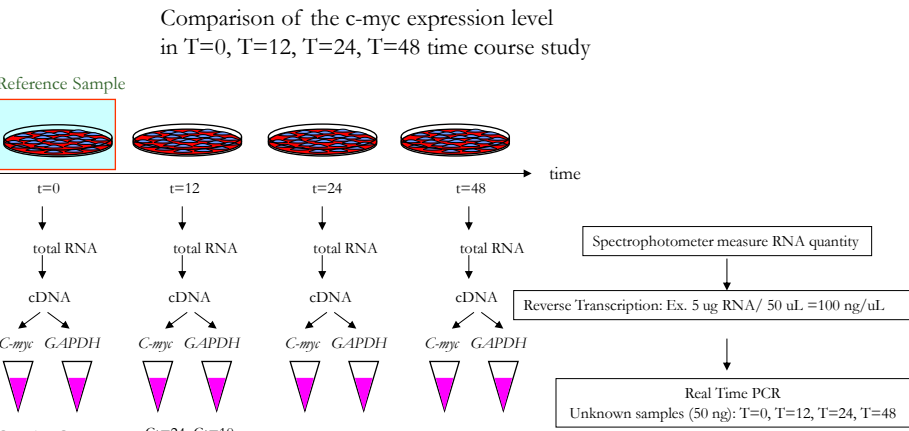
$$\text{So, Assay Efficiency} = 10^{(-1/m)} - 1 = 10^{(-1/-3.696)} - 1 = 86.438\%$$

For a 10-fold dilution series, a slope of -3.32 = 100% reaction efficiency

Commonly accepted standard curve slopes are between -3.58 and -3.1 (equivalent to 90 – 110% assay efficiency)

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

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



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).

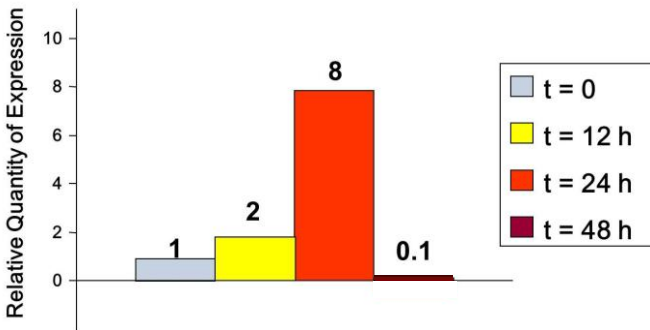
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相對定量 (Relative Quantitation): Comparative Ct Method ($\Delta\Delta C_t$)

	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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Data Analysis – 4 check points

- 完整的擴增曲線
 - Baseline
 - Exponential phase
 - Linear phase
 - Plateau
- 每個target gene的Threshold設置在各自正確的位置
 - Exponential phase
- Melt curve plot (for SYBR Green only)
 - Single peak
 - no primer-dimer
- 技術性重複(Technical Replicate)
 - 三重複為佳
 - 排除(Omit)異常樣本

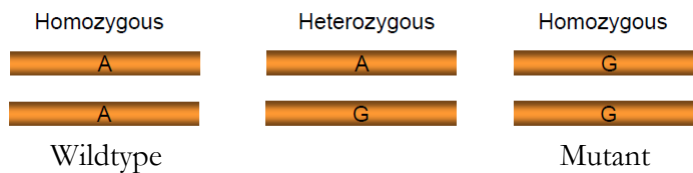
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Single Nucleotide Polymorphisms (SNPs)

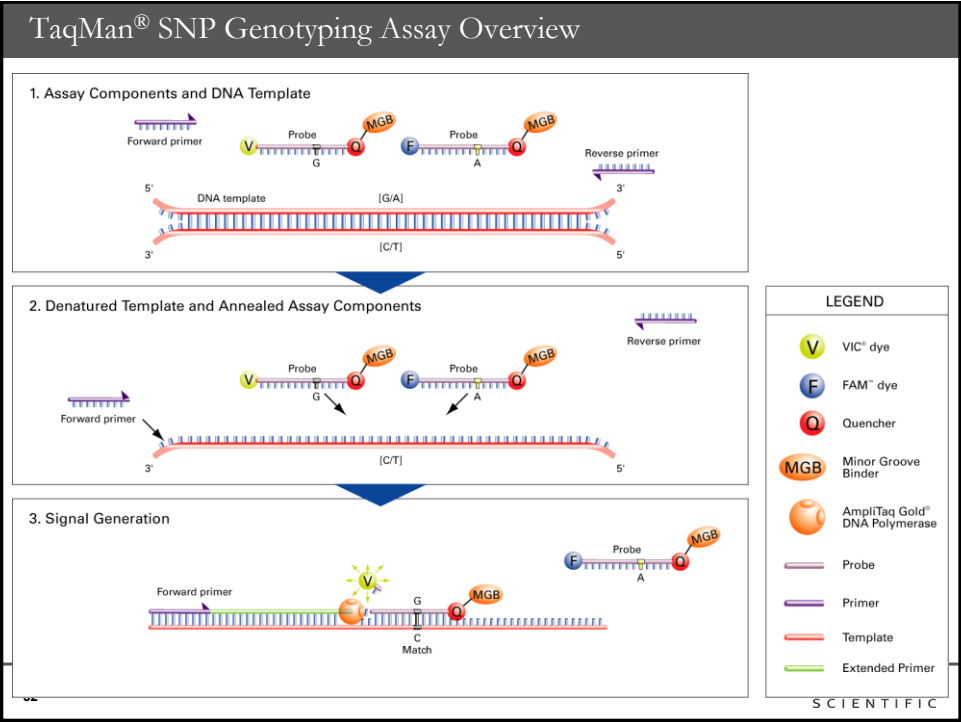
- SNPs are single base changes in genomic DNA
- SNPs may cause diseases, alter metabolisms or act as markers
- SNPs in diploid loci commonly have 3 genotypes..



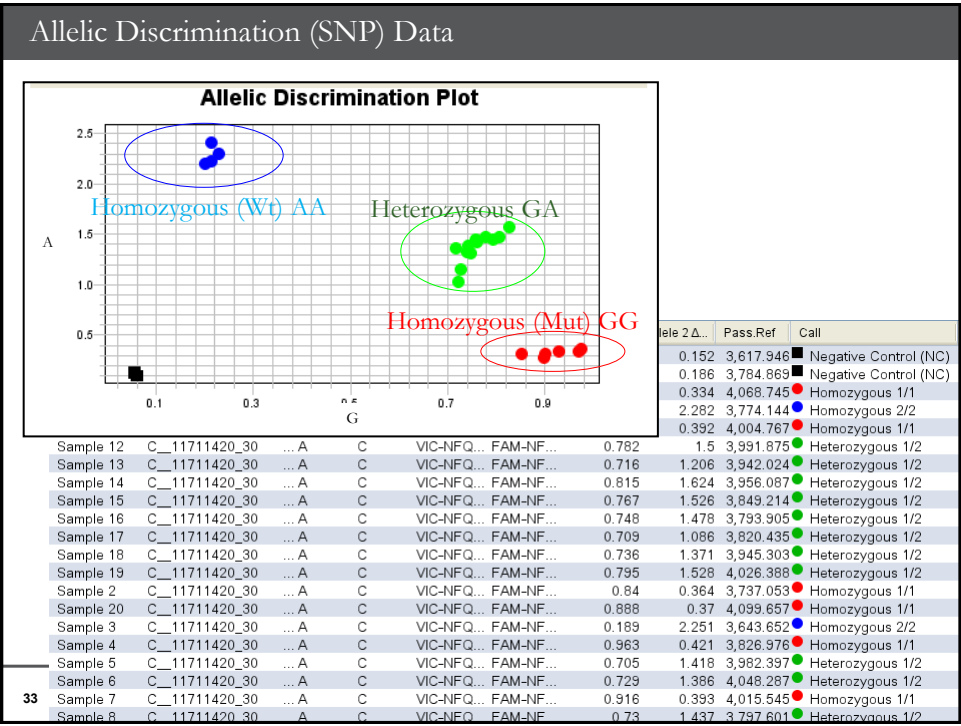
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Assay Design

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

☐ H. sapiens	☐ A. thaliana
☐ R. norvegicus	☐ D. melanogaster
☐ M. musculus	☐ C. elegans
☐ M. mulatta (Rhesus)	☐ C. familiaris (Canine)
☐ D. rerio (Zebrafish)	☐ B. taurus (Cow)
☐ G. gallus (Chicken)	☐ O. cuniculus (Rabbit)
☐ S. scrofa (Pig)	☐ E. caballus (Horse)
☐ O. sativa (Rice)	☐ 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



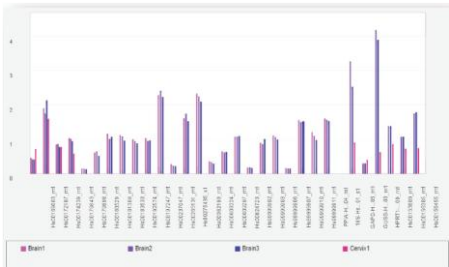
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Preconfigured TaqMan Array Plates – Partial list

- Antioxidant mechanisms
- Apoptosis
- Drug transporters
- Endogenous controls
- Extracellular matrix and adhesion molecules
- Inflammation
- Lipid-regulated genes
- Molecular mechanisms of cancer
- Phagocytosis of microbes
- Rheumatoid arthritis pathogenesis
- Ubiquitin-proteasome proteolysis
- WNT pathway

★Using real-time PCR to know your gene expression profile!



Pre-Designed TaqMan® Assay

Search and order all TaqMan assays

TaqMan Assays

Gene Expression

Microbe & ABR Detection

SNP Genotyping

Copy Number

miRNA

Mutation Detection

Search TaqMan® Assays and Arrays

Search

Applications

assays for qPCR applications, including gene expression, miRNA analysis, genetic variation, and mutation detection

qPCR assays and applications

We offer over 20 million pre-designed assays for qPCR applications, including gene expression, miRNA analysis, genetic variation, and mutation detection

Gene expression analysis

• Assays help detect the highest number of transcript variants possible

• 2.8 million assays, >32 species

• Pre-designed gene expression assays

• Custom gene expression assays

Explore: Gene expression analysis

MicroRNA analysis

• Detect targets with as few as 60 copies of input microRNA

• Compatible with tissue & biofluids including serum & plasma

• 30K assays

• Pre-designed microRNA assays

• Custom microRNA assays

Explore: MicroRNA analysis

Genetic variation analysis

• Pre-designed assays for SNP genotyping & drug metabolism (DME): 17 million assays

• 1.8 million copy number variation (CNV) assays

• Pre-designed SNP genotyping assays

• Custom SNP genotyping assays

Explore: Genetic variation analysis

Mutation detection analysis

• Enhance your specificity, sensitivity, and workflow efficiency with TaqMan mutation detection assays: 900 assays

• Pre-designed mutation detection assays

Explore: Mutation detection analysis

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

Pre-Designed TaqMan[®] Assay – Gene expression

Search and order TaqMan gene expression assays

Learn more: [How to search TaqMan gene expression assays using search tool](#)

TaqMan Assays

Gene Expression

Search TaqMan[®] Assays and Arrays

Try Advanced Search

Search

Search within TaqMan[™] Assays and Arrays

gapdh

Application

Gene Expression (94)

Search

Assays (94)

Arrays (2,893)

94 results for "gapdh"

Species

Attributes

Genes

Assay type

Remove cross-reactivity

Assay ID	Gene	Species	Transcripts	Amplicon	Dye / Size	Price (TWD)
Recommended						
Hg05064520_gH	Gapdh	Naked mole-rat	2	59	FAM-MGB S: 360 rxns	Price not available Catalog # 4361372 Made to order

Details

Related products

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Pre-Designed TaqMan[®] Assay - SNP

Search and order all TaqMan assays

Learn more: [How to search TaqMan assays using search tool](#)

TaqMan Assays

SNP Genotyping

RS6542095

Try Advanced Search

Search

1 results for "RS6542095"

Species

Attributes

Genes

SNP type

Assay type

SNP ID	Gene	Assay ID	Allele [VIC/FAM]	Size
Functionally tested				
rs6542095		C__11725602_10	C/T	S: 300 rxns

Details

Allele frequency

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Primer Design: Bioinformatics Evaluations

- **Bioinformatics**

- BLAST tool: Sequence specificity (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>)
- SNPMasker: N masked SNPs (<http://bioinfo.ebc.ee/snpmasker/>)
- RepeatMasker: N masked repeat sequence (<http://www.repeatmasker.org>)

- Custom TaqMan® Assay Design Tool

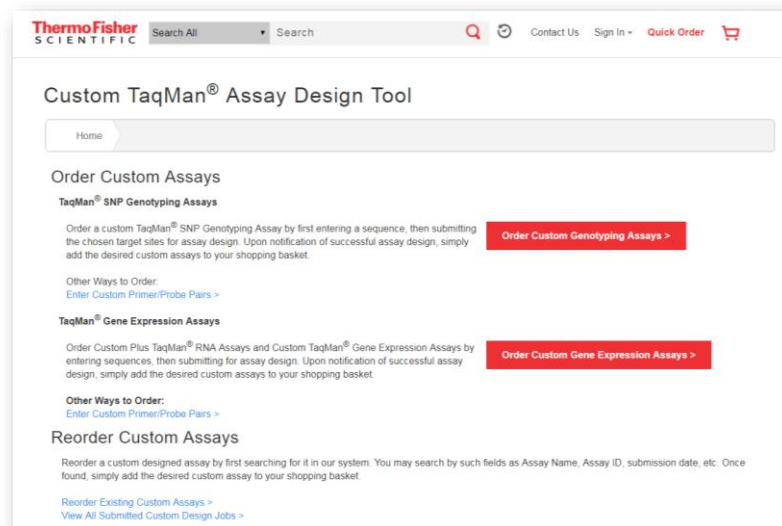
(<https://www.thermofisher.com/order/custom-genomic-products/tools/cadt/>)

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Custom TaqMan® Assay Design Tool



<https://www.thermofisher.com/order/custom-genomic-products/tools/cadt/>

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Assay Design Hub

The one-stop shop for all your custom assay design needs

Assay Design Hub

The one-stop shop for all your custom assay design needs

Tools

Services

Leverage our proprietary Bioinformatics algorithms to design your own customized assay. Input your target sequence(s) below into our self-service Design Tools and get an assay design right away.

Choose the tool that suits your request

Gene expression
(2 primers + 1 probe)

Genotyping
(2 primers + 2 probes)

Custom Probes
(sequencing, ordnance, probes)

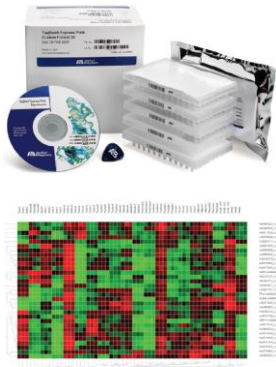
Small RNA (Classic)
(2 primers + 1 probe + RT primer)

<https://www.thermofisher.com/tw/zt/home/life-science/pcr/real-time-pcr/real-time-pcr-assays/assay-design.html#menu1to>

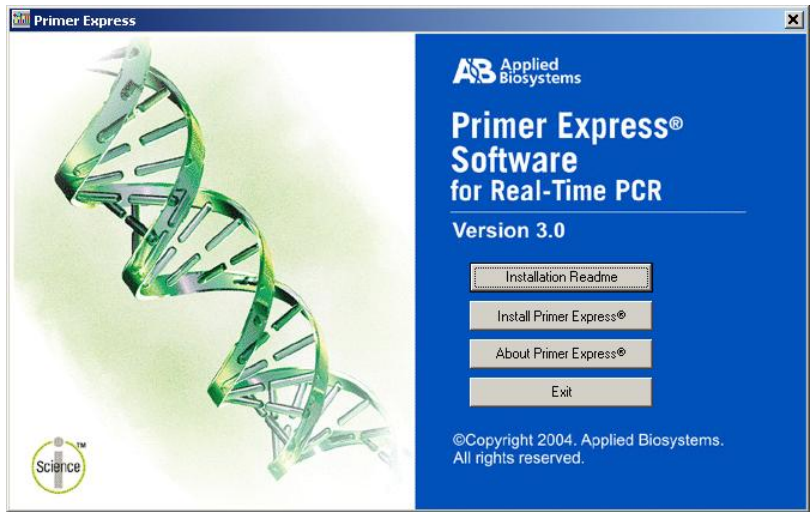
Configure Custom TaqMan® Array Plate

Ready-to-use plates with preloaded TaqMan Assays!

	Preconfigured TaqMan Array Plates	Flexible-content TaqMan Array Plates	Custom TaqMan Array Plates	Custom formatting service
Definition	Fixed, predefined gene panels	Modifiable predefined gene panels	Configurable predesigned assays	Client-requested designs

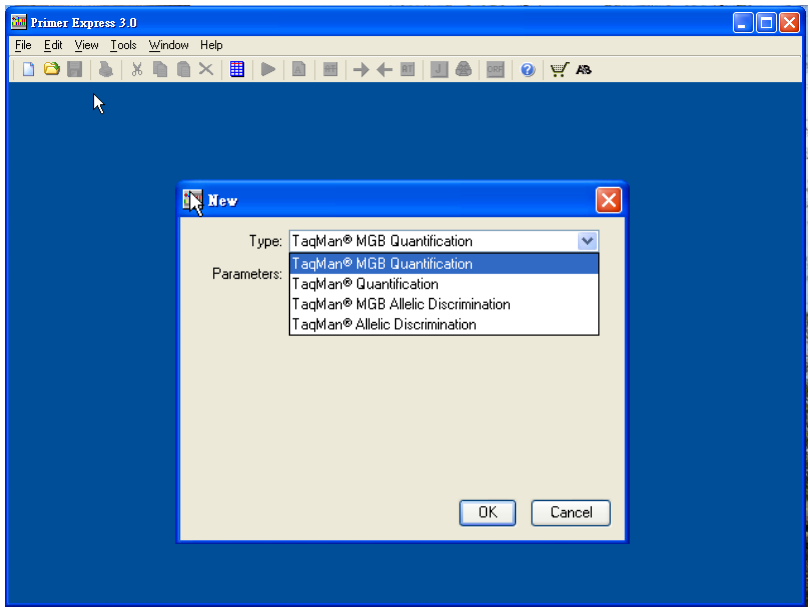


- **Convenient setup**
 - dried TaqMan Assays in a 96- or 384-well plate; just add master mix and your cDNA sample
- **Flexible format**
 - choose from preconfigured to customizable TaqMan Array Plates in fast or standard formats
- **Easy data analysis**
 - Applied Biosystems™ analysis modules help provide rapid cloud-based data interpretation



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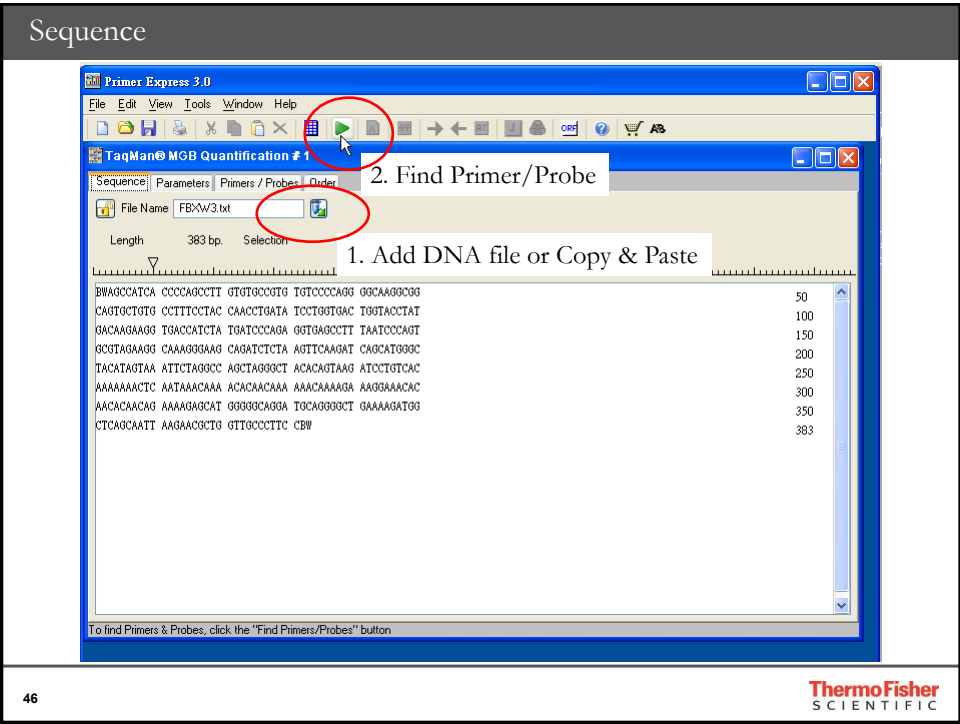
44



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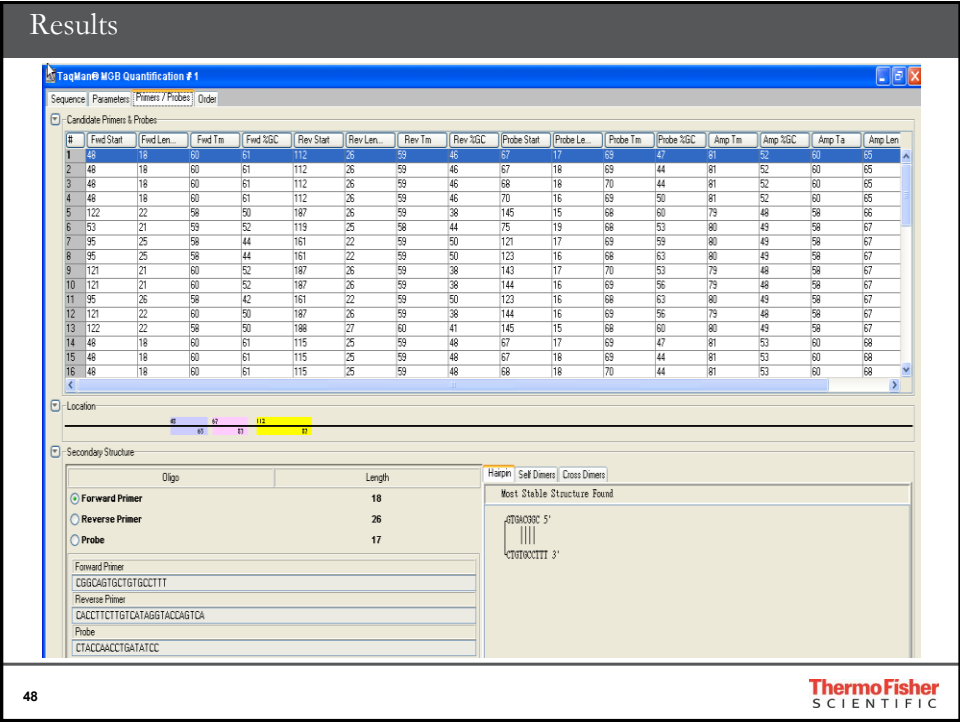
Sequence



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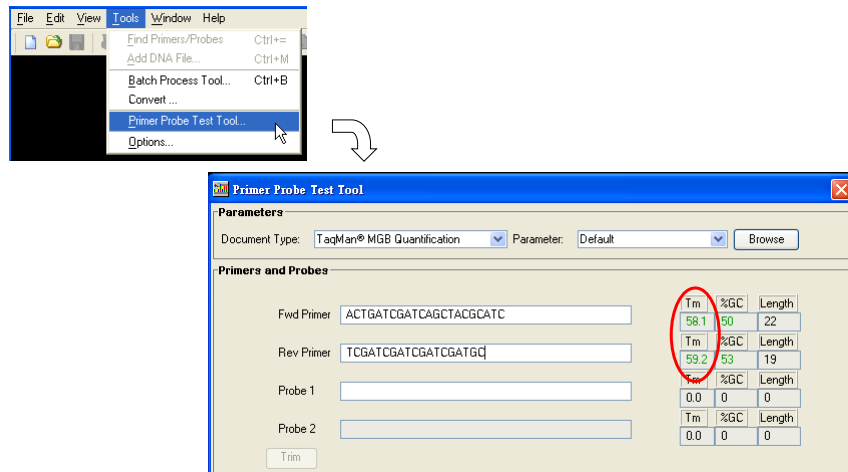
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Results



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Check Tm of Primers



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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 QuantStudio™ 1 Real-Time PCR System

The world leader in serving science

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QuantStudio™ 1 Real-Time PCR Systems: Front

8.5 inch touch screen
(stand-alone capabilities, PIN-protected user accounts, and dye calibration/RNaseP functionality)

USB outlet
for template upload and data download

Manual pull drawer
(controlled by touchscreen)



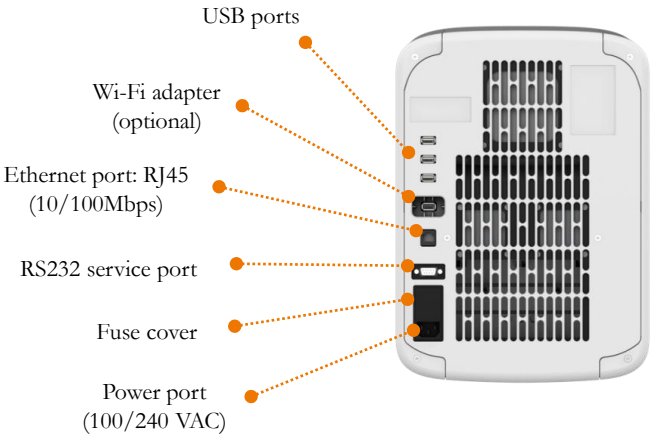
Power button (back)

96-well **0.2 mL** isothermal block

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QuantStudio™ 1 Real-Time PCR Systems: Rear



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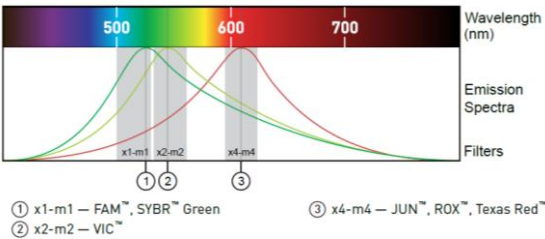
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Multiplexing Capabilities

- White LED for long lasting performance
- 3-color instrument
 - FAM™/SYBR® Green dyes
 - VIC®/JOE™ dyes
 - ROX™ dye
- Factory calibrated

Peak filter	Color	Filter wavelength (nm) ^[1]		Factory-calibrated dyes	Example custom dyes
		Excitation	Emission		
x1-m1	Blue	470 ± 15	520 ± 15	FAM™, SYBR™ Green	SYT09
x2-m2	Green	520 ± 10	558 ± 12	VIC™	JOE™, HEX™, TET™ ^[2]
x4-m4	Orange	580 ± 10	623 ± 14	ROX™, JUN™	Texas Red™

^[1] The central wavelengths are the optimized wavelengths.
^[2] The HEX™ and TET™ dyes from Thermo Fisher Scientific fall within the emission wavelength range of the system, therefore they can be added and adapted for use on the instrument.



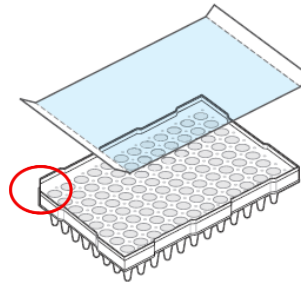
55

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QuantStudio™ 1 Real-Time PCR System: Consumables

- 様品量多時
 - MicroAmp® 96-Well Reaction Plate (0.2ml) - 10 plates (P/N N8010560)
 - MicroAmp® Optical Adhesive Film - 25 films (P/N 4311971)
- 様品量少時
 - MicroAmp® 8-Tube Strip (0.2ml) - 125 strips (P/N 4316567)
 - MicroAmp® Optical 8-Cap Strip - 300 strips (P/N 4323032)



★ Load at least 16 tubes with tray

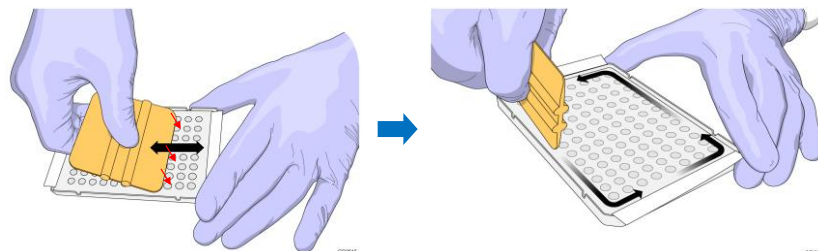
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Sealing the Plate

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

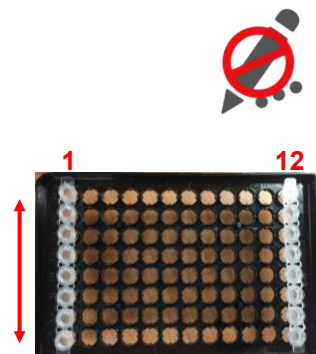
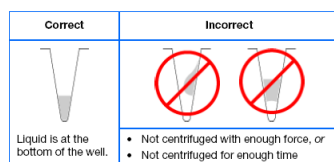
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QuantStudio™ 1 Real-Time PCR System: Operation Notes

- 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

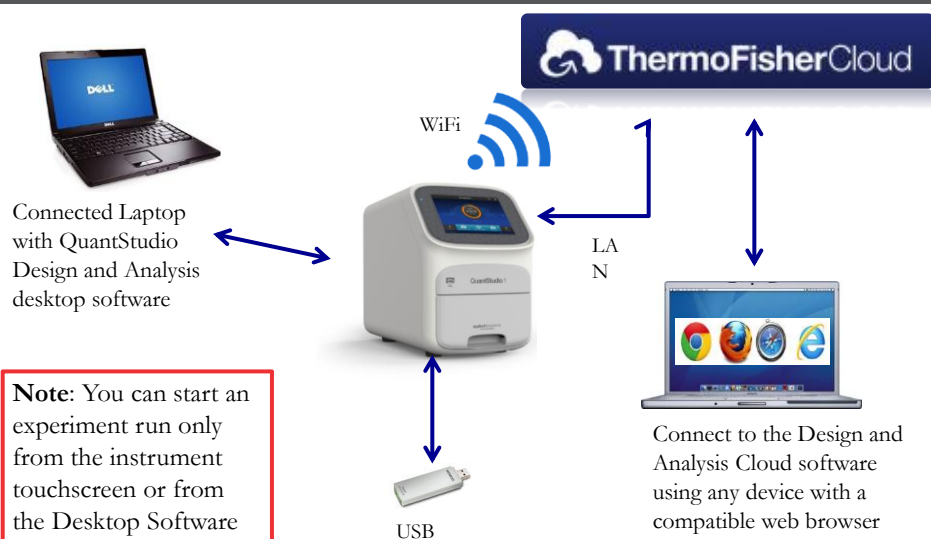


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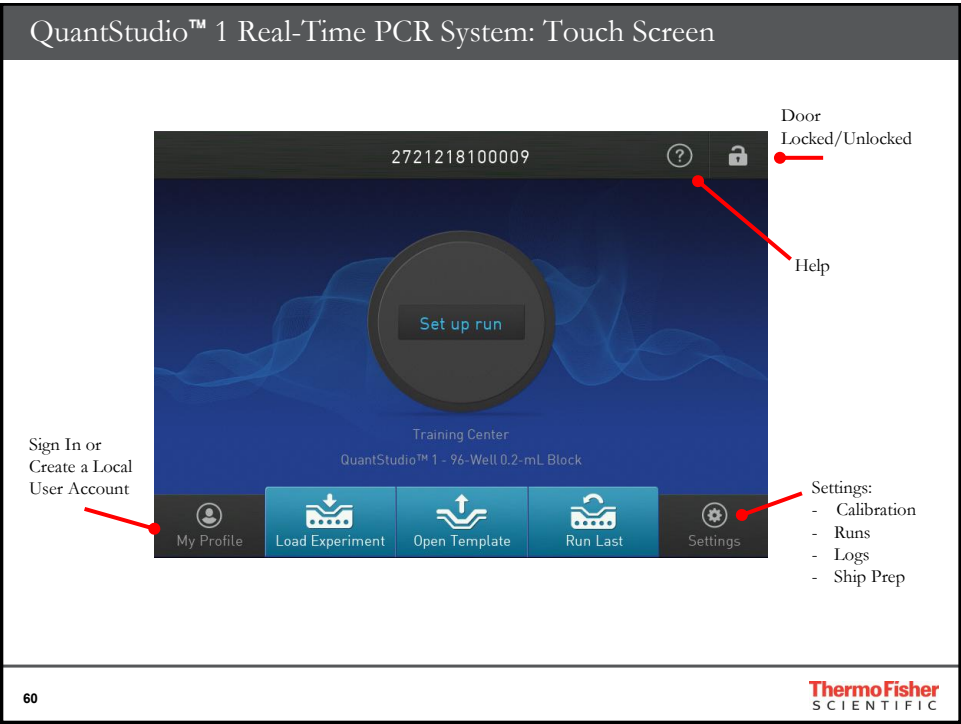
Stand-alone, Desktop, or Online



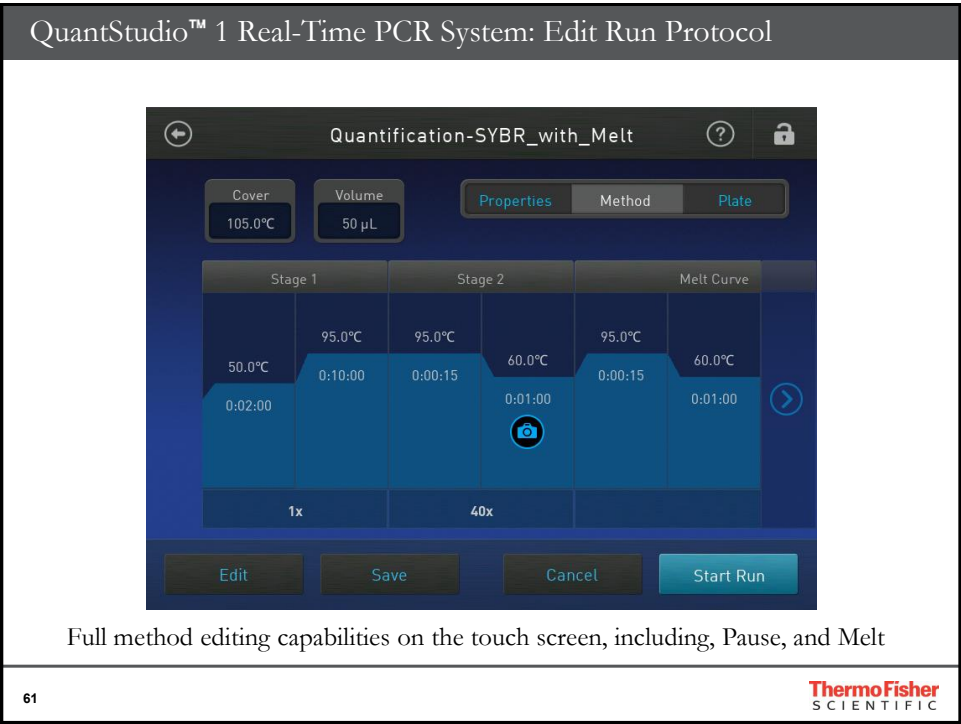
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Monitor Progress During the Run

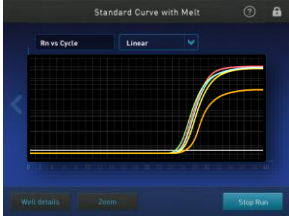
Time Remaining

Thermal Protocol Status

Live Amplification Curves







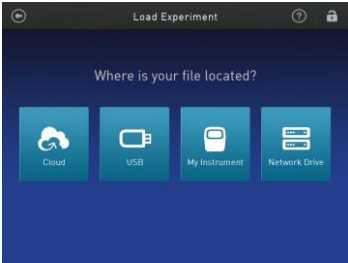
62

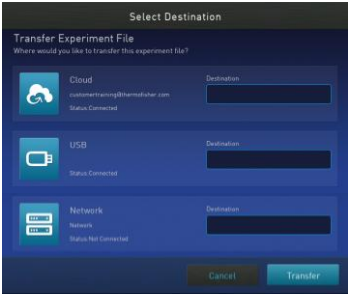
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Data Transfer

- Ability to transfer run data from/to a ‘Network Location’





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QuantStudio™ Design and Analysis Software

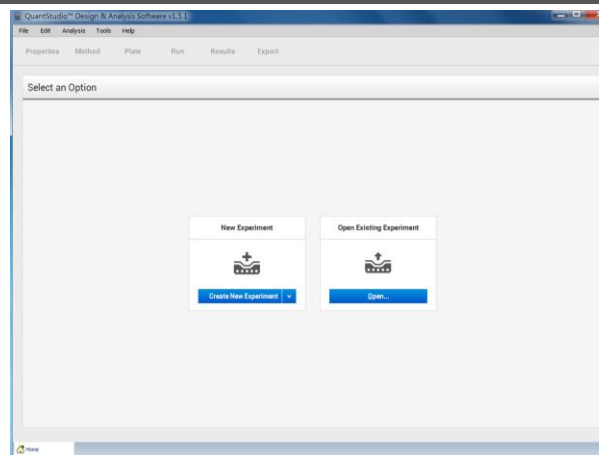
- QuantStudio™ Design and Analysis Software supports a variety of analysis methods, including:
 - Absolute Quantitation
 - Standard Curve
 - Relative Quantitation
 - Relative Standard Curve
 - Comparative CT ($\Delta\Delta$ CT)
 - Melt curve analysis
 - Genotyping (including real-time amplification)
- 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

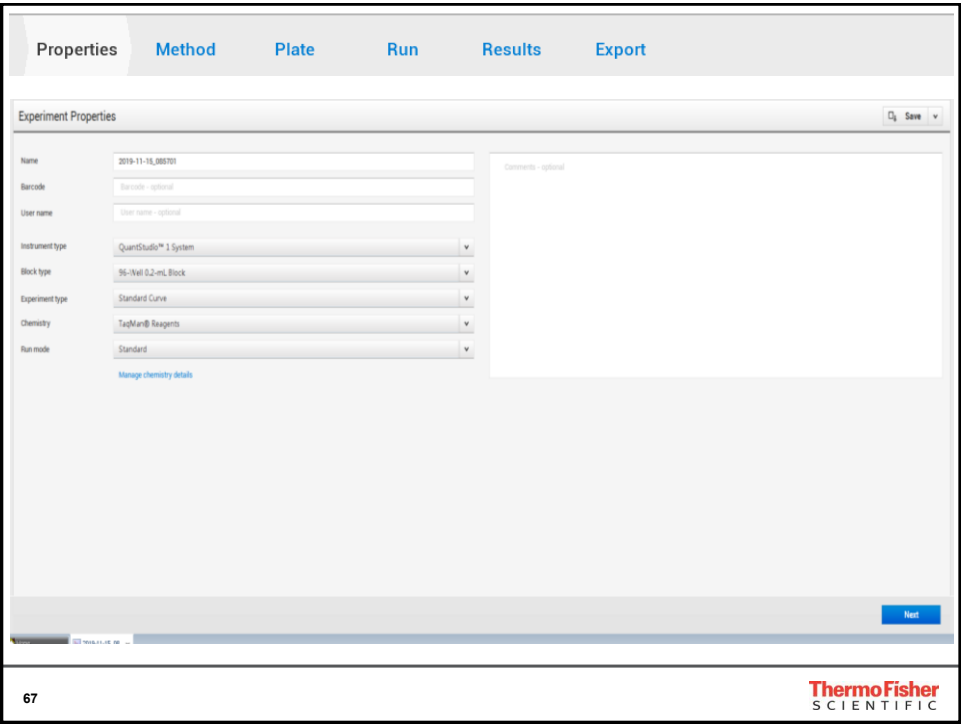


- Similar look and feel as online software
- <https://www.thermofisher.com/tw/en/home/global/forms/life-science/quantstudio-software-download.html>

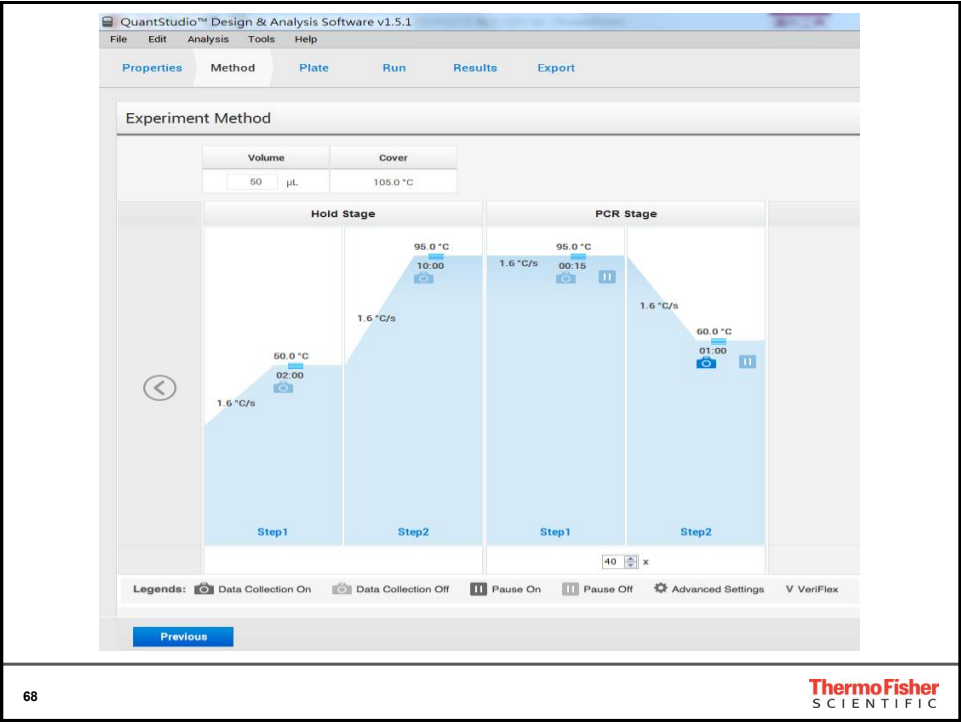
66

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PropertiesMethodPlateRunResultsExport

Assign Targets and Samples

Quick SetupAdvanced Setup

Well Attributes

Sample

New Sample

Target

New Target

Well Comments

Well Comments

Plate Attributes

Select well and type sample names

View

	1	2	3
A			
B			
C			
D			
E			
F			
G			
H			

Wells: U 0 S 0 N 0

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PropertiesMethodPlateRunResultsExport

Assign Targets and Samples

Quick SetupAdvanced Setup

Targets

Name

Reporter

Quencher

Comments

Task

Quantity

Target 1

FAM

NFQ-MGB

Samples

Sample Name

Comments

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

PropertiesMethodPlateRunResultsExport

Assign Targets and Samples

Quick SetupAdvanced Setup

Well Attributes

Sample

New Sample

Target

New Target

Well Comments

Well Comments

Plate Attributes

ActionSave

Select Sample

Select Target

Select Task

Define and Set Up Standards

View

12345678

A

B

C

D

E

F

G

H

Wells: 1100000096 Empty

PreviousNext

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Define and Set Up Standards

Select a target

Model: SingleplexSelect 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

Select and arrange wells for the standards

Arrange standards in: ColumnsRows

Use Wells: Automatically Select Wells for MeLet Me Select Wells

123456789101112

A

B

C

D

E

15 Required Wells / 15 Selected Wells
A1,A2,A3,A4,A5,A6,A7,A8,A9,A10,A11,A12,B1,B2,B3

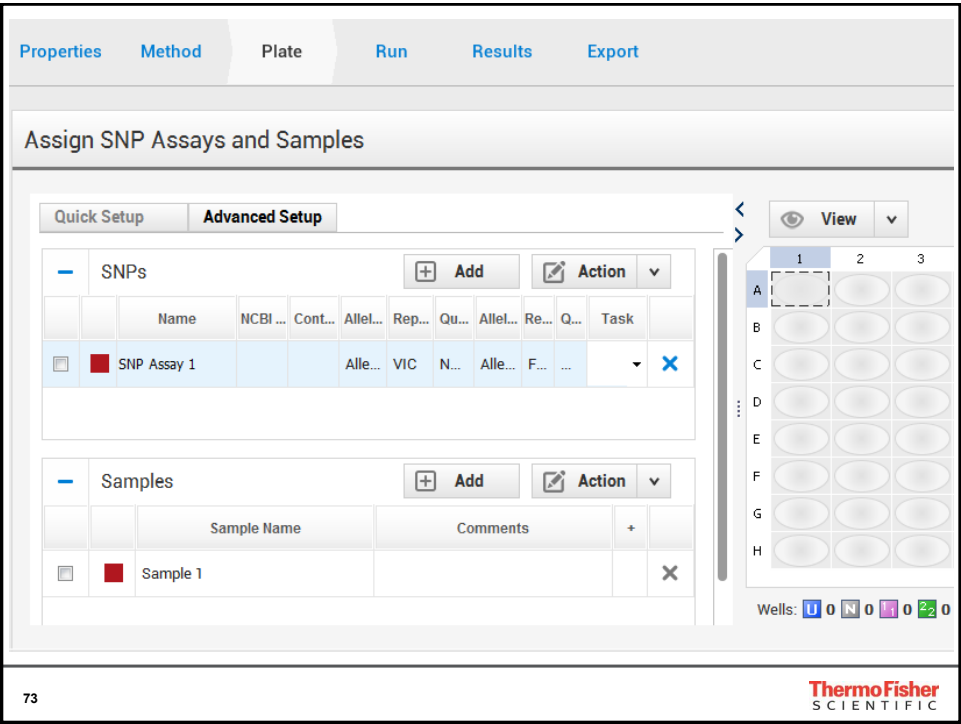
ApplyResetClose

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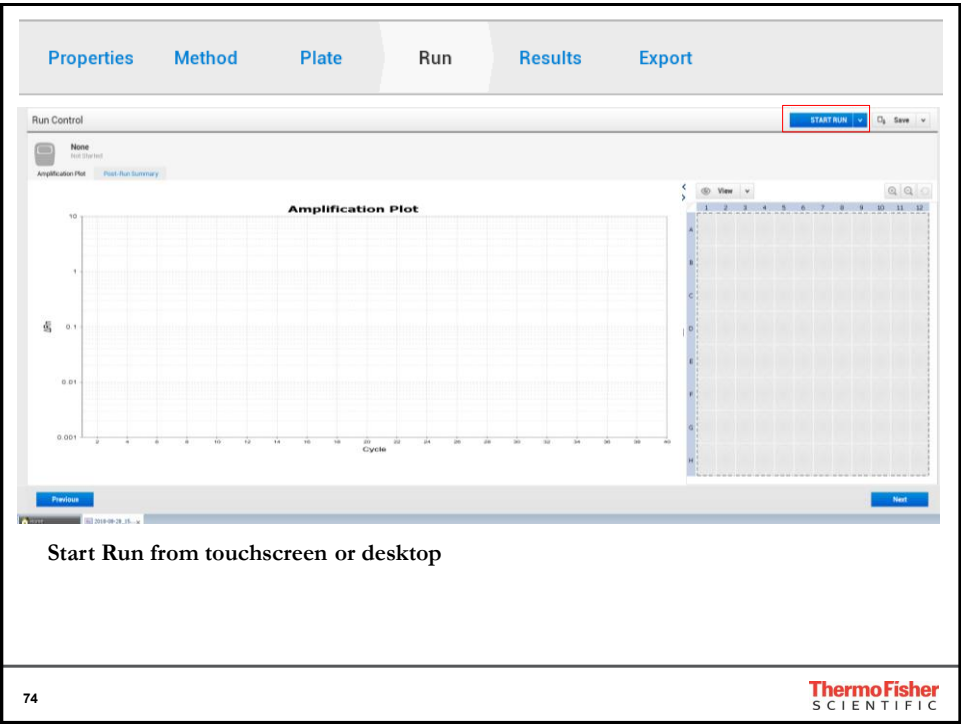
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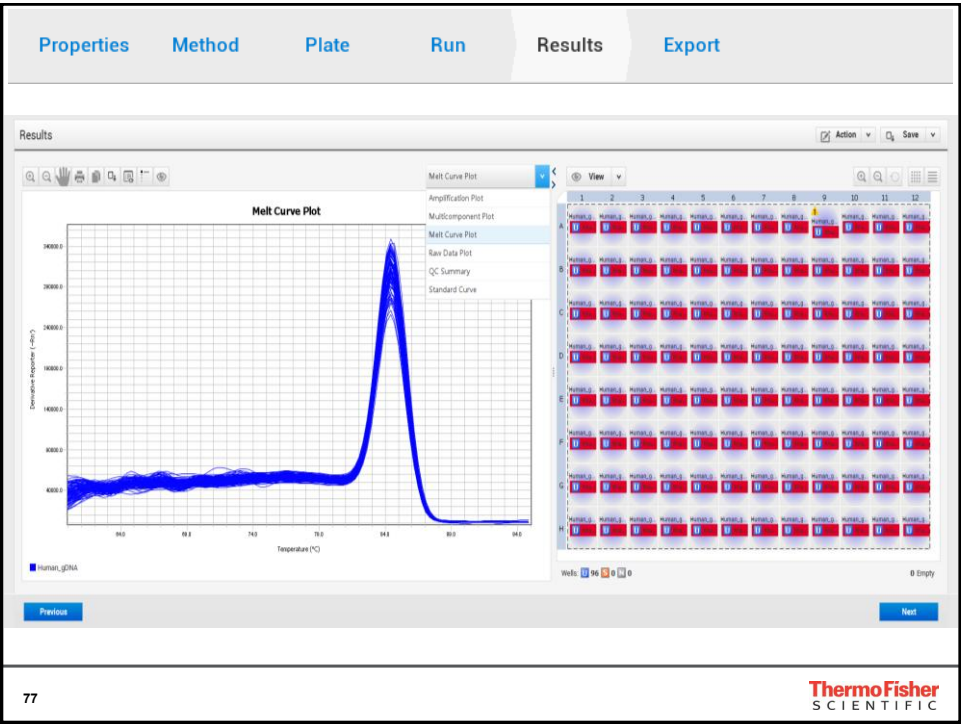
34



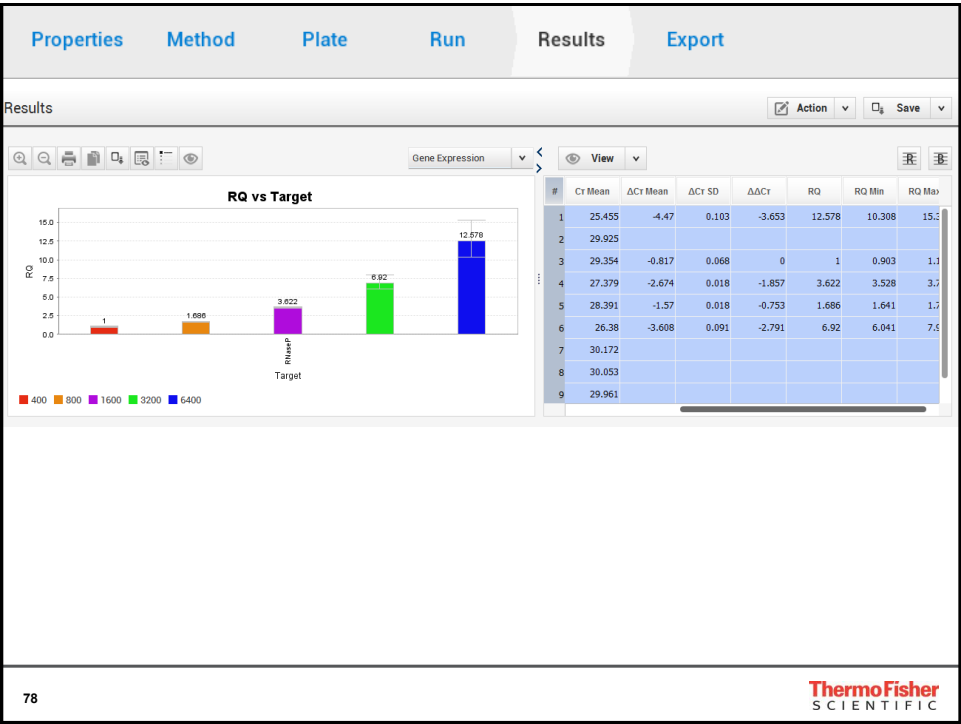
73



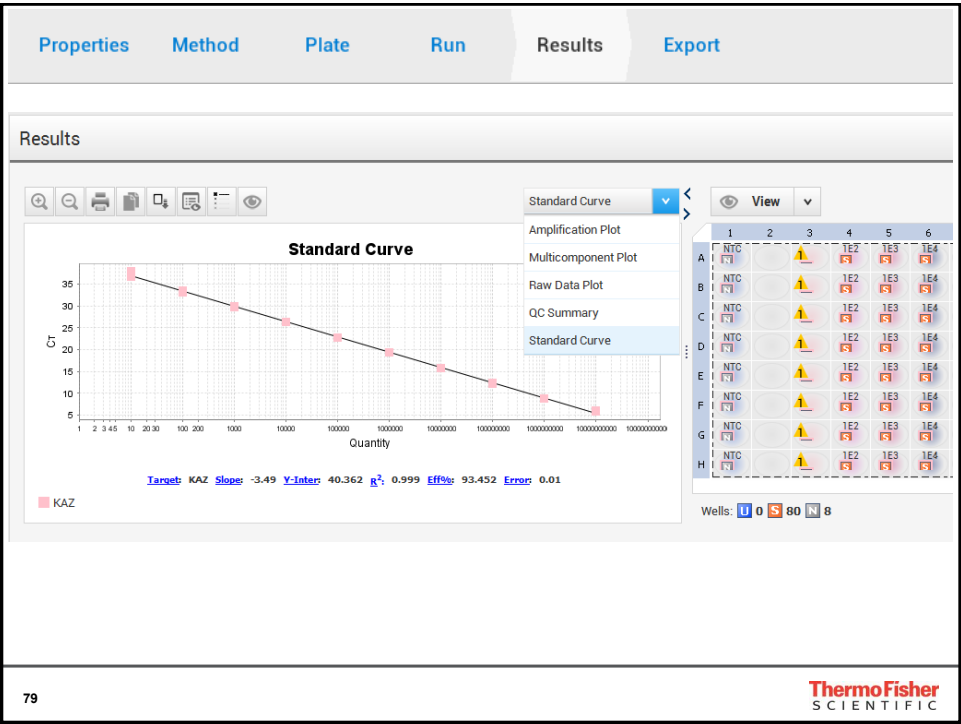
74



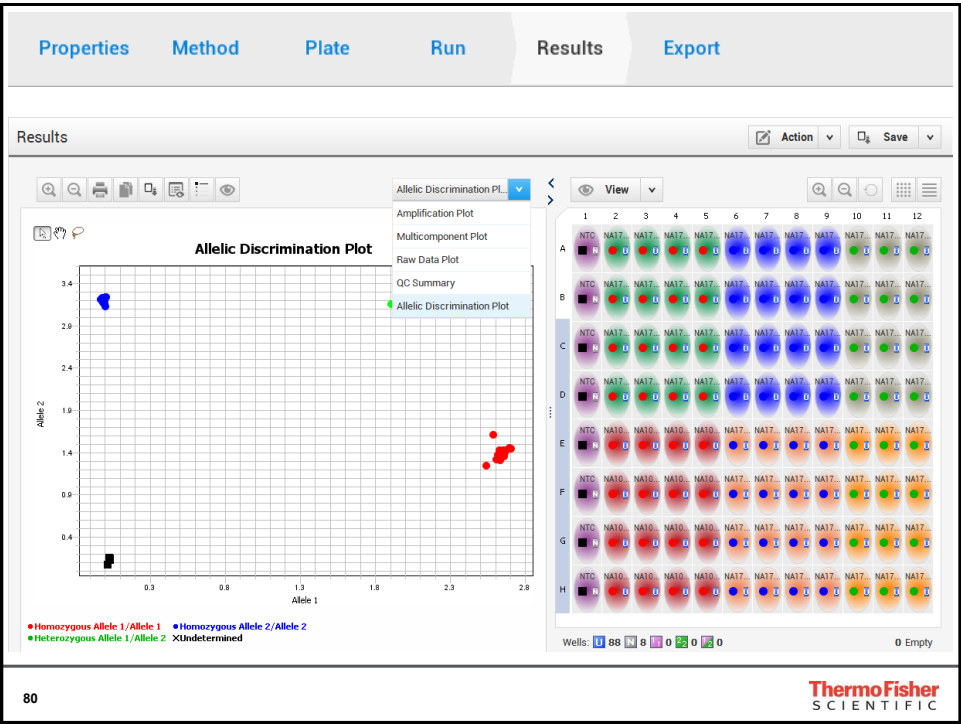
77



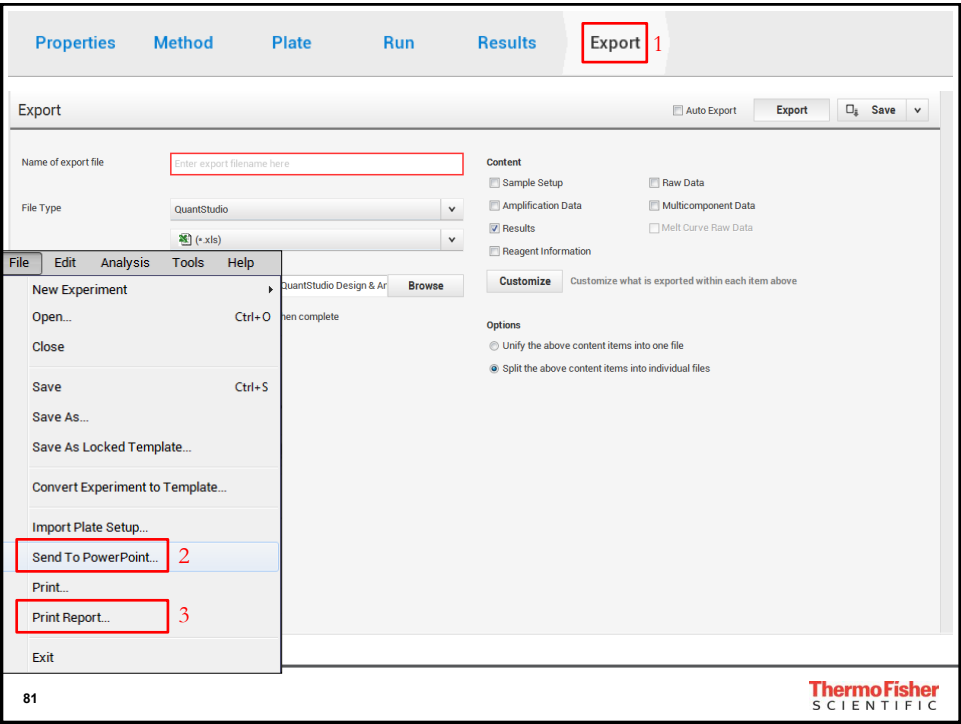
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Diagram illustrating the ThermoFisher Cloud capabilities across a world map:

- Monitor global outbreaks
- Access data anywhere
- Centralize data with localized projects
- Share with labs in remote areas
- Upload new SOPs and protocols

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Thermo Fisher Cloud: Dashboard

The front page of Thermo Fisher

ThermoFisher SCIENTIFIC Search All Search Contact Us Sign In Quick Order

<https://apps.thermofisher.com/apps/spa/#/dashboard>

Home Powered by Thermo Fisher Cloud

Create projects, upload files, monitor your instruments, see available apps

Recent projects and files Tutorials View my files

Welcome to your dashboard, Nai-Chen
What do you want to do?

Upload files (You can drag and drop)

Create a new project

Connect an instrument

My Apps

All Apps

- Run Reporter
- PCR Designer
- Bright Image
- Design and Analysis Application
- Genotyping
- Presence/Absence Analysis
- Relative Quantification
- Standard Curve
- Next-generation Confirmation
- Quality Check

Quick access to Files, Applications, and Instruments

Upload files and create new projects

Check the status of connected instruments

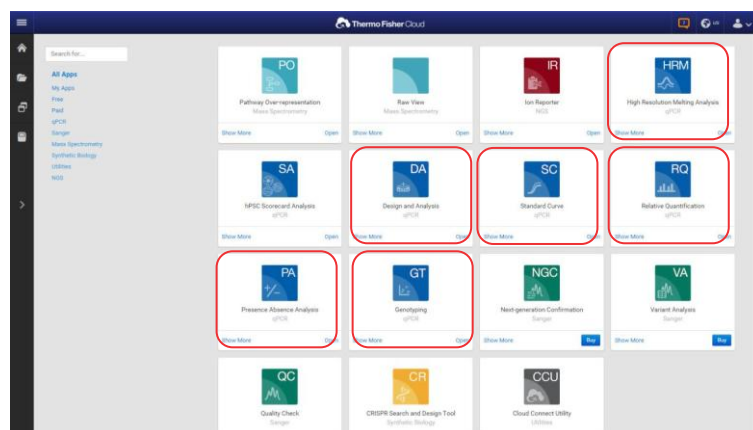
Manage Profile
Switch to Region
Get Notifications

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Applied Biosystems qPCR Analysis Modules on Thermo Fisher Cloud



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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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

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

- QuantStudio™ 3 and QuantStudio™ 5 Real-Time PCR Systems Support

Protein Electrophoresis and Western Blotting Support

Real-Time PCR and Digital PCR Applications Support Center

How-To and Educational Videos
How-To and

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™ 3 and 5 Real-Time PCR Systems Support

View articles and troubleshooting

StepOne™ and StepOnePlus™ 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.

7900HT Fast Real-Time PCR System Support

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.

ViiA™ 7 Real-Time PCR System Support

Find useful resources and

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.

QuantStudio™ 3D Digital PCR System Support

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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

Applications

One resource for all 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.

TaqMan™ Protein Assay Support

Find information and help about analyzing protein-protein interactions through TaqMan™ assays.

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 assays and analyzing your data.

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

125 Real-Time PCR and Digital PCR

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125

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感謝您的聆聽!

針對不曾使用過 Applied Biosystems qPCR master mix 的用戶，我們邀請您免費申請樣本試用。完成樣品評估後，上傳結果將獲得一張免費飲品兌換券。

https://www.thermofisher.com/qPCR-SwitchSip

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Complete your sample evaluation and get a Free drink voucher!

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Thank You!

- 技術服務E-mail:
support.tw@thermofisher.com
- 訂貨及維修服務專線: 0800-251-326 #3



您的推薦是對我們最大的鼓勵！

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