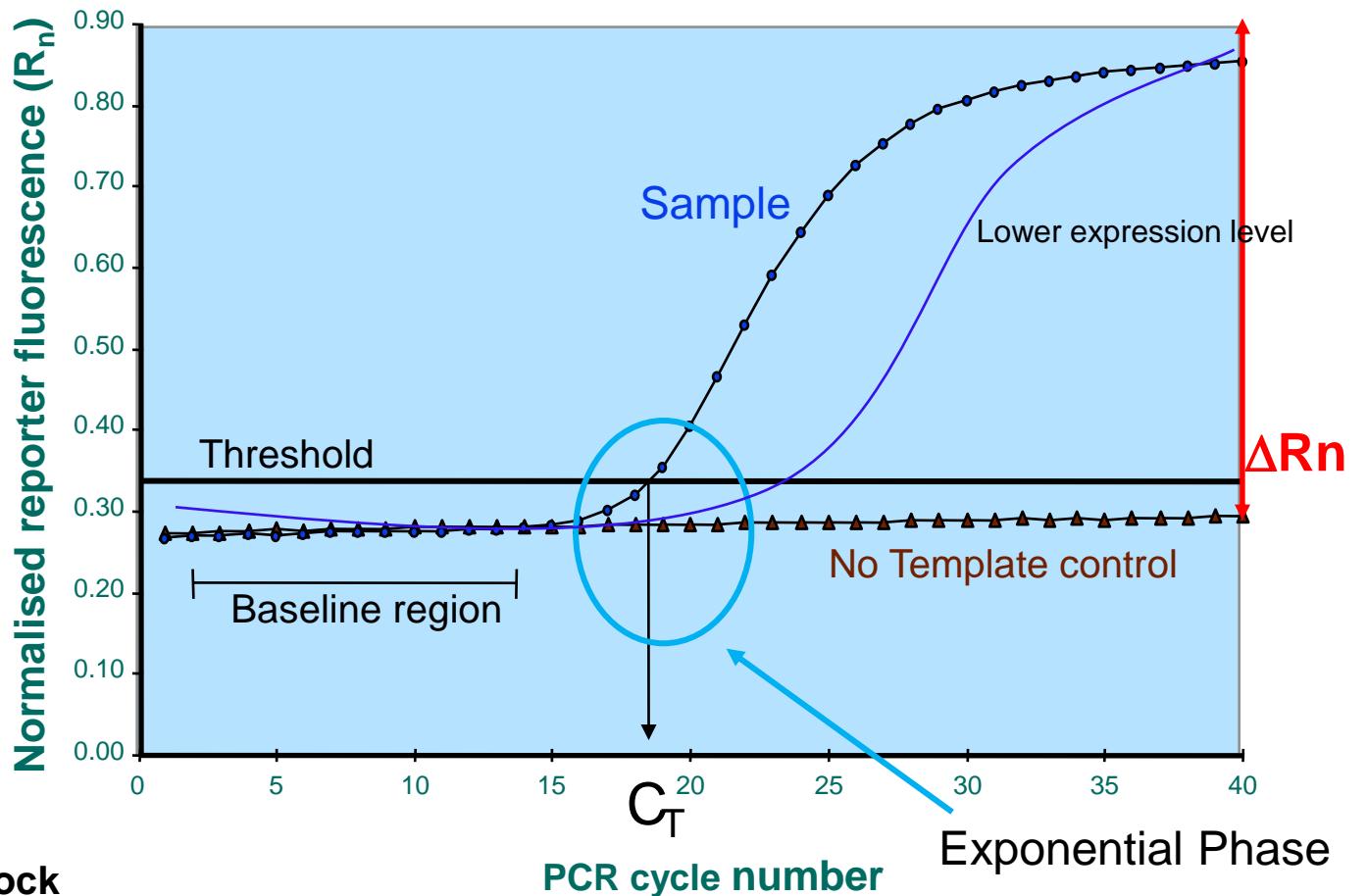
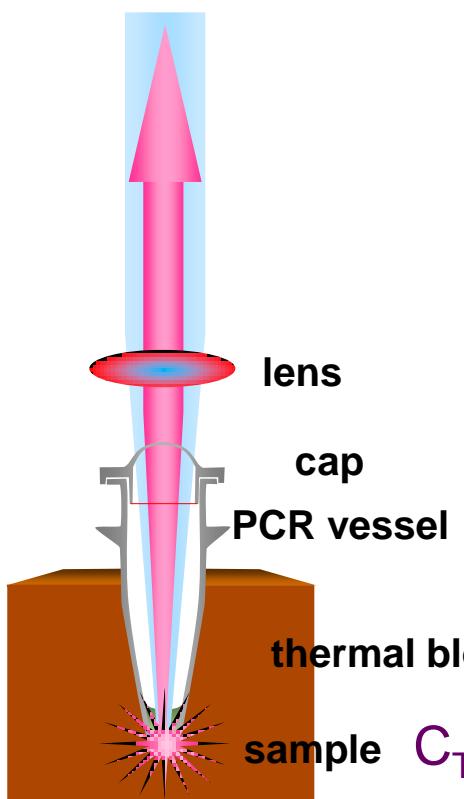




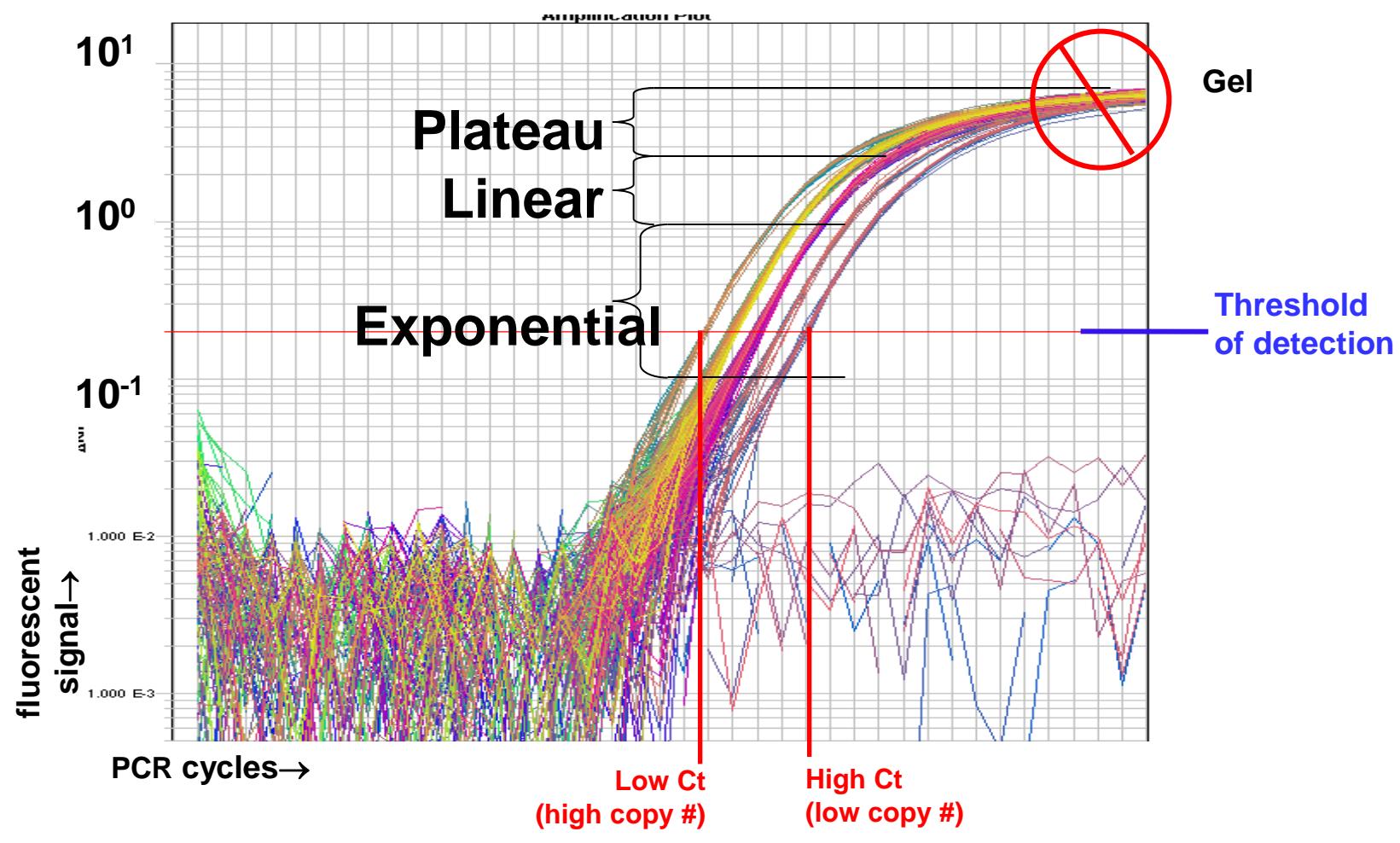
# Applied Biosystems StepOnePlus™ Real-time PCR System之原理與應用介紹

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

# Principle of Real-time PCR



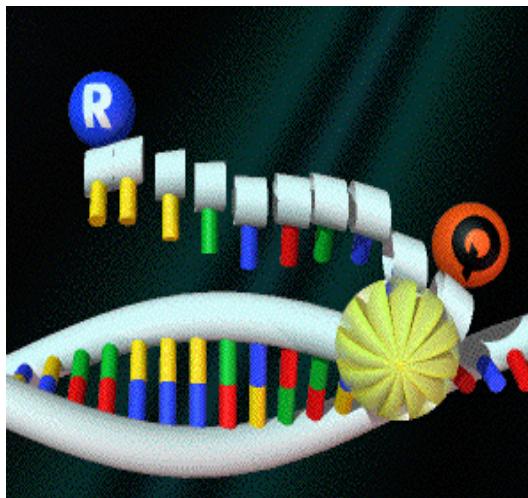
# Real-time PCR Signal Detection: Exponential Phase



$Y = No \ 2^n$ ,  $C_T$  與起始濃度之對數值成反比

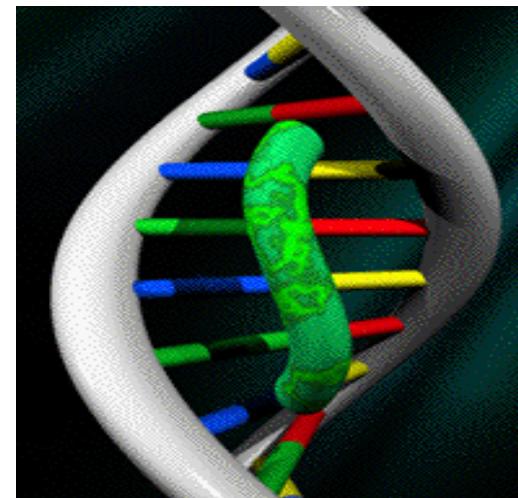
# Real-time PCR Chemistries

TaqMan® and TaqMan® MGB



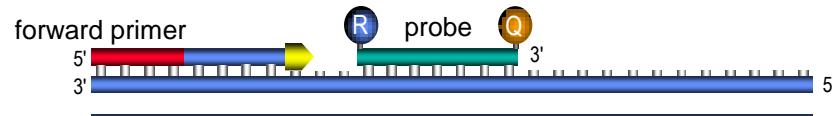
Fluorogenic 5' Nuclease  
Assay

SYBR® Green I dye

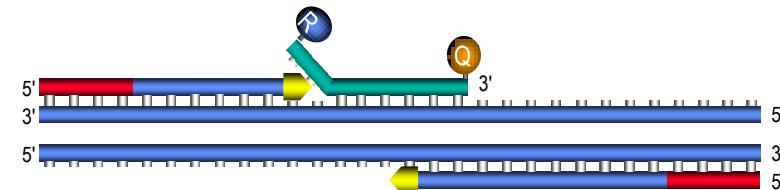


Binds Double-  
stranded DNA

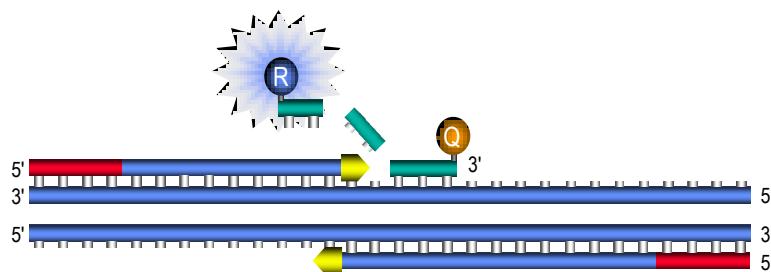
# TaqMan® Assay: Fluorogenic 5'-nuclease Assay



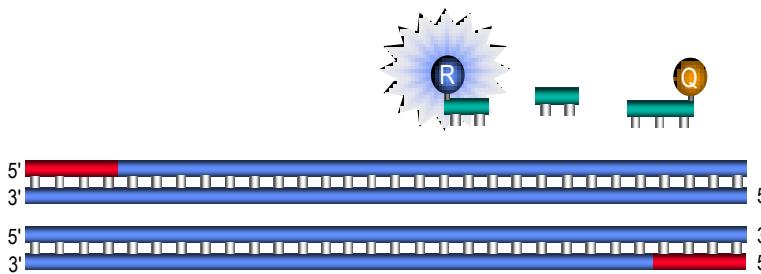
1. Polymerization



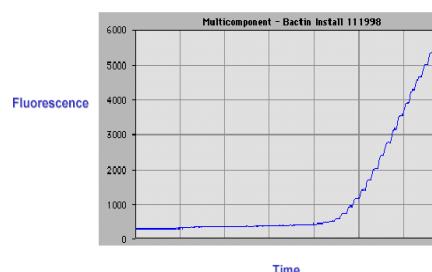
2. Strand displacement



3. Cleavage



4. Polymerization completed

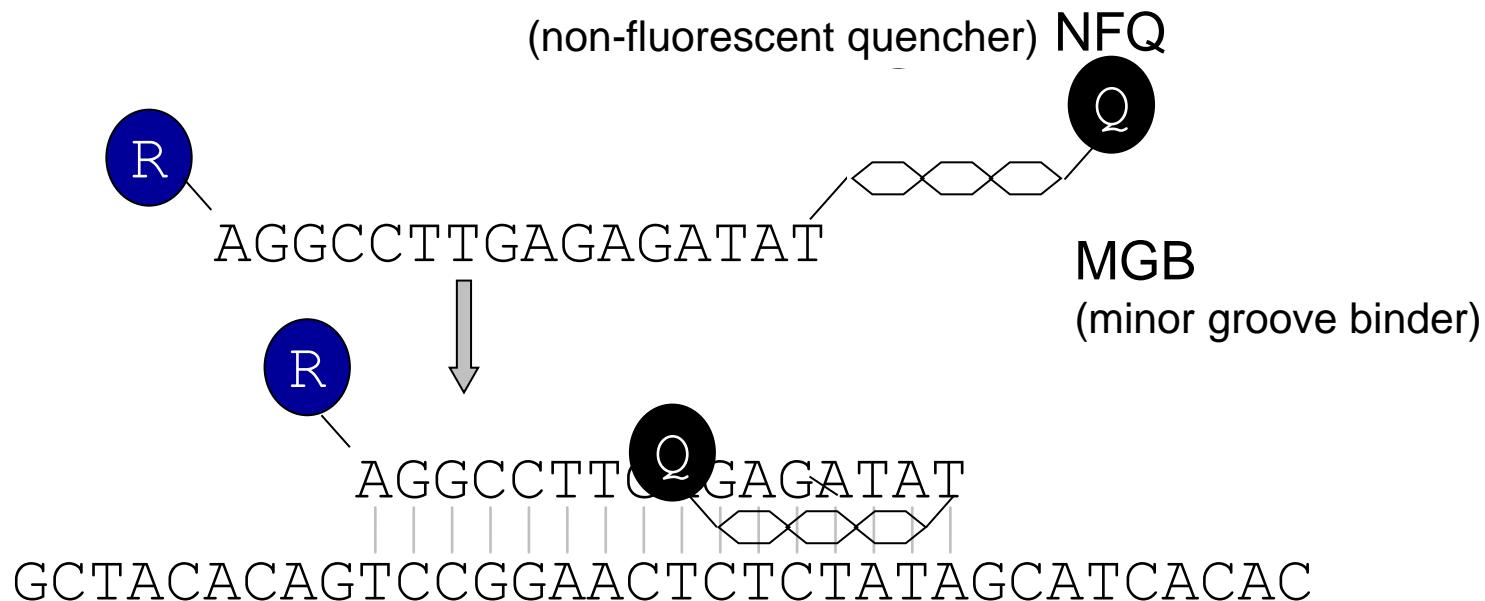


R = Reporter (FAM, VIC, etc.)

Q = Quencher (NFQ/MGB, etc.)

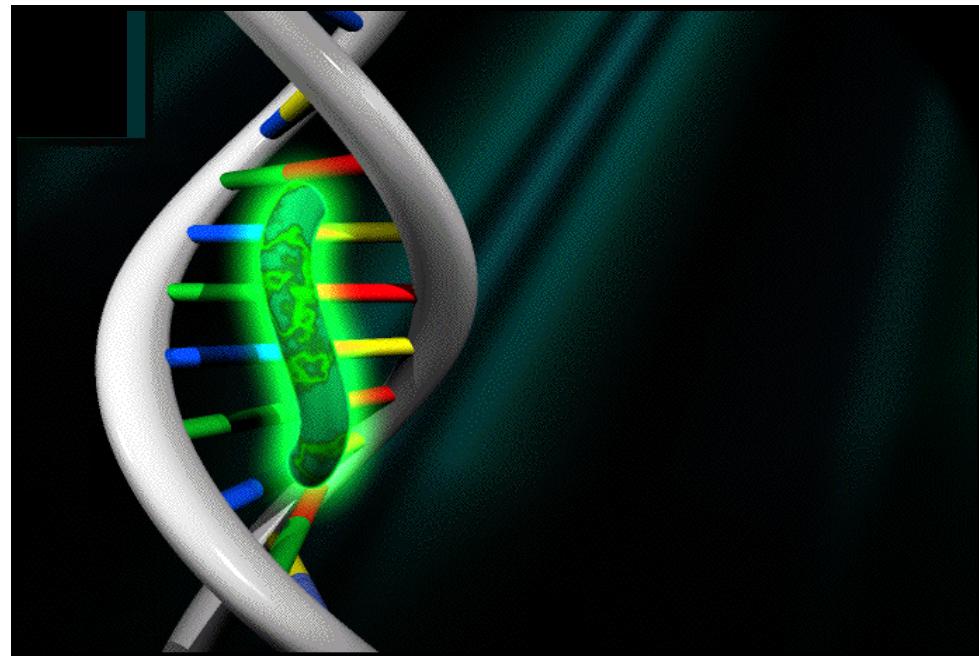
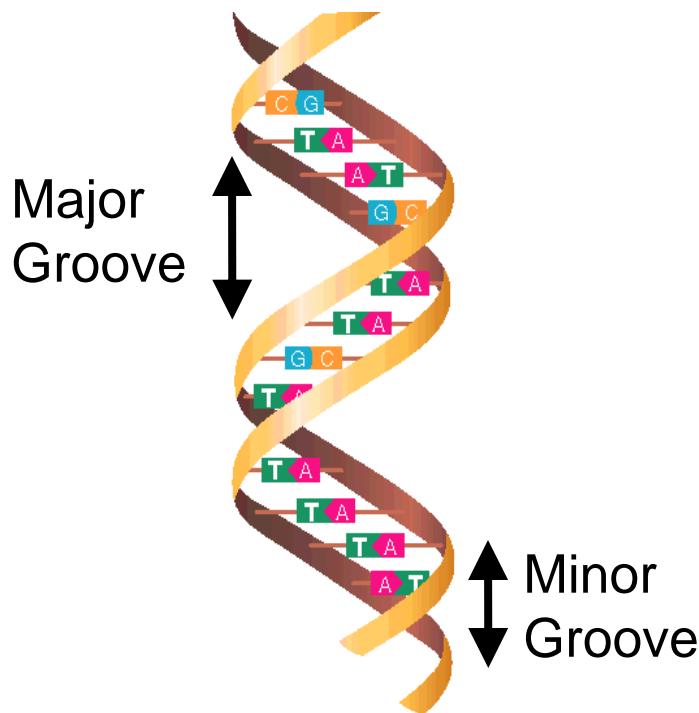
# TaqMan® Probe: TaqMan® MGB/NFQ Probes

- 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)

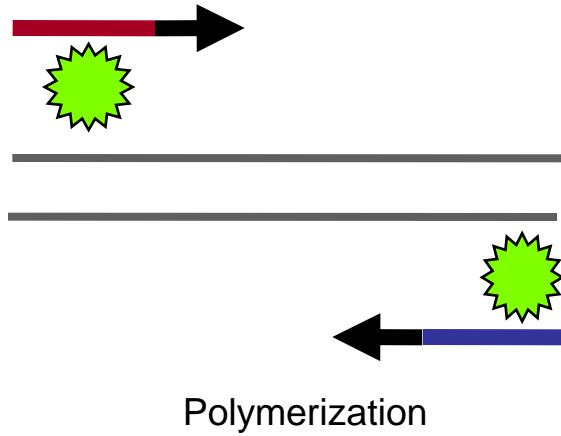


# Real-time PCR Chemistries: SYBR® Green I Dye

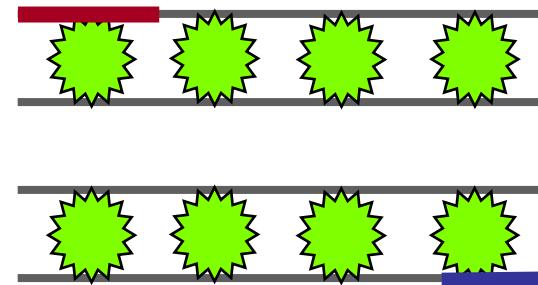
- A ‘minor groove’-binding molecule specific to the minor groove of double-stranded DNA
- Fluoresces at an increased intensity when bound



# SYBR® Green I Dye: Melting Curve Analysis

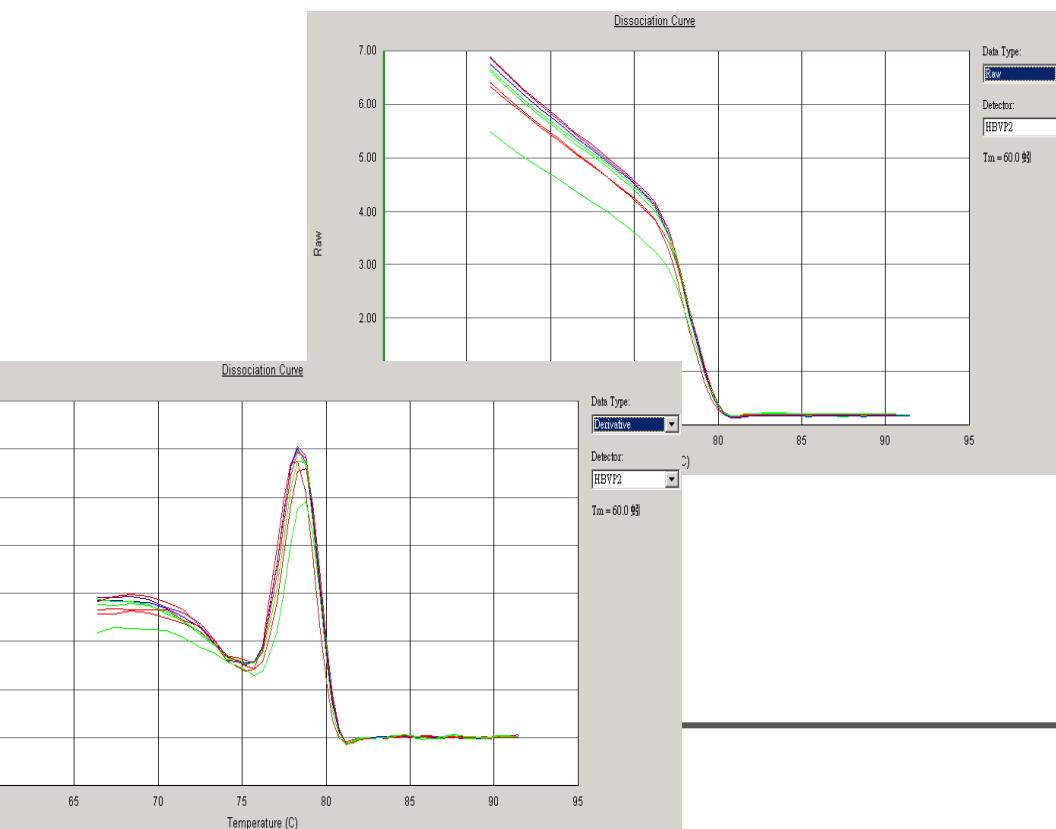


PCR



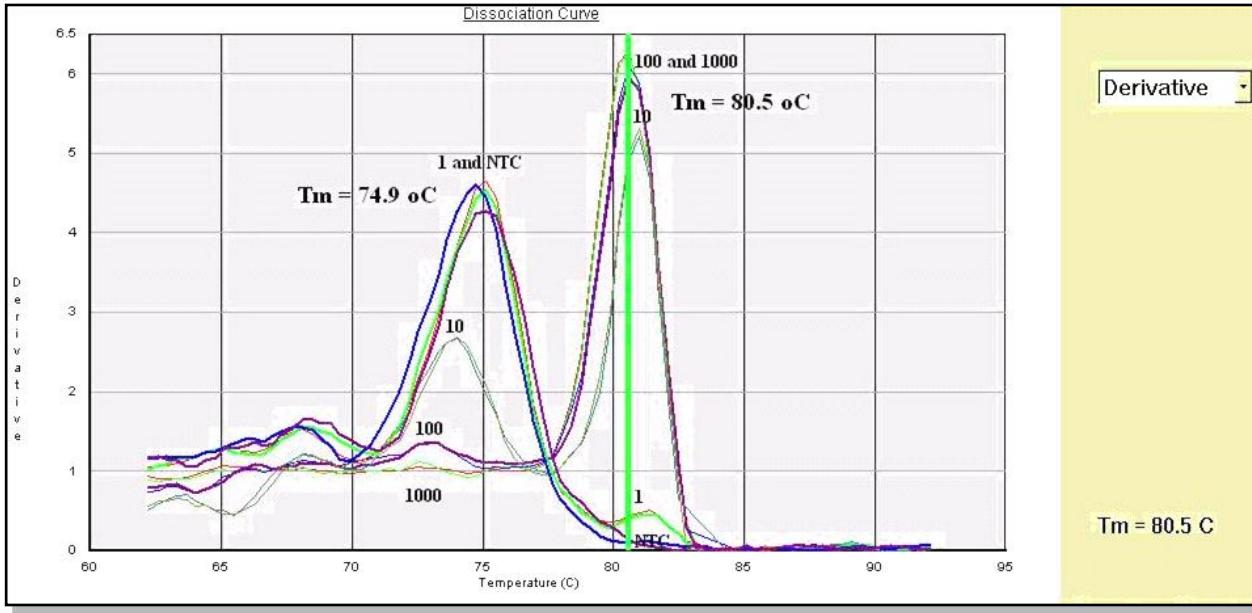
Polymerization Complete

Polymerization



Denaturation

# 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

# Real-time PCR Chemistries

	TaqMan® Assay	SYBR® Green I Dye
<b>Specificity</b>	More specific	Less specific
	Probe hybridization	
<b>Sensitivity</b>	Very high	Very high
<b>Flexibility</b>	Multiplex PCR	No probe required
	SNP detection	Screening tool
	+/- application	
<b>Optimization</b>	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

## **Reverse Transcription and Real-time PCR Reaction**

# One-step vs Two-step Workflows

- One-step Technology

- RT and PCR are performed in single buffer system

- ✓ One tube, one step

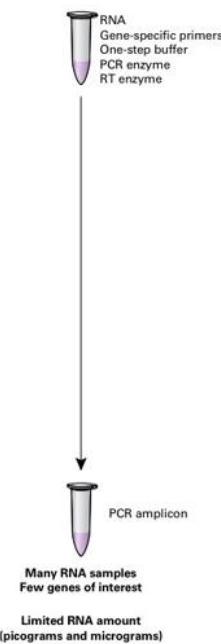
- ✓ Reduce chance of cross-contamination

- ✓ Easy for high throughput workflow

- ✓ Cost effective when few targets/sample analyzed

- ✓ Uses gene-specific primers

- X cDNA can not be stored



- Two-step Technology

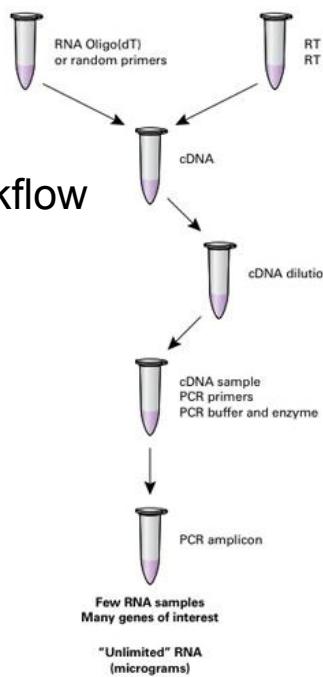
- RT and PCR are performed in two separate reactions

- ✓ Cost advantaged when interrogating multiple targets

- ✓ cDNA can be stored and used for further experiments

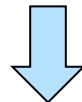
- ✓ Best choice if RNA is limiting

- X Multiple steps, longer time to result



# 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.
<b>Total volume per reaction</b>	<b>20 µL</b>	—



For sample volumes ≤30 µL

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

<sup>†</sup> 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).

<sup>‡</sup> Reverse transcription works best between 48 °C and 55 °C.

# Two-step Workflow: Real-time PCR Reactions

## Reverse Transcription : High Capacity RNA-to-cDNA Kit

2X RT Buffer	10µl
20X RT Enzyme Mix	1µl
Sample (up to 2µg)	Up to 9µl
Nuclease-Free water	To 20µl



	Step 1	Step 2	Step 3
Temperature (°C)	37	95	4
Time	60 min	5 min	∞

## Real-time PCR:

### TaqMan Chemistry

2x TaqMan Master Mix	1x	10µl
20x Probe/primer Assay Mix	1x	1µl
Water		NA
cDNA	1-100 ng	5-10µl

2x Power SYBR Master Mix	1x	10µl
F Primer	optimized	NA
R Primer	optimized	NA
Water		NA
cDNA	1-100 ng	5-10µl

20µl

20µl

### 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

# Two-step Workflow: Master Mixes

## Standard Mode

- TaqMan® Chemistry
  - TaqMan® Universal Master Mix II (PN 4440038)
  - TaqMan® Gene Expression Master Mix (PN 4369016)
- SYBR® Green Chemistry
  - Power SYBR® Green PCR Master Mix (PN 4367659)

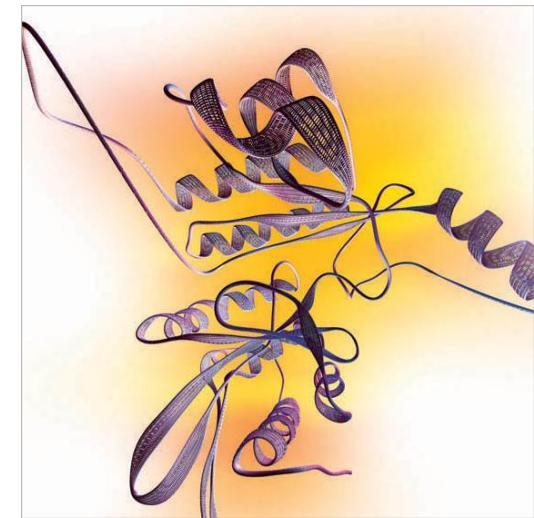
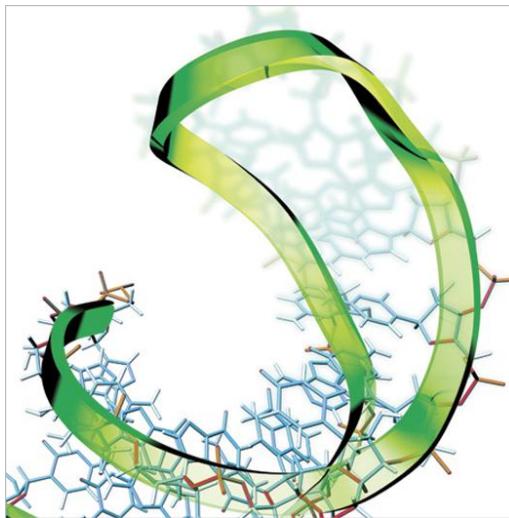
## Fast Mode

- TaqMan® Chemistry
  - TaqMan® Fast Universal Master Mix (PN 4366072)
  - TaqMan® Fast Advanced Master Mix (PN 4444557)
- SYBR® Green Chemistry
  - Fast SYBR® Green Master Mix (PN 4385612)
  - PowerUp™ SYBR® Green Master Mix (PN A25742)



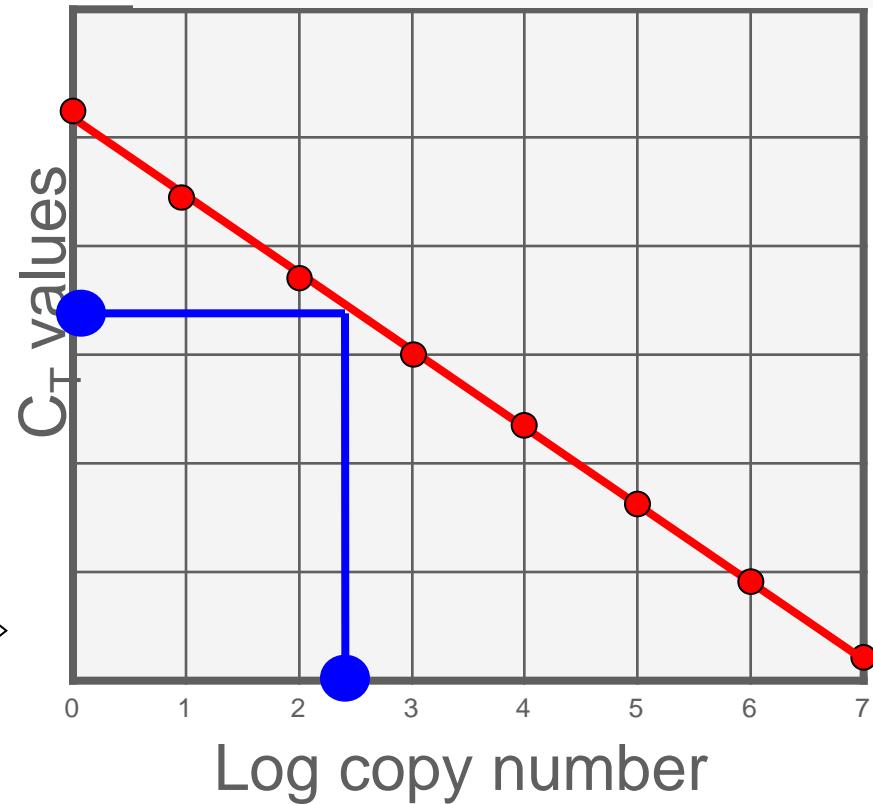
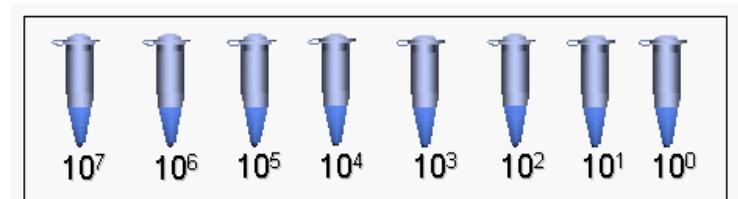
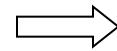
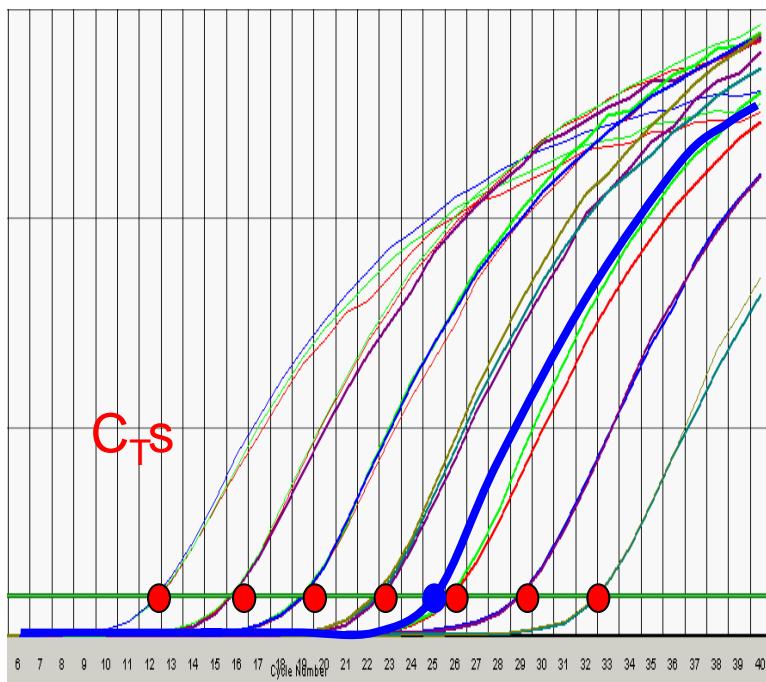
# Real-time PCR Quantitation Methods

- Absolute Quantitation vs. Relative Quantitation



# 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.



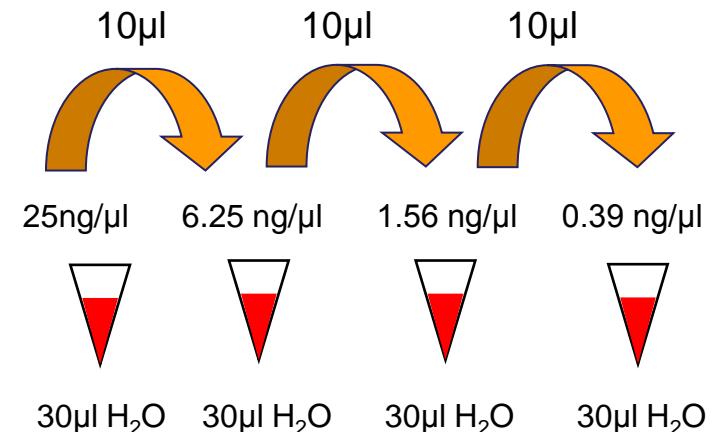
$C_T$  is directly proportional to log of amount of input template

# 相對定量 (Relative Quantitation)

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

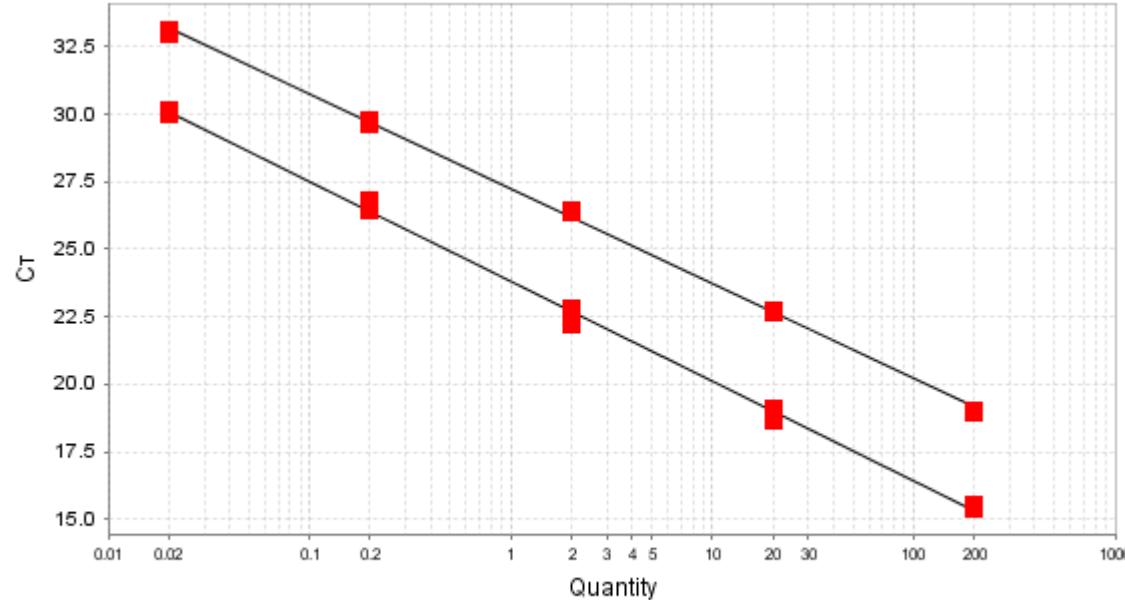
# 相對定量 (Relative Quantitation): PCR Efficiency Validation

- 2 $\mu$ g RNA in 20 $\mu$ l RT = 100ng cDNA/ $\mu$ l
- Gene name: C-Myc and GAPDH
- cDNA 4-fold serial dilution: 10 $\mu$ l cDNA + 30 $\mu$ l H<sub>2</sub>O (25ng/ $\mu$ l)
  - 1. 25ng/ $\mu$ l
  - 2. 6.25 ng/ $\mu$ l
  - 3. 1.56 ng/ $\mu$ l
  - 4. 0.39ng/ $\mu$ l
- 5. NTC (duplicate for each sample)
- 每個濃度點各做二重複



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

# 相對定量 (Relative Quantitation): PCR Efficiency Validation



Target: GAPDH Slope: -3.506 Y-Inter: 27.226 R<sup>2</sup>: 0.999 Eff%: 92.853

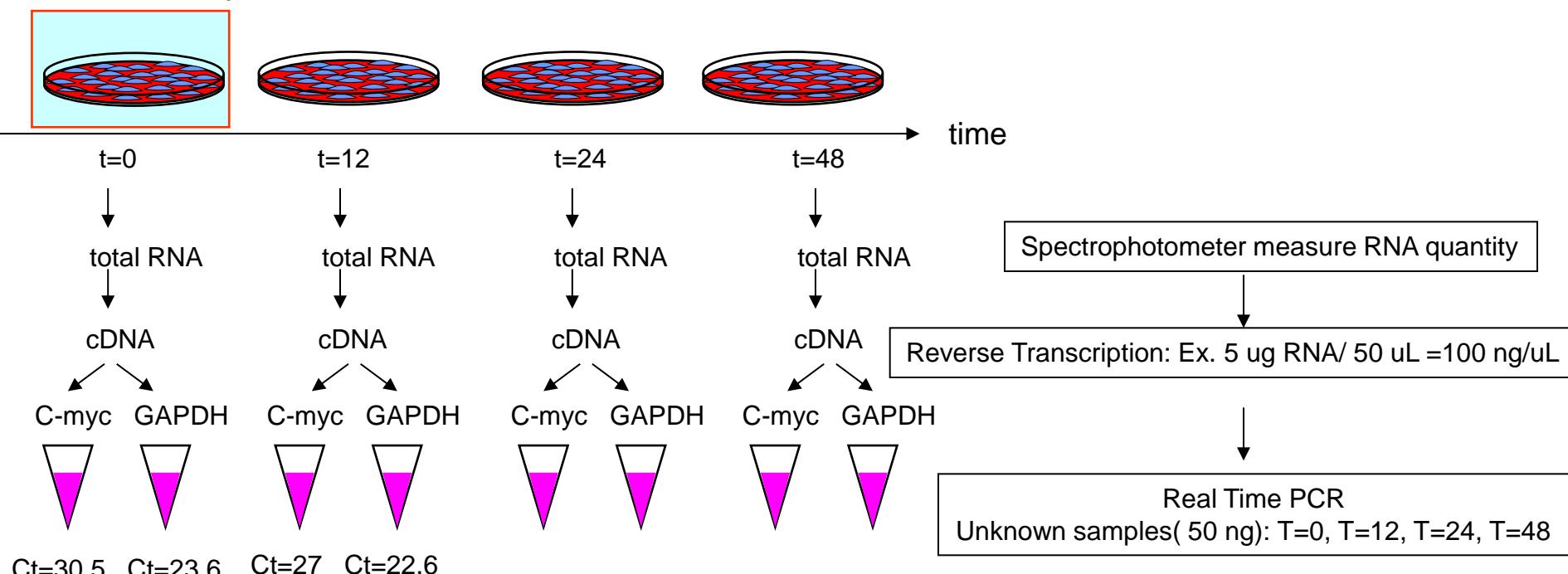
Target: c-myc Slope: -3.696 Y-Inter: 23.82 R<sup>2</sup>: 0.998 Eff%: 86.438

90 ≤ Eff% ≤ 110 → ΔΔ Ct  
Eff% < 90 → Relative standard curve

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

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

Reference Sample



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

*step 1: Normalization to endogenous control*

Sample:  $Ct_{c-Myc} - Ct_{GAPDH} = \Delta Ct_{sample}$

Reference:  $Ct_{c-Myc} - Ct_{GAPDH} = \Delta Ct_{reference\ sample}$

*step 2: Normalization to reference sample*

$\Delta Ct_{sample} - \Delta Ct_{reference\ sample} = \Delta\Delta Ct$

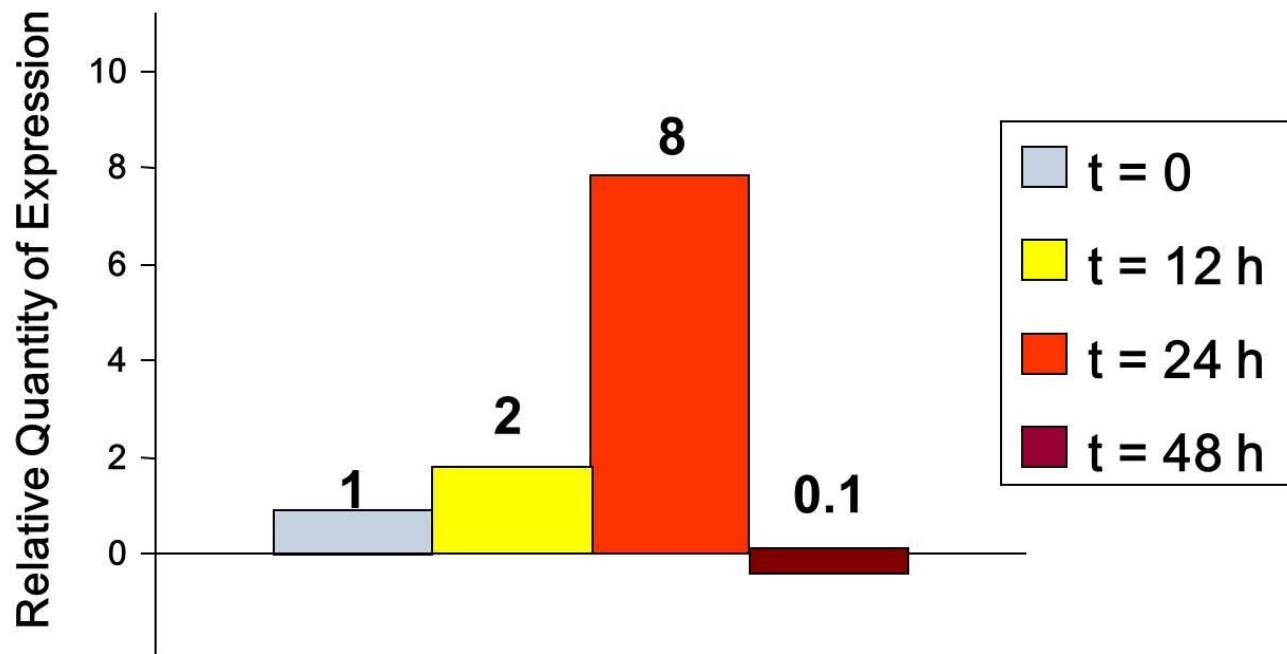
*step 3: use the formula*

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

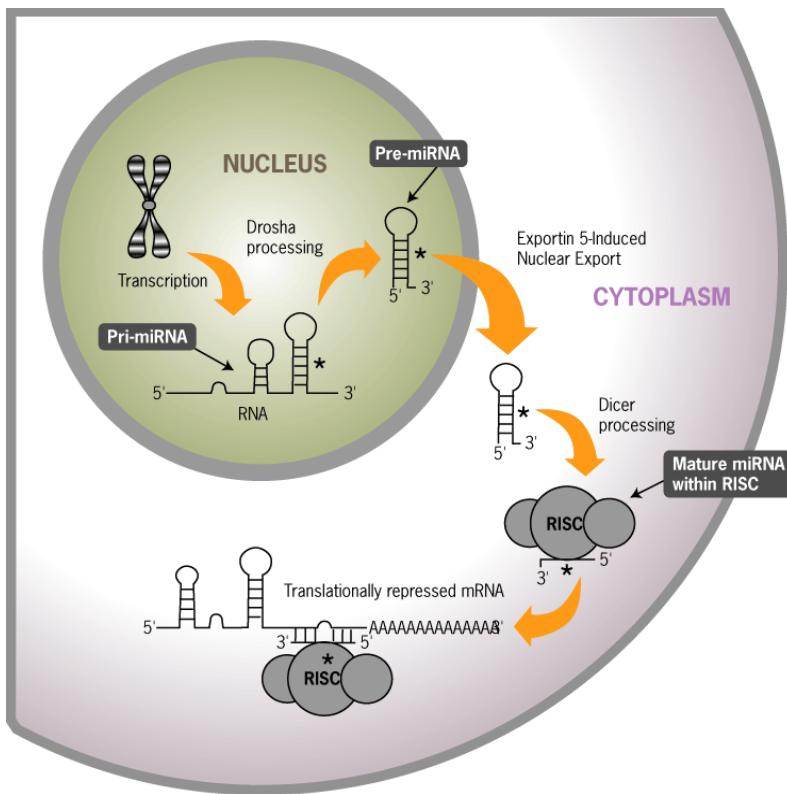
A reference sample is a sample to which unknown samples are compared (e.g. untreated sample or control).

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

	c-Myc	GAPDH	$\Delta C_t$	$\Delta\Delta C_t$	$2^{-\Delta\Delta Ct}$
T=0 (Reference)	25	10	15	0	1.0
T=12hr	24	10	14	-1	2.0
T=24hr	23	11	12	-3	8.0
T=48hr	28	10	18	3	0.1



# Introducing TaqMan™ Advanced miRNA Assays



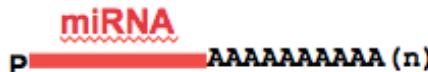
Mature  
miRNA

- Excellent sensitivity in biological samples (serum/plasma, tissue)
- Low input amount ( $2\mu\text{l}$ ) saves precious samples; no need to run multiple RTs and split sample
- Universal cDNA can be used for any miRNA assay
- cDNA can be archived for future miRNA studies

- TaqMan™ advanced miRNA assays
  - SKU A25576, Size S, 250 reactions
  - TaqMan™ advanced miRNA cDNA synthesis kit

# TaqMan™ Advanced miRNA Assays: How it Works

## Step 1. Poly A Tailing



## Step 2. Ligation

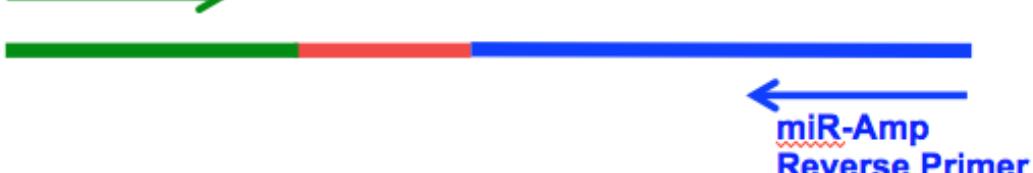


## Step 3. Universal RT

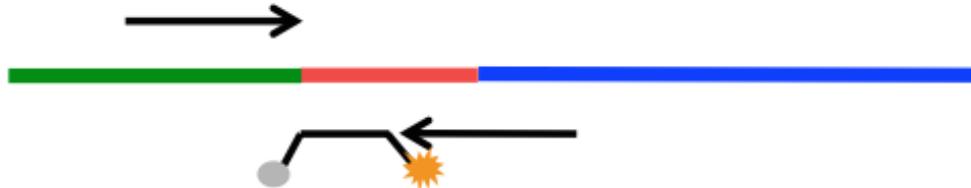


miR-Amp  
Forward Primer

## Step 4. miR-Amp



## Step 5. TaqMan qPCR



# TaqMan™ Advanced miRNA Assays: High Specificity

miRNA Name	miRNA Sequence
hsa-let-7a-5p	UGA GGU AGU AGG UUG UAU AGU U
hsa-let-7b-5p	UGA GGU AGU AGG UUG UGU GGU U
hsa-let-7c-5p	UGA GGU AGU AGG UUG UAU GGU U
hsa-let-7d-5p	AGA GGU AGU AGG UUG CAU AGU U
hsa-let-7e-5p	UGA GGU AGG AGG UUG UAU AGU U
hsa-let-7f-5p	UGA GGU AGU AGA UUG UAU AGU U
hsa-let-7g-5p	UGA GGU AGU AGU UUG UAC AGU U
hsa-let-7i-5p	UGA GGU AGU AGU UUG UGC UGU U
	*
	*
	*
	***
	*

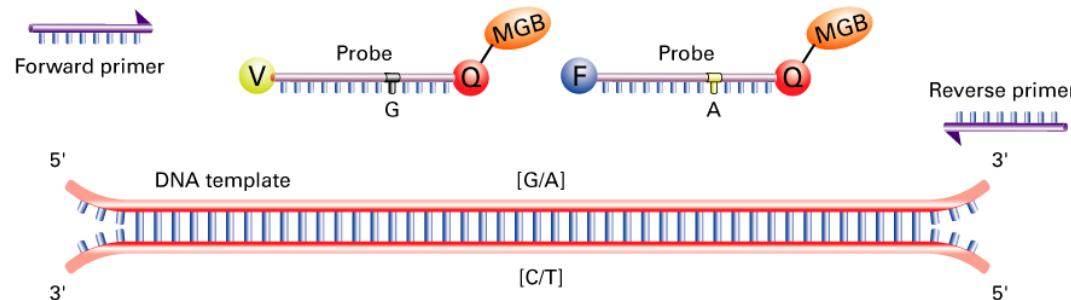
Let-7 miRNA family:  
differences as small  
as single base  
mismatches

TaqMan Advanced miRNA Assays	Synthetic Template							
	Let7a	Let7b	Let7c	Let7d	Let7e	Let7f	Let7g	Let7i
Let7a	100%	0%	0%	0%	4%	2%	0%	0%
Let7b	0%	100%	3%	0%	0%	0%	0%	0%
Let7c	1%	2%	100%	0%	0%	0%	0%	0%
Let7d	0%	0%	0%	100%	0%	0%	0%	0%
Let7e	0%	0%	0%	0%	100%	0%	0%	0%
Let7f	1%	0%	0%	0%	0%	100%	0%	0%
Let7g	0%	0%	0%	0%	0%	0%	100%	4%
Let7i	0%	1%	0%	0%	0%	0%	0%	100%

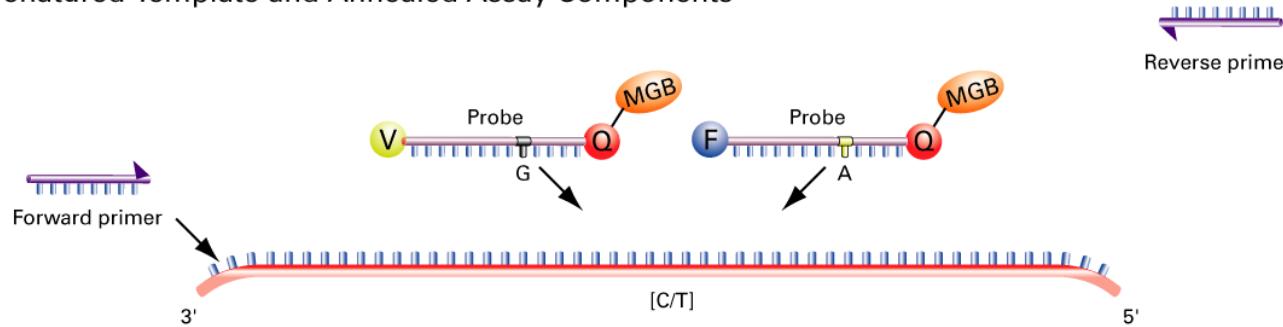
Extremely low  
cross-reactivity,  
usually 1% or lower

# TaqMan® SNP Genotyping Assay Overview

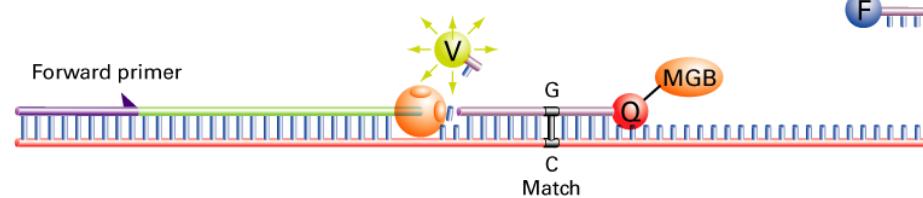
## 1. Assay Components and DNA Template



## 2. Denatured Template and Annealed Assay Components



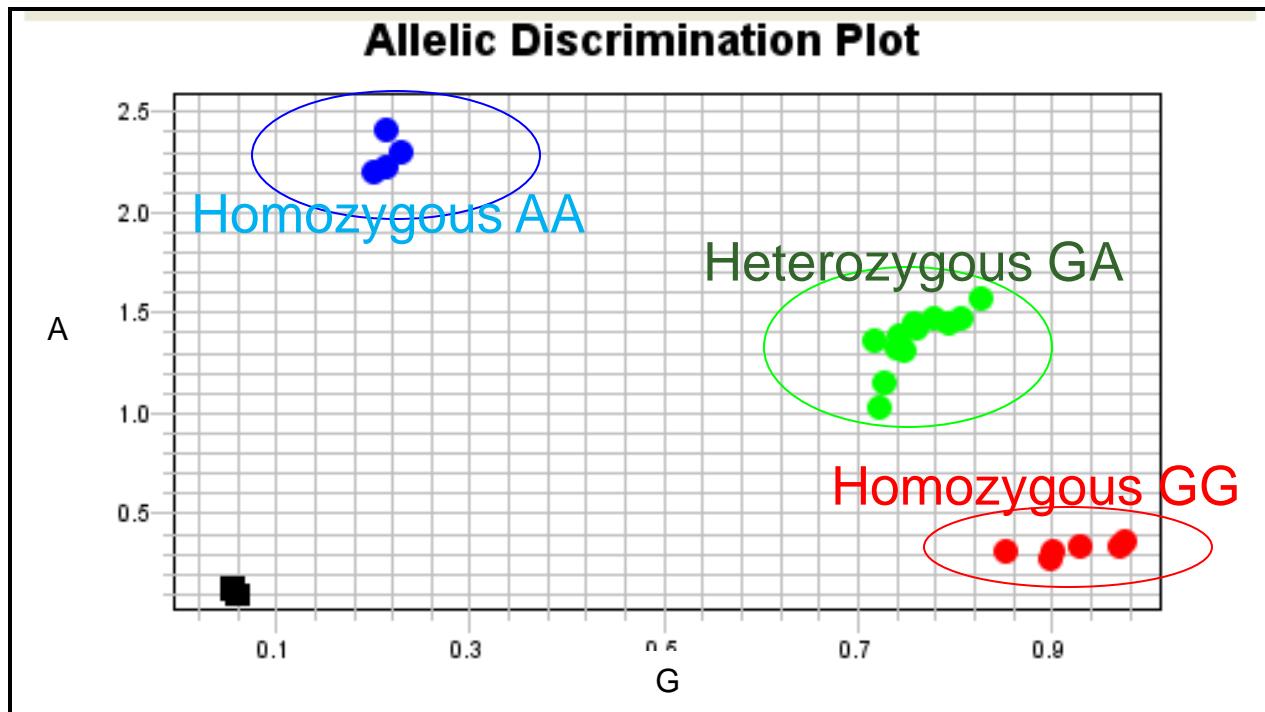
## 3. Signal Generation



## LEGEND

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

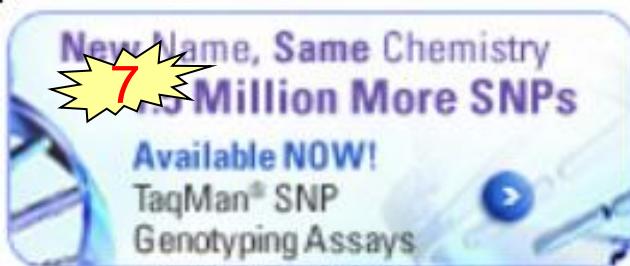
# Allelic Discrimination (SNP) Data



						Allele 2 Δ...	Pass.Ref	Call
						0.152	3,617.946	■ Negative Control (NC)
						0.186	3,784.869	■ Negative Control (NC)
						0.334	4,068.745	● Homozygous 1/1
						2.282	3,774.144	● Homozygous 2/2
						0.392	4,004.767	● Homozygous 1/1
							1.5	3,991.875
							1.206	3,942.024
							1.624	3,956.087
							1.526	3,849.214
							1.478	3,793.905
							1.086	3,820.435
							1.371	3,945.303
							1.528	4,026.388
							0.364	3,737.053
							0.37	4,099.657
							2.251	3,643.652
							0.421	3,826.976
							1.418	3,982.397
							1.386	4,048.287
							0.393	4,015.545
							1.437	3,797.601

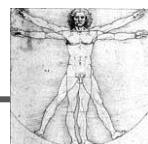
# Applied Biosystems 提供Primers/Probe設計的全方位解決方案

<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



**ThermoFisher**  
SCIENTIFIC

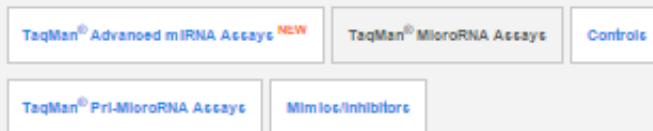
# Finding the Right Assay for Your Research

## Assay Search Tool - Find & Buy Your Single Tube TaqMan® Assays:

What type of experiment are you conducting?



Which miRNA product(s) are you interested in using?



What species do you want to target? (Select one or more)



Enter target information

e.g., Assay ID, miRBase ID, miRBase Accession #

Enter / Upload Multiple Targets

Enter Single Sequence

Select a single species to search by sequence

- Search for the assay you need quickly and easily
  - Powerful search engine
  - Streamlined search interface
  - Flexibility to search by gene name, gene alias or assay ID

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

# TaqMan® Gene Expression Array Plates

<https://www.thermofisher.com/tw/en/home/life-science/pcr/real-time-pcr/real-time-pcr-assays/taqman-gene-expression/real-time-pcr-taqman-arrays.html>

## Pre-configured (fixed content) TaqMan® Array cards and plates

TaqMan® arrays are pre-configured with the most relevant TaqMan® Gene Expression Assays that target relevant genes for specific biological pathways, processes, or diseases. Click below to explore our offering of preconfigured arrays.

### Browse for pre-configured (fixed content) TaqMan® Array cards and plates

#### By disease

Alzheimer's, cancer,  
diabetes, more >

#### By pathway

ABC transporters, CREB,  
more >

#### By biological process

Angiogenesis, DNA repair,  
more >

#### Endogenous controls

Human, mouse,  
eukaryotic, more >

### Search for pre-configured TaqMan® Array cards and plates

#### What Array format do you want?

All Arrays

96-well standard

96-well Fast

384-well

OpenArray

#### What species do you want to target? (Select one or more)



Human



Mouse



Rat

#### Enter target information

Enter Pathway, Disease, Gene Target

Enter / Upload Multiple Targets

Search

ThermoFisher  
SCIENTIFIC

# Targets and Pathway Information

**Narrow Your Results**

**TaqMan® Array Human Alzheimer's Disease 96-well plate, Fast**

The TaqMan® Array Human Alzheimer's Disease Plate contains 92 assays to Alzheimer's associated genes and 4 assays to candidate endogenous control genes.

Species	Samples/Plate	Supported Applied Biosystems Instruments	Inventoried   Cat. #
Human	1	7500 Fast System StepOnePlus™ System ViiA™ 7 System 7900HT System QuantStudio™ 12K Flex System	4418715 96-well Fast

[My Price](#) [新增到購物車](#)

[Plate Details](#) [Plate Layout](#)

**Panel Description**

The panel of assays in the TaqMan® Array 96-well Human Alzheimer's Disease Plate is based on the 'amyloid hypothesis'. The 92 genes are involved in APP processes that generate beta-amyloid and included genes implicated in multiple secondary steps of beta-amyloid aggregation, tau hyperphosphorylation, excitotoxicity, inflammation, oxidation and microglial activation. We also include assays for genes involved in cholesterol biosynthesis due to the correlation between high cholesterol and increased risk of Alzheimer's. Genes associated with Alzheimer's disease pathology, biochemistry and genetics are also included.

[View Less...](#)

**Targets**

ABCA1, ACHE, ADAM10, ADAM17, ADAM9, AGER, APBA1, APBA2, APBA3, APBB1, APBB2, APBB3, APCS, APH1A, APH1B, APLP1, APLP2, APOE, APP, BACE1, BACE2, BCHE, BPTF, CAPN1, CAPNS1, CAPNS2, CASP3, CASP8, CDC2, CDK5, CDK5R1, CHRM1, CHRM3, CHRNA4, CHRNAT, CSNK1A1, CSNK1D, CTSB, CTSC, CTSD, CTSG, CYP46A1, GAL, GAP43, GJB1, GLS, GRIN1, GRIN2A, GRIN2B, GRIN2C, GRIN2D, GSK3B, HSD17B10, IDE, IFNG, IL1A, IL1B, IL6, INS, INSR, LRP1, LRP2, LRPAP1, MAPK1, MAPK3, MAPT, MME, NAE1, NCSTN, PDE8B, PKN1, PLD1, PPP2CA, PRKACB, PRKCA, PRKCB1, PRKCE, PRKCG, PSEN1, PSEN2, PSENEN, SERPINA3, SLC18A3, SLC30A3, SNCA, SOAT1, SOD2, ST6GAL1, TNF, UBQLN1, UCHL1, VSNL1

[View Less...](#)

**Controls**

18S, GAPDH, GUSB, HPRT1

**Pathway Information**

Alzheimer's disease is a progressive and fatal neurodegenerative disorder. The disease has a characteristic neuropathology—cerebral plaques containing beta-amyloid deposits and neurofibrillary tangles composed of the microtubule-associated protein tau. There is strong evidence that generation and deposition of beta-amyloid has a pivotal role in pathogenesis.

[View Less...](#)

# Plate Layout and Assay ID

**Narrow Your Results**

**Species**

 Human  
 Mouse

TaqMan® Array Human Alzheimer's Disease 96-well plate, Fast

The TaqMan® Array Human Alzheimer's Disease Plate contains 92 assays to Alzheimer's associated genes and 4 assays to candidate endogenous control genes.

Species	Samples/Plate	Supported Applied Biosystems Instruments	Inventoried   Cat. # 4418715
Human	1	7500 Fast System StepOnePlus™ System ViiA™ 7 System 7900HT System QuantStudio™ 12K Flex System	96-well Fast

[My Price](#) [新增到購物車](#)

[Plate Details](#) [Plate Layout](#)

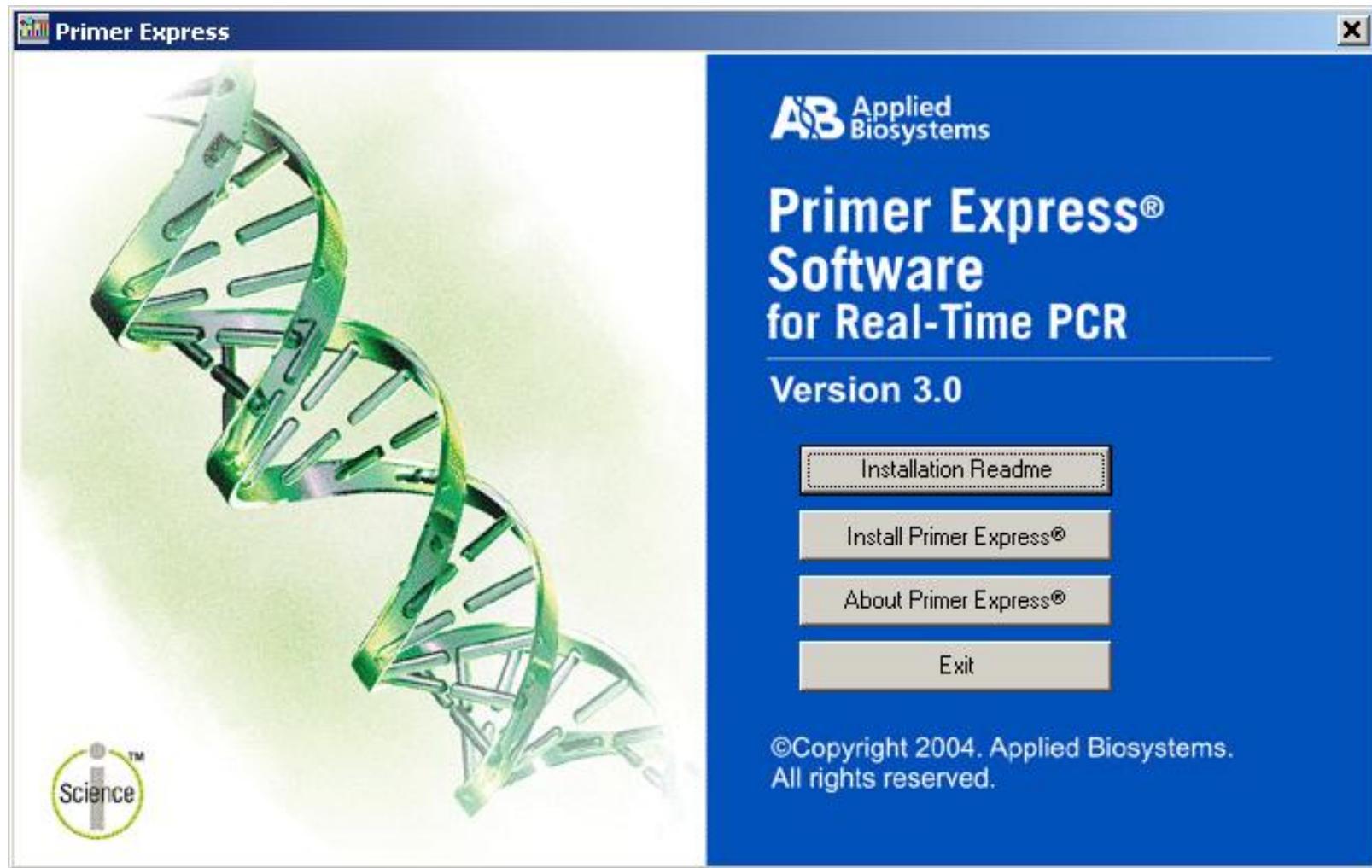
[Export to Excel](#)

	1	2	3	4	5	6	7	8	9	10	11	12
A	18S	GAPDH	HPRT1	GUSB	ABCA1	ADAM10	ADAM17	ADAM9	APBA1	APBA2	APBA3	APBB1
B	APBB2	APBB3	APCS	APH1A	APH1B	APLP1	APLP2	APOE	APP	BACE1	BACE2	CAPN1
C	CASP3	CASP6	CDC2	CDK5	CDK5R1	SLC18A3	CHRM1	CHRM3	CHRNA4	CSNK1A1	CTSB	CTSC

Assay ID	1	2	3	4	5	6	7	8	9	10	11	12
A	Hs99999901_s1	Hs99999905_m1	Hs99999909_m1	Hs99999908_m1	Hs00194045_m1	Hs00153853_m1	Hs00234224_m1	Hs00177638_m1	Hs00154104_m1	Hs00194072_m1	Hs00191660_m1	Hs00377427_m1
B	Hs00300268_m1	Hs00195923_m1	Hs00356632_g1	Hs00211268_m1	Hs00229911_m1	Hs00193069_m1	Hs00155778_m1	Hs00171168_m1	Hs00169098_m1	Hs00201573_m1	Hs00273238_m1	Hs00559804_m1
C	Hs00263337_m1	Hs00154250_m1	Hs00364293_m1	Hs00358991_g1	Hs00243655_s1	Hs00268179_s1	Hs00265195_s1	Hs00265216_s1	Hs00181247_m1	Hs00793391_m1	Hs00157194_m1	Hs00175188_m1
D	Hs00157205_m1	Hs00175195_m1	Hs00189461_m1	Hs00702141_s1	Hs00248163_m1	Hs00609557_m1	Hs00168219_m1	Hs00168230_m1	Hs00181352_m1	Hs00275656_m1	Hs00189576_m1	Hs00610438_m1
E	Hs00174143_m1	Hs00174092_m1	Hs00174097_m1	Hs00174131_m1	Hs00355773_m1	Hs00169631_m1	Hs00233856_m1	Hs00189742_m1	Hs00158875_m1	Hs00177066_m1	Hs00385075_m1	Hs00213491_m1
F	Hs00153519_m1	Hs00299716_m1	Hs00405493_m1	Hs00708570_s1	Hs00160118_m1	Hs00427259_m1	Hs00176944_m1	Hs00176973_m1	Hs00176998_m1	Hs00178455_m1	Hs00177010_m1	Hs00177028_m1
G	Hs0097789_m1	Hs01577197_m1	Hs00153674_m1	Hs00240906_m1	Hs00162077_m1	Hs00167309_m1	Hs00260517_s1	Hs00174128_m1	Hs00188233_m1	Hs00374305_m1	Hs00544355_m1	Hs01085739_g1
H	Hs00542592_g1	Hs01000370_m1	Hs00992319_m1	Hs00998426_m1	Hs01063373_m1	Hs01017895_m1	Hs01042347_m1	Hs00967138_m1	Hs01016626_m1	Hs00900696_m1	Hs00949382_m1	Hs00923840_m1

Gene Symbol	1	2	3	4	5	6	7	8	9	10	11	12
A	18S	GAPDH	HPRT1	GUSB	ABCA1	ADAM10	ADAM17	ADAM9	APBA1	APBA2	APBA3	APBB1
B	APBB2	APBB3	APCS	APH1A	APH1B	APLP1	APLP2	APOE	APP	BACE1	BACE2	CAPN1
C	CASP3	CASP6	CDC2	CDK5	CDK5R1	SLC18A3	CHRM1	CHRM3	CHRNA4	CSNK1A1	CTSB	CTSC
D	CTSD	CTSG	BPTF	GJB1	GLS	GRIN1	GRIN2A	GRIN2B	GSK3B	HSD17B10	IDE	
E	IFNG	IL1A	IL1B	IL6	INS	INSR	LRP1	LRP2	LRPAP1	MAPK1	MAPK3	MAPT
F	ORC3L	NCSTN	PDE8B	PSENEN	PLD1	PPP2CA	PRKACB	PRKCA	PRKCB1	PRKCE	PRKCG	PKN1
G	PSEN1	PSEN2	SERPINA3	SNCA	SOAT1	SOD2	CAPNS2	TNF	UCHL1	VSNL1	GAL	ACHE
H	AGER	NAE1	BCHE	CAPNS1	CHRNA7	CSNK1D	CYP46A1	GAP43	GRIN2C	SLC30A3	ST6GAL1	UBQLN1

# 定量PCR Primers/ Probe設計軟體

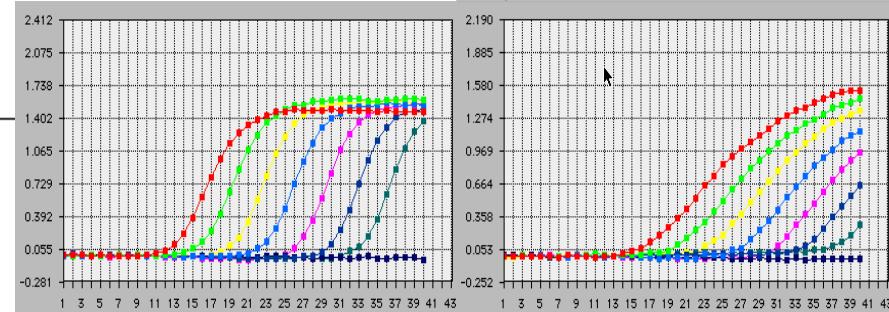


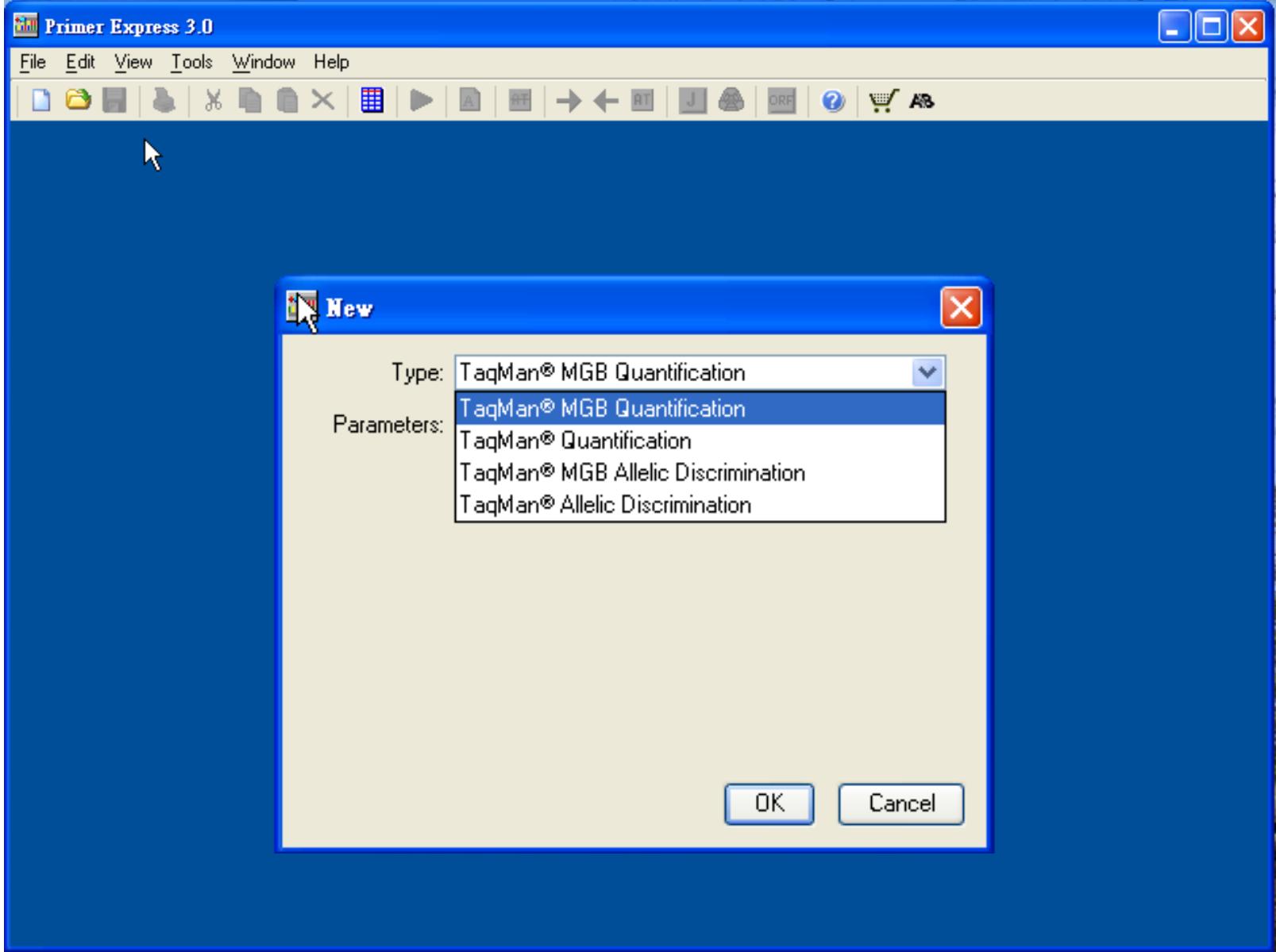
# 清楚明確的 TaqMan Probe and Primer 設計規範

TaqMan Probe	Primer
Probe 與 Primer 的距離愈近愈好, PCR 產物大小建議在 50-150 bp 為最佳	
G/C % 為 30-80 %	
避免有重複序列的出現，尤其避免 4 個以上 G 的出現	
Tm 值: 68-70°C (Quantification assay) 65-67°C (Allelic Discrimination assay)	Tm 值: 58-60°C
Probe 長度: 13~25 bases (TaqMan MGB probe) 13~30 bases (TaqMan probe)	Primer 長度: 20 bases (Optimal)
避免連續 6 個 A 的序列出現	3' 端的前五個序列裡不能超過 2 個 C+G
5' 端第一個序列不能為 G (如果選擇 FAM-dye 在 5' 端第二個序列也不能為 G)	
選擇 C 比 G 多的 strand 當作 probe <sup>b</sup>	
避免 3' 端的前 4 個序列裡含有 3 個或以上 G (GGG-MGB-3' or GGAG-MGB-3') <sup>a</sup>	200 bp amplicon
避免 probe 的中間區域含有 2 個或以上的 CC di-nucleotides <sup>a</sup>	500 bp amplicon

a: 針對 TaqMan MGB probe

b: 參數可選擇設定





# Sequence

The screenshot shows the Primer Express 3.0 software interface. At the top, there is a menu bar with File, Edit, View, Tools, Window, and Help. Below the menu is a toolbar with various icons. A red circle highlights the 'Find' button in the toolbar. Another red circle highlights the 'File Name' input field in the main window, which contains the text 'FBXw3.txt'. The main window title is 'TaqMan® MGB Quantification #1'. The tab bar at the top of the main window includes Sequence, Parameters, Primers / Probes, and Order, with 'Primers / Probes' currently selected. The main area displays a DNA sequence with its length listed as 383 bp. To the right of the sequence, a vertical scale from 50 to 383 is shown. A large text box in the center of the main window contains the following DNA sequence:

BWAGCCATCA CCCCAGCCTT GTGTGCCGTG TGTCCCCAGG GGCAAGGCAG  
CAGTGCTGTG CCTTTCTAC CAACCTGATA TCCCTGGTAC TGTTACCTAT  
GACAAGAAGG TGACCATCTA TGATCCCAGA GGTGAGCCTT TAATCCCAGT  
GCGTAGAAGG CAAAGGGAAAG CAGATCTCTA AGTTCAAGAT CAGCATGGC  
TACATAGTAA ATTCTAGGCC AGCTAGGGCT ACACAGTAAG ATCCTGTCAC  
AAAAAAAACTC AATAAACAAA ACACAAACAAA AAACAAAAGA AAGGAAACAC  
AACACAAACAG AAAAGAGCAT GGGGGCAGGA TGCAGGGGCT GAAAAGATGG  
CTCAGCAATT AAGAACGCTG GTTGCCTTC CBW

Below the sequence, a message reads: 'To find Primers & Probes, click the "Find Primers/Probes" button'. The title '2. Find Primer/Probe' is displayed above the sequence area.

1. Add DNA file or Copy & Paste

# Design Parameter

TaqMan® MGB Quantification #1	
Parameter	Value
Max Primer Length	40
Optimal Primer Length	20
Primer Composition	
Max Primer G Repeats	3
Max Num Ambig Residues in Primer	0
Primer Secondary Structure	
Max Primer Consec Base Pair	4
Max Primer Total Base Pair	8
Primer Site Uniqueness	
Max % Match in Primer	75
Max Consec Match in Primer	9
Max 3' Consec Match in Primer	7
Probe Tm	
Min Probe Tm	68
Max Probe Tm	70
Probe GC Content	
Min Probe %GC Content	30
Max Probe %GC Content	80
Probe Length	
Min Probe Length	13
Max Probe Length	25
Probe Composition	
Max Probe G Repeats	3
Max Num Ambig Residues in Probe	0
No G at 5' End in Probe	<input checked="" type="checkbox"/>
Select Probe with more C's than G's	<input type="checkbox"/>
Probe Secondary Structure	
Max Probe Consec Base Pair	4
Max Probe Total Base Pair	8
Amplicon	
Min Amplified Region Tm	0
Max Amplified Region Tm	85
Min Amplified Region Length	50
Max Amplified Region Length	150
General	

# Results

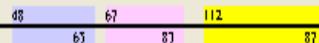
## TaqMan® MGB Quantification #1

Sequence Parameters Primers / Probes Order

### Candidate Primers & Probes

#	Fwd Start	Fwd Len...	Fwd Tm	Fwd %GC	Rev Start	Rev Len...	Rev Tm	Rev %GC	Probe Start	Probe Le...	Probe Tm	Probe %GC	Amp Tm	Amp %GC	Amp Ta	Amp Len
1	48	18	60	61	112	26	59	46	67	17	69	47	81	52	60	65
2	48	18	60	61	112	26	59	46	67	18	69	44	81	52	60	65
3	48	18	60	61	112	26	59	46	68	18	70	44	81	52	60	65
4	48	18	60	61	112	26	59	46	70	16	69	50	81	52	60	65
5	122	22	58	50	187	26	59	38	145	15	68	60	79	48	58	66
6	53	21	59	52	119	25	58	44	75	19	68	53	80	49	58	67
7	95	25	58	44	161	22	59	50	121	17	69	59	80	49	58	67
8	95	25	58	44	161	22	59	50	123	16	68	63	80	49	58	67
9	121	21	60	52	187	26	59	38	143	17	70	53	79	48	58	67
10	121	21	60	52	187	26	59	38	144	16	69	56	79	48	58	67
11	95	26	58	42	161	22	59	50	123	16	68	63	80	49	58	67
12	121	22	60	50	187	26	59	38	144	16	69	56	79	48	58	67
13	122	22	58	50	188	27	60	41	145	15	68	60	80	49	58	67
14	48	18	60	61	115	25	59	48	67	17	69	47	81	53	60	68
15	48	18	60	61	115	25	59	48	67	18	69	44	81	53	60	68
16	48	18	60	61	115	25	59	48	68	18	70	44	81	53	60	68

### Location



### Secondary Structure

Oligo	Length
-------	--------

Forward Primer

18

Reverse Primer

26

Probe

17

Forward Primer

CGGCAGTGCTGTGCCCTT

Reverse Primer

CACCTTCTTGTCAAGGTACCAAGTC

Probe

CTACCAACCTGATATCC

Hairpin Self Dimers Cross Dimers

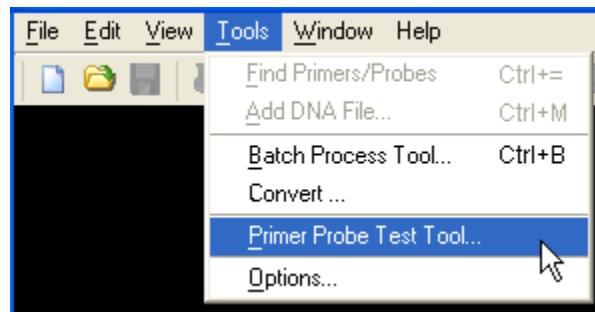
Most Stable Structure Found

GIGACGGC 5'

|||

CTGTGCCCTT 3'

# Check Tm of Primers



**Primer Probe Test Tool**

**Parameters**

Document Type: TaqMan® MGB Quantification Parameter: Default Browse

**Primers and Probes**

Fwd Primer	ACTGATCGATCAGCTACGCATC
Rev Primer	TCGATCGATCGATCGATGC
Probe 1	
Probe 2	

Trim

Tm	%GC	Length
58.1	50	22
59.2	53	19
0.0	0	0
0.0	0	0

life  
technologies™

# 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

# StepOnePlus™ Real-time PCR System

簡易三步驟！



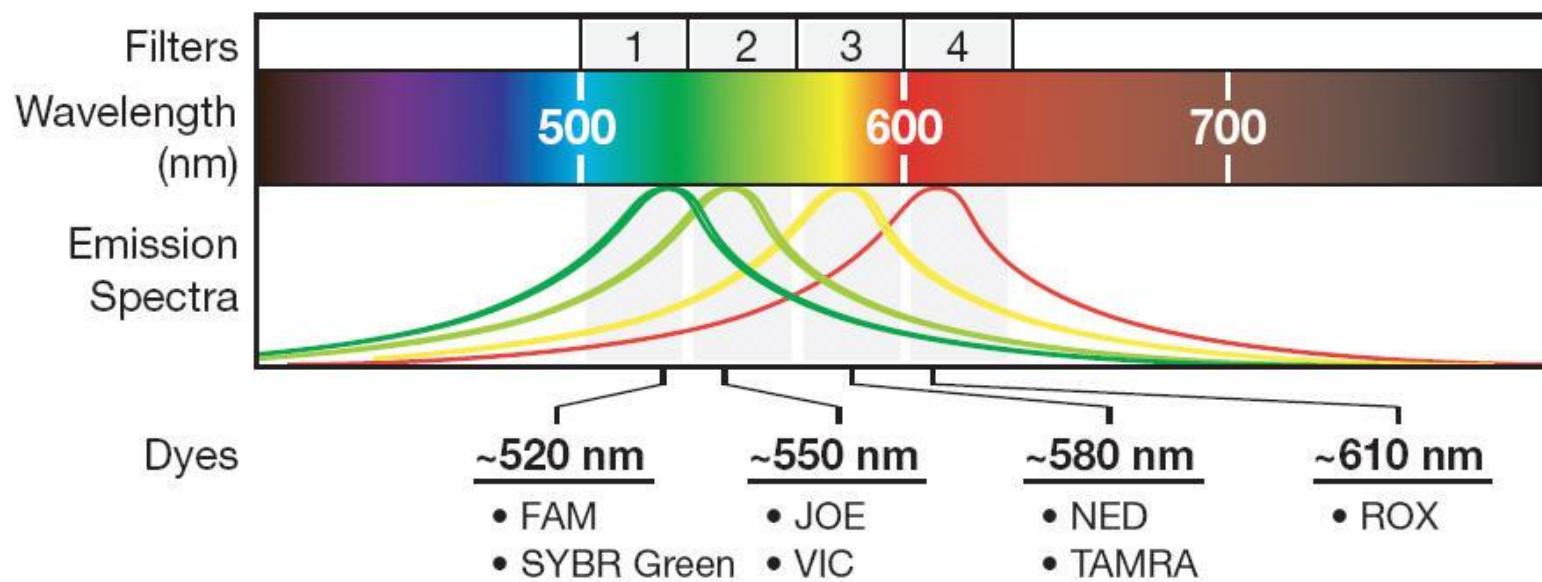
# StepOnePlus™ Real-time PCR System: the Basics

- Hardware Specifications
  - Power switch in the back
  - Automatically enters standby mode after 4hr
  - During standby mode, the touchscreen can be activated with a simple touch
  - Entire system becomes activated by pressing the solid blue button
- 96-Well Block
  - One block, 2 speeds
  - **Fast cycling: 40 cycles in ~40 minutes**
  - Standard cycling: 40 cycles in under 2 hours
  - 10-30µl reaction volume



# StepOnePlus™ Real-time PCR System: the Basics

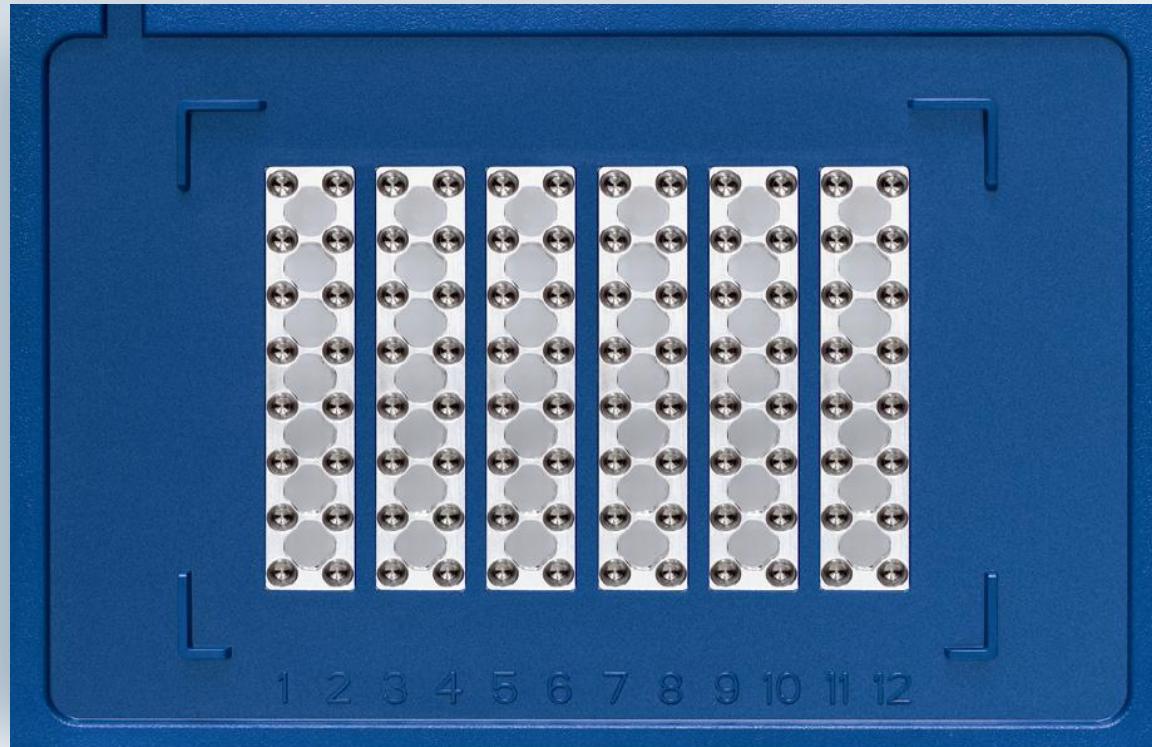
- 4-color instrument
  - FAM™/SYBR® Green dyes
  - VIC®/JOE™ dyes
  - ROX™ dye
  - NED™/TAMRA™ dye



# StepOnePlus™: Veriflex™ Block

- **Veriflex™ Block**

- One block, Six Zones
- The same “Better than gradient” feature from Veriti™ 96-well Thermal Cycler



\*Image from Veriti  
Thermal Cycler

# StepOnePlus™: Consumables

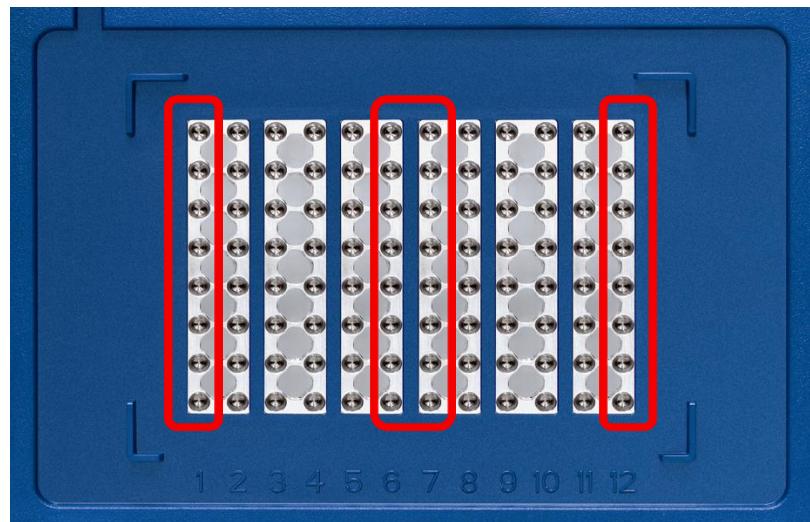
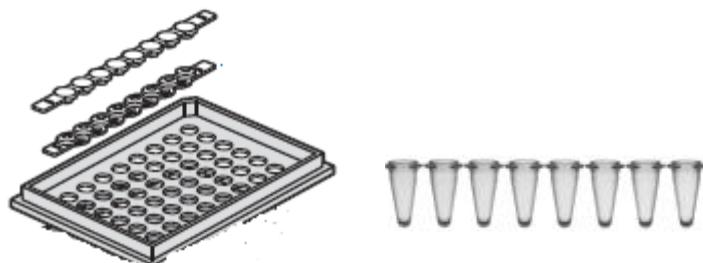
- 樣品量多時

- MicroAmp® Fast 96-Well Reaction Plate (0.1 ml) - 10 plates (P/N 4346907)
- MicroAmp® Optical Adhesive Film - 25 films (P/N 4360954)



- 樣品量少時

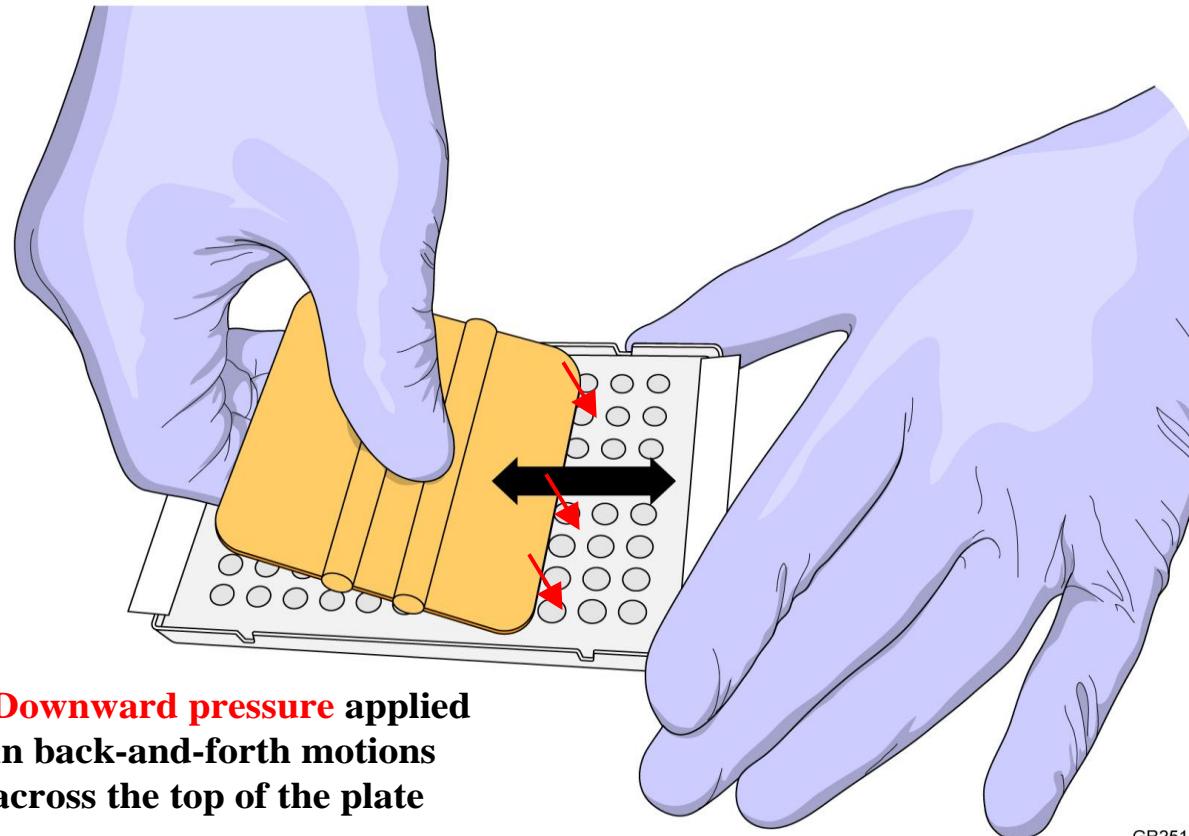
- MicroAmp® Fast 8-Tube Strip (0.1 ml) - 125 strips (P/N 4358293)
- MicroAmp® Optical 8-Cap Strip - 300 strips (P/N 4323032)



★ Place the tray containing the tube, Load at least 16 tube

# Sealing the Plate

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



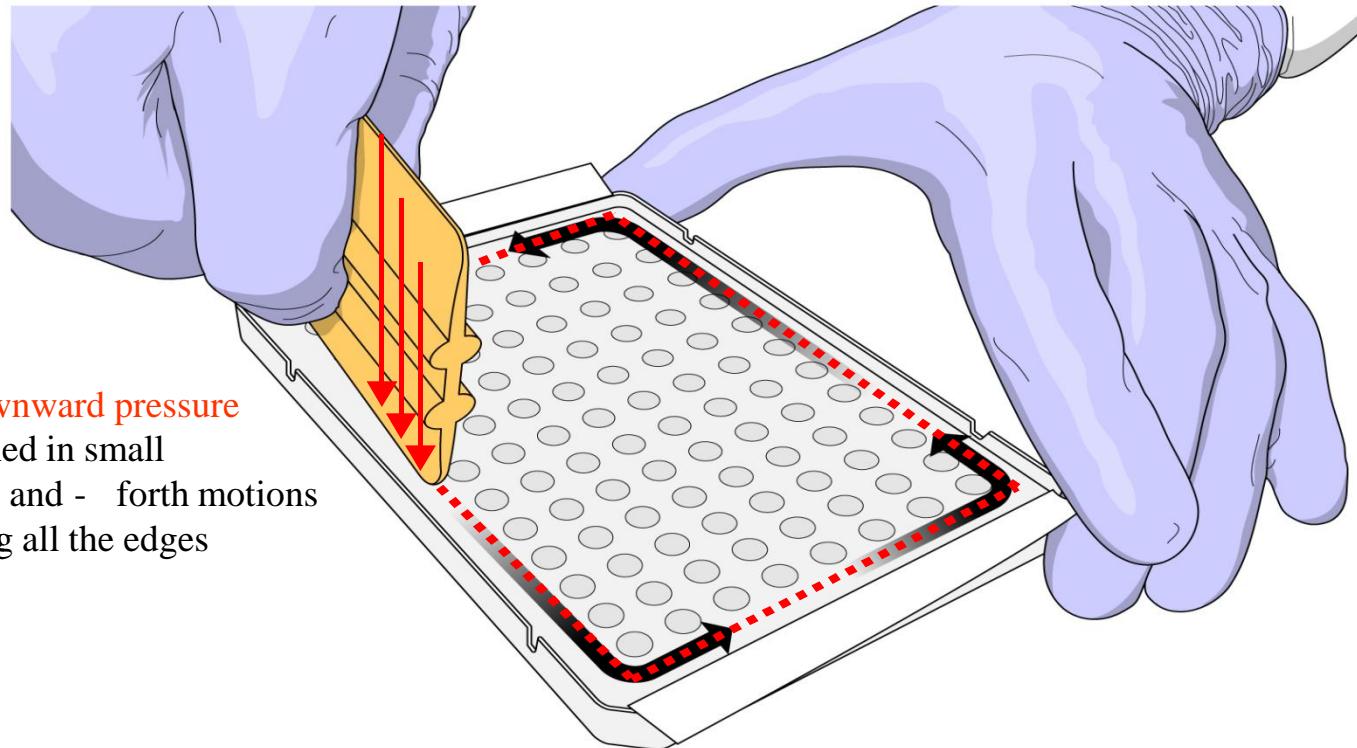
**Downward pressure applied  
in back-and-forth motions  
across the top of the plate**

GR2515

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

# Sealing the Plate

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



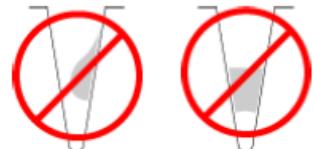
Downward pressure  
applied in small  
back - and - forth motions  
along all the edges

GR2516

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

# StepOnePlus™: Operation Notes

- Directly load fast optical 96-well plate into the instrument
  - If using 8-tube strips, load strips with fast 96-well tray
- Save your data with USB device after each run (standalone)
- Do not label on the consumables
  - This may increase the background signal
- Avoid bubbles when pipetting into each well
  - Centrifuge samples

Correct	Incorrect
 Liquid is at the bottom of the well.	 <ul style="list-style-type: none"><li>• Not centrifuged with enough force, or</li><li>• Not centrifuged for enough time</li></ul>

# Standalone (PC-free) Operation

只需輕按觸控螢幕 不需電腦連線也能上機!!



1. Start run from touch-screen
2. After run, download the file (.eds) to your PC
3. Analyze your data



Main Menu

## Browse/New Experiments: New

Browse / New Experiments	Settings Menu	Tools Menu	Collect Results
TaqMan cDNA (Fast)	TaqMan cDNA (Standard)	SYBR Green (Standard)	
Create New Experiment	Shortcut 6		

2007-07-07 | 11:45

**Browse Last Accessed Experiments (4)**

Experiment	Folder	Last Used
TaqMan_Std	lily	2007-04-19
20070401-LILY	AB	2007-04-19
TaqMan_Fast	AB	2007-04-19
TaqMan_Std	AB	2007-04-08

Selected: 0

Touch an experiment to select it, then touch any of the buttons to perform an action.  
Touch a column title to sort the table.



## Create an Experiment



## Select Experiment : Save

Last Used ▾

SYBR\_GREEN\*

Template

2007-04-08



GTYPE\_FST\*

Template

2007-02-12

PRES\_ABS\_FST\*

Templ

1\_STP\_RT\_PCR\*

Templ

AB\_RNASE\_P\*

Templ



Select

Touch a template experiment to Select to make it the selected t

File View Help

## Edit Experiment: SYBR\_GREEN



Stage 1

x 1

95.0

10:00

Step 1

Stage 2

x 40

95.0

0:15

Step 1

Melt Curve

95.0

0:15

Step 1

95.0

0:15

Step 3



Add



Delete



Options



Save

Touch a stage or step to insert a stage/step. Touch a time or temperature to edit it. Touch Options to create AutoDelta, to show ramp rates, to add a melt curve or collection point.

?



## Save Experiment



Run Experiment:

SYBR\_GREEN

Folder:

Default



Reaction Volume:

20

File View Help



## Browse Last Accessed Experiments (5)



## Experiment

## Folder

## Last Used

SYBR\_GREEN

lily

2007-07-07

TaqMan\_Std



## Warning

There are uncollected results.  
Do you want to collect them now?

Collect

Overwrite

Cancel

20070401-LIL'

TaqMan\_Fast

TaqMan\_Std



Start Run



New



View/Edit



Copy



Delete

Page  
1 / 1Selected:  
1

Enter a name, reaction volume, and folder for this experiment.  
Touch 'Save' to save.

Touch an experiment to select it, then touch any of the buttons to perform an action.  
Touch a column title to sort the table.

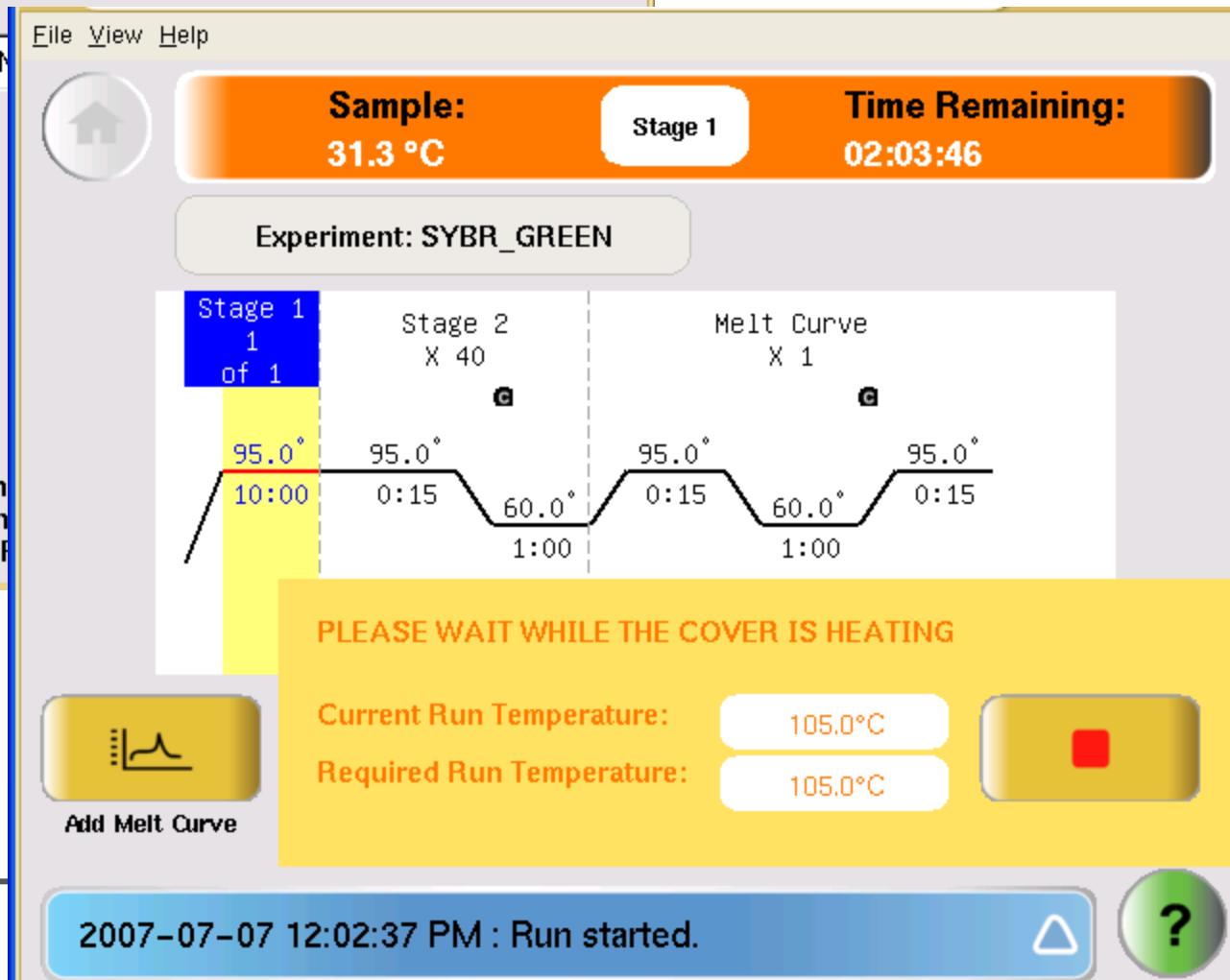




## Experiment Parameters

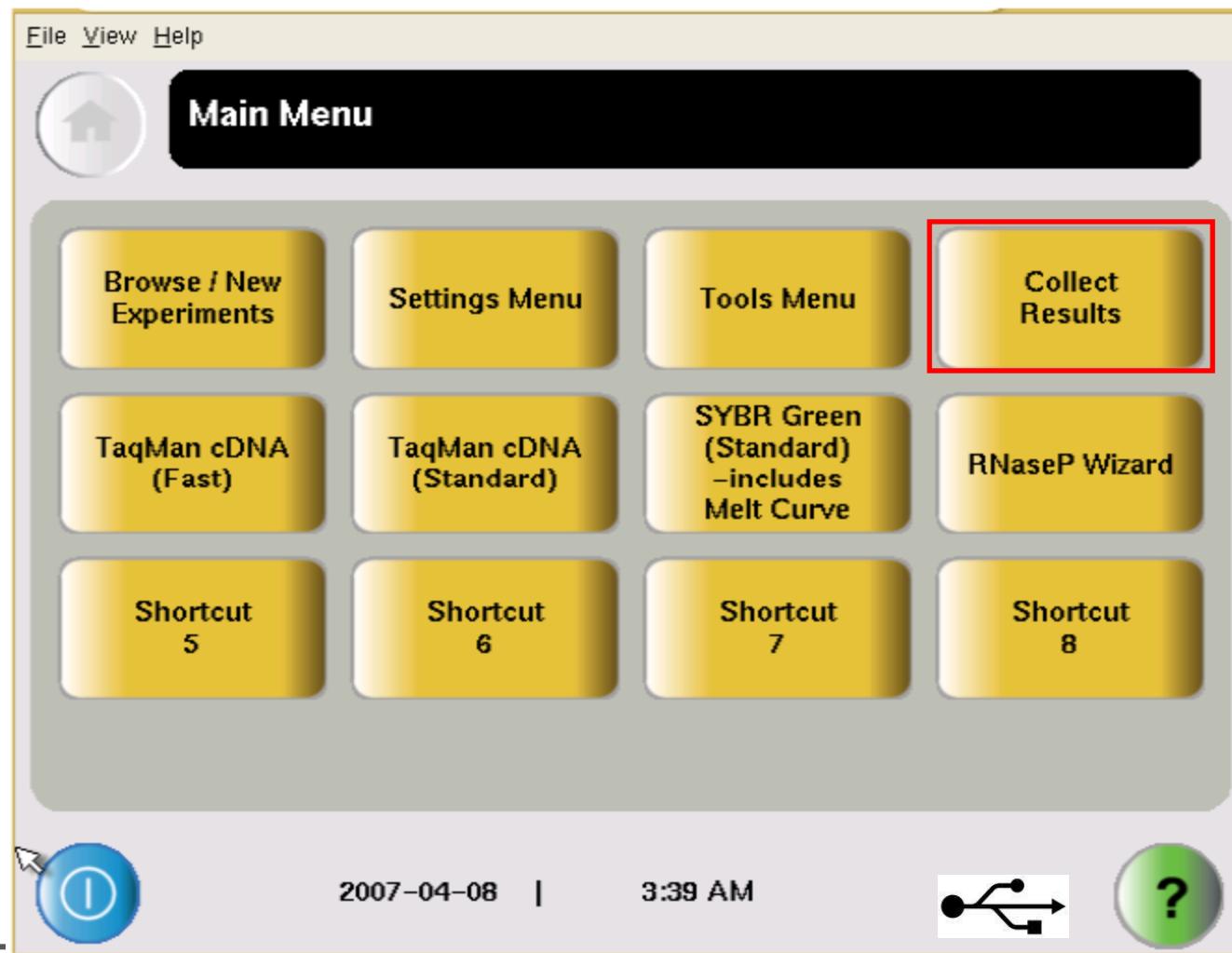
Reaction Volume:  uLCover Temperature:  °CExperiment Name: 

Touch each field then  
to edit the contents. When  
touch Start Run.



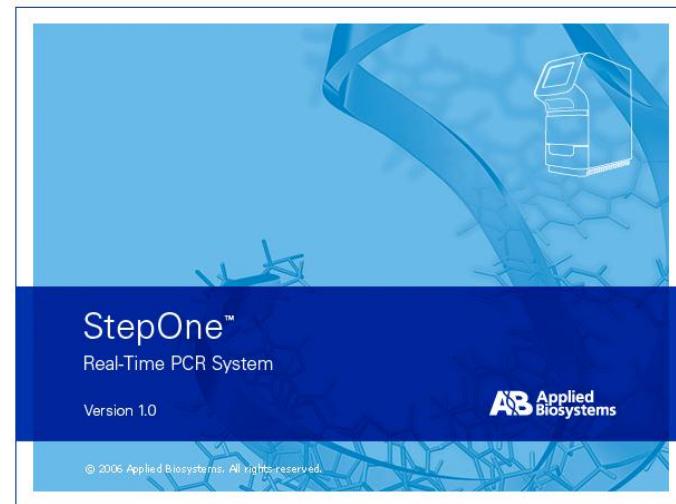
# Standalone: 只能暫存一個檔案

- 插入USB, 待 icon 出現在右下角, 即可點選 Collect Results 檔案會自動存到 USB 中

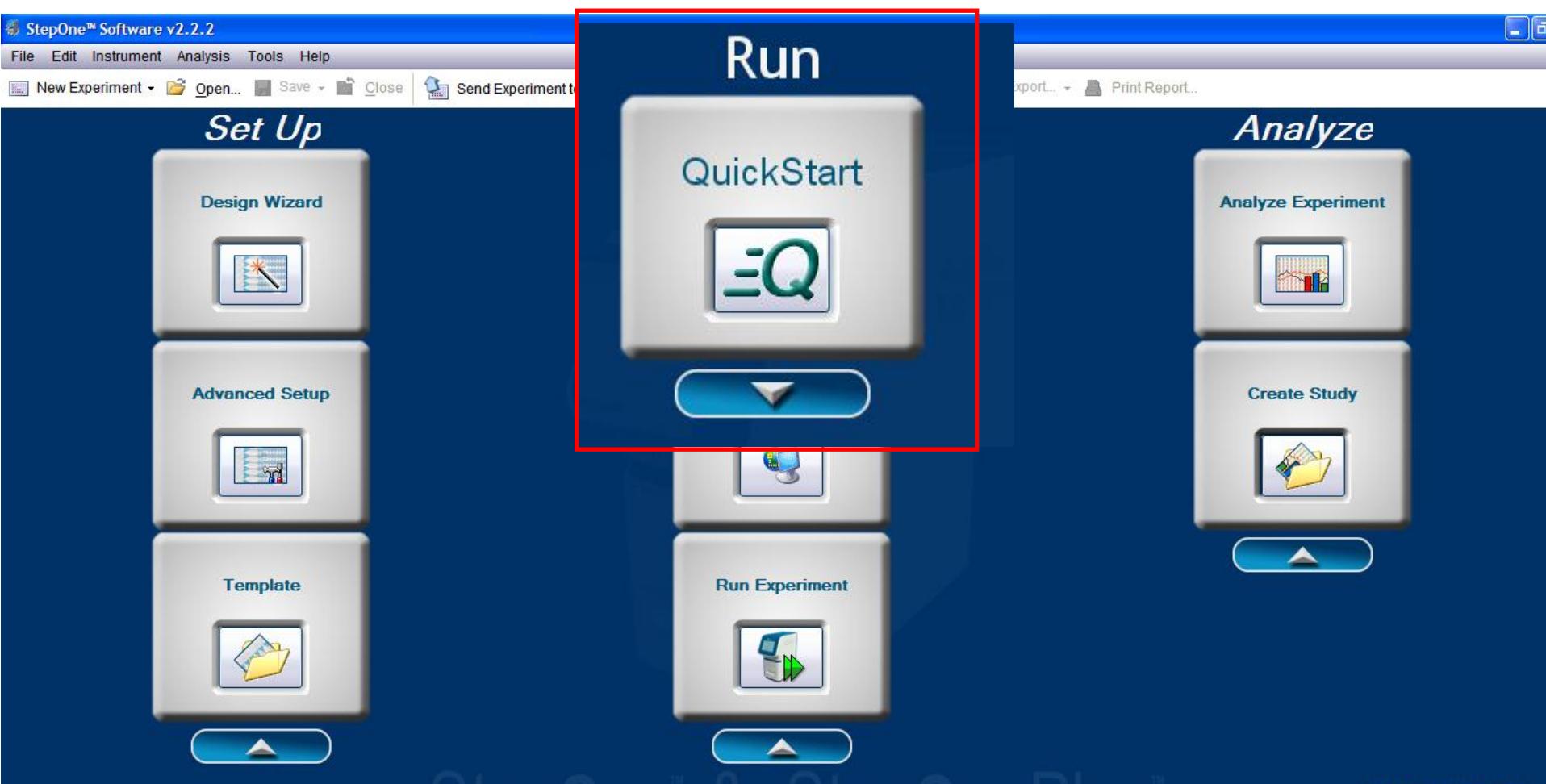


# StepOne™ v2.3 Software

- 一套軟體可以符合全方位的應用 (1280x1024 pixel resolution)
- 絶對定量 Quantification - Standard Curve
- 相對定量 Quantification – Comparative Ct ( $\Delta\Delta Ct$ )
- 相對定量 Quantification - Relative Standard Curve
- Melting Curve Analysis
- Genotyping
- Presence/Absence



# 1. Run: QuickStart



## 2. Setup: Experiment Properties

### a. Experiment Name 及檔案儲存位置

Enter Experiment Name and Location

• Enter Experiment Name: AB      • Location: D:\Applied Biosystems\StepOne System\experiments\AB.eds     

### b. 選擇 Experiment Type

• What type of experiment do you want to set up?

Quantitation - Standard Curve      Quantitation - Relative Standard Curve      Quantitation - Comparative Ct ( $\Delta\Delta Ct$ )  
 Melt Curve       Genotyping       Presence/Absence

### c. 選擇使用螢光系統

Select Reagents

TaqMan® Reagents      SYBR® Green Reagents (No Melt Curve)      SYBR® Green Reagents (With Melt Curve)  
 Other

### d. 選擇 Ramp Speed

Which ramp speed do you want to include in the instrument run?

Standard (~ 2 hours to complete a run)      Fast (~ 40 minutes to complete a run)

### e. 選擇 實驗樣品種類

What type of template do you want to use in the real-time PCR reactions?

cDNA (complementary DNA)      RNA      gDNA (genomic DNA)

### 3. Setup: Run Method

Setup

- Experiment Prop...
- Plate Setup
- Run Method
- Reaction Setup
- Materials List

Set 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.

abular View

We 20  $\mu\text{L}$

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

Holding Stage	Cycling Stage	Melt Curve Stage
	<p>Number of Cycles: 40</p> <p><input type="checkbox"/> Enable Auto Δ</p> <p>Starting Cycle: 2</p> <p>The graph displays a temperature scale from 0 to 100 °C and a time scale from 0 to 12 hours. The profile starts at 25°C, rises to 95.0°C at 10:00, remains constant through 40 cycles, and then falls to 60.0°C at 01:00. It then rises to 95.0°C at 00:15, remains constant through another set of cycles, and finally rises to 95.0°C at 00:15.</p>	

4. **START RUN** ➤

## 5. Setup: Plate Setup 定義基因和樣品名稱

The screenshot shows the Thermo Fisher QPCR software interface. On the left, a vertical menu bar lists: Setup, Experiment Prop..., Plate Setup, Run Method, Reaction Setup, Materials List, Run, and Analysis. The 'Plate Setup' option is selected. The main window title is 'Define Targets and Sample'. A red box highlights the title bar, and a red arrow points from the Chinese text '輸入偵測的基因及使用的螢光' (Input detection genes and used fluorescence) to the 'Define Targets' section. Another red arrow points from the Chinese text '輸入樣品名稱' (Input sample name) to the 'Define Samples' section. The 'Reagents: TaqMan® Reagents' label is at the top right, along with a 'START ...' button and a help icon. The 'Define Targets' section contains buttons for 'Add New Target', 'Add Saved Target', 'Save Target', and dropdown fields for 'Target Name' (Target 1), 'Reporter' (FAM), 'Quencher' (NFQ-MGB), and 'Co...'. The 'Define Samples' section contains buttons for 'Add New Sample', 'Add Saved Sample', 'Save Sample', and a dropdown field for 'Sample Name' (Sample 1). A large button at the bottom right says 'Assign Targets and Samples'.

Define Targets and Sample

Reagents: TaqMan® Reagents

START ...

Define Targets and Samples Assign Targets and Samples

Instructions: Define the targets to quantify and the samples to test in the reaction plate.

Define Targets

Add New Target Add Saved Target Save Target ►

Target Name	Reporter	Quencher	Co...
Target 1	FAM	NFQ-MGB	

Define Samples

Add New Sample Add Saved Sample Save Sample ►

Sample Name
Sample 1

Assign Targets and Samples

輸入偵測的基因及  
使用的螢光

輸入樣品名稱

## 6. Setup: Plate Setup 決定基因和樣品位置

### Assign Targets and Samples

#### I Instructions:

To set up unknowns: select wells, assign target(s), select "Unknown (double-click U icon)" as the task for each target assignment, then assign a sample.

To set up negative controls: select wells, assign target(s), then select "Negative Control (double-click N icon)" as the task for each target assignment.

Assign target(s) to the selected wells.

Assign	Target	Task
<input type="checkbox"/>	U	<input type="checkbox"/>
*	Mixed	<input checked="" type="checkbox"/> Unknown

Assign sample(s) to the selected wells.

Assign	Sample
<input type="checkbox"/>	1

Select relative quantitation settings.

Reference Sample:

Endogenous Control:

Select the dye to use as the passive reference.

ROX

[View Plate Layout](#) [View Well Table](#)

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

	1	2	3	4	5	6	7	8
A	<input checked="" type="checkbox"/>							
B								
C								
D								
E								
F								

Wells:  0 Unknown  0 Negative Control

48 Empty

圈選樣品擺放位置，再從左邊勾選樣品名稱與偵測的基因

選擇Reference Sample & Endogenous Control Gene

# 6. Setup: Plate Setup 決定標準品位置



## Automatic Standard Curve Setup

Define Targets and Samples    Assign Targets and Samples

To set up standards: Click "Define and Set Up Standards."

- Instructions:** To set up unknowns: Select wells, assign target(s), select "U" (Unknown) as the task for each target assignment, then assign a sample.  
To set up negative controls: Select wells, assign target(s), then select "N" (Negative Control) as the task for each target assignment.

Assign target(s) to the selected wells.

Assign	Target	Task	Quantity
<input type="checkbox"/>	IL5	<span style="background-color: blue; color: white;">U</span> <span style="background-color: orange; color: white;">S</span> <span style="background-color: grey;">N</span>	

\* Mixed U Unknown S Standard

**Define and Set Up Standards**

Assign sample(s) to the selected wells.

Assign	Sample
<input type="checkbox"/>	Sample 1

Select the dye to use as the passive reference.

ROX

< 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	
A									
B									
C									
D									
E									
F									

圈選樣品擺放位置，再從左邊勾選樣品名稱與偵測的基因

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

48 Empty

## 6. Setup: Plate Setup 決定標準品位置

**Define and Set Up Standards**

Select a target from the list of targets in the reaction plate. Define the standard curve, select wells for the standards, then click "Apply." Repeat for each standard curve in the reaction plate, then click "Close" to return to plate setup.

Select a target • = Required

• Select a target for the standards IL5

Define the standard curve • = Required

- # of Points: 5 5 Recommended
- # of Replicates: 3 3 Recommended
- Starting Quantity: 100.0 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 = ... Required Wells

Standard Curve Preview

Select and arrange wells for the standards

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

15 Required Wells / 15 Selected Wells

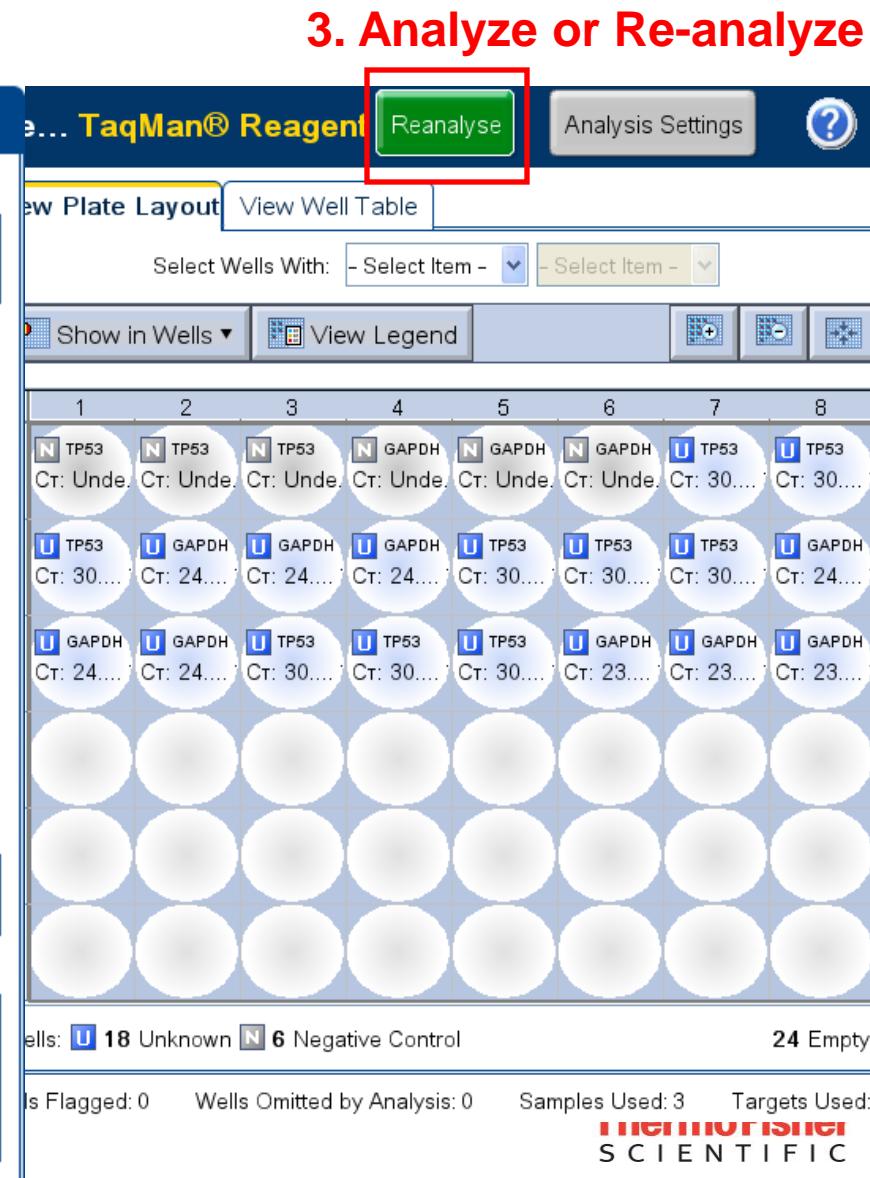
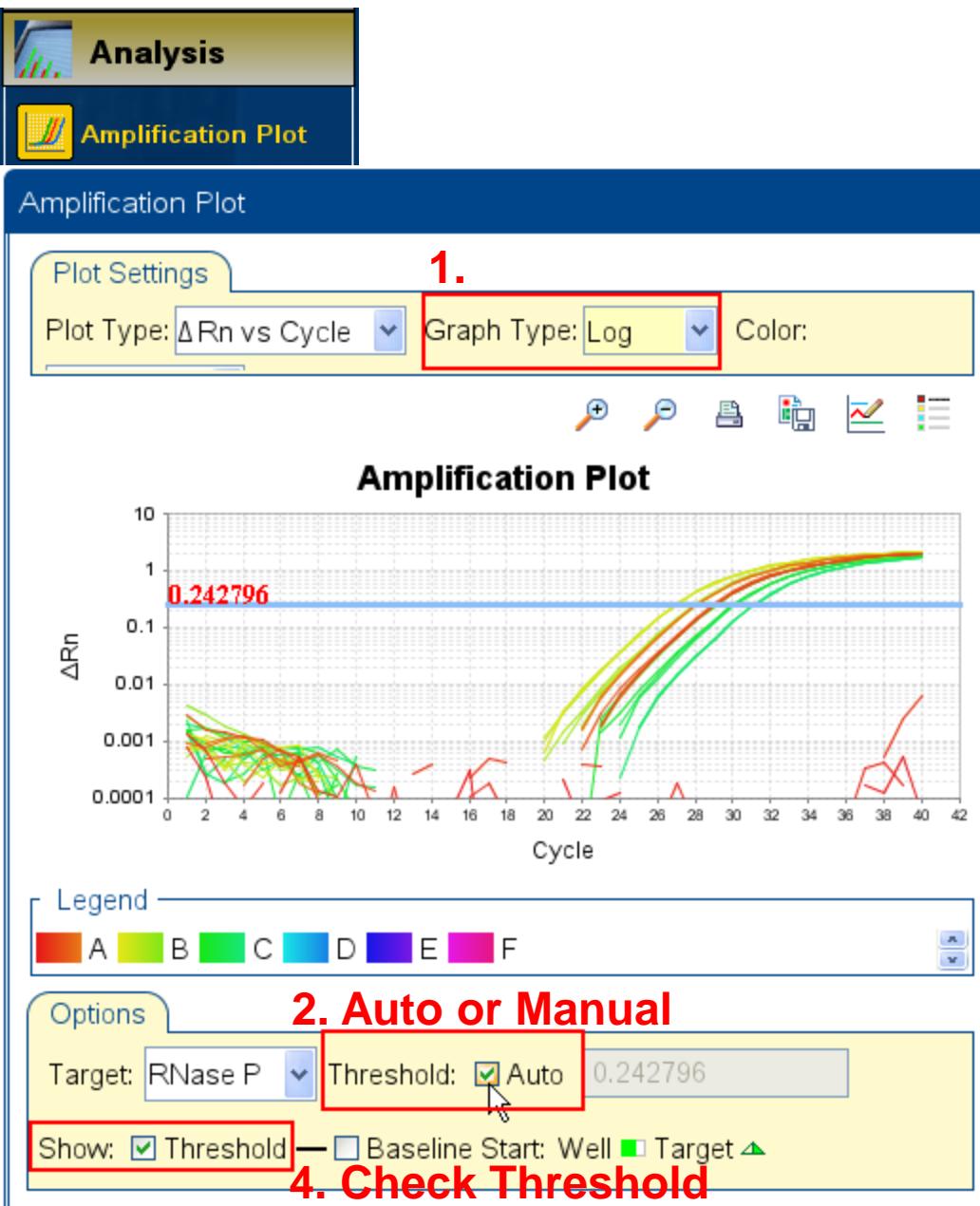
B8,C1,C2,C3,C4,C5,C6,C7,C8,D1,D2,D3,D4,D5,D6

	1	2	3	4	5	6	7	8
A	—	—	—	—	—	—	—	—
B	—	—	—	—	—	—	—	—
C	—	—	—	—	—	—	—	—
D	—	—	—	—	—	—	—	—
E	—	—	—	—	—	—	—	—
F	—	—	—	—	—	—	—	—

Arrange standards in:  Columns  Rows

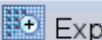
**Apply** **Reset Fields** **Close**

# 7. Analysis: Amplification Plot



# Analysis: View Well Table

Group By ▾



Target Name

Sample Name

## Task

### Replicate N

Dye

Flag

C1

## Comments

### WELL POSITION (ROW)

### WCH Position (Column)

None

## [View Plate Layout](#)

## **View Well Table**

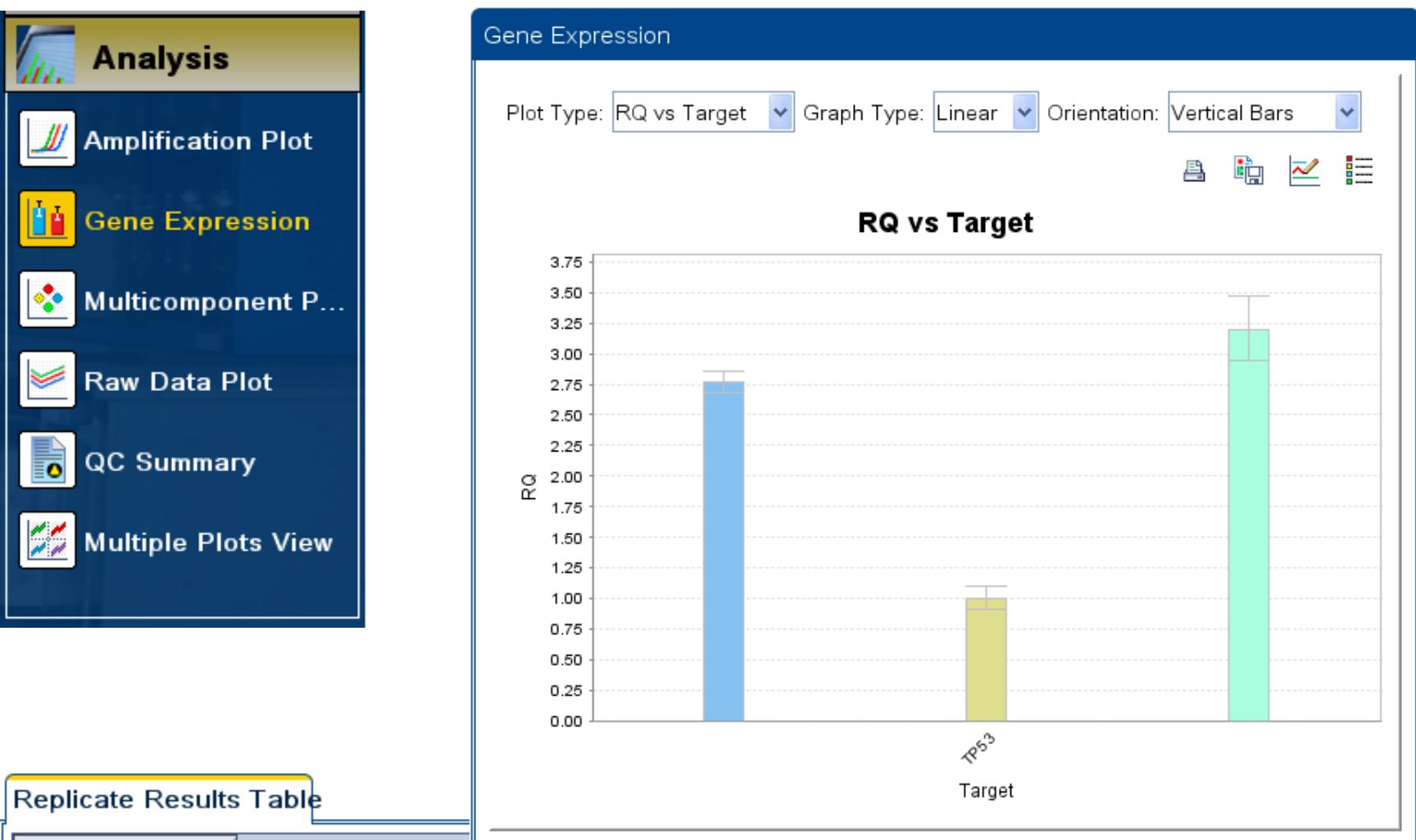
Select Wells With: - Select Item - ▾ - Select Item - ▾

Show in Table ▾

Group By ▾



# Analysis: Gene Expression

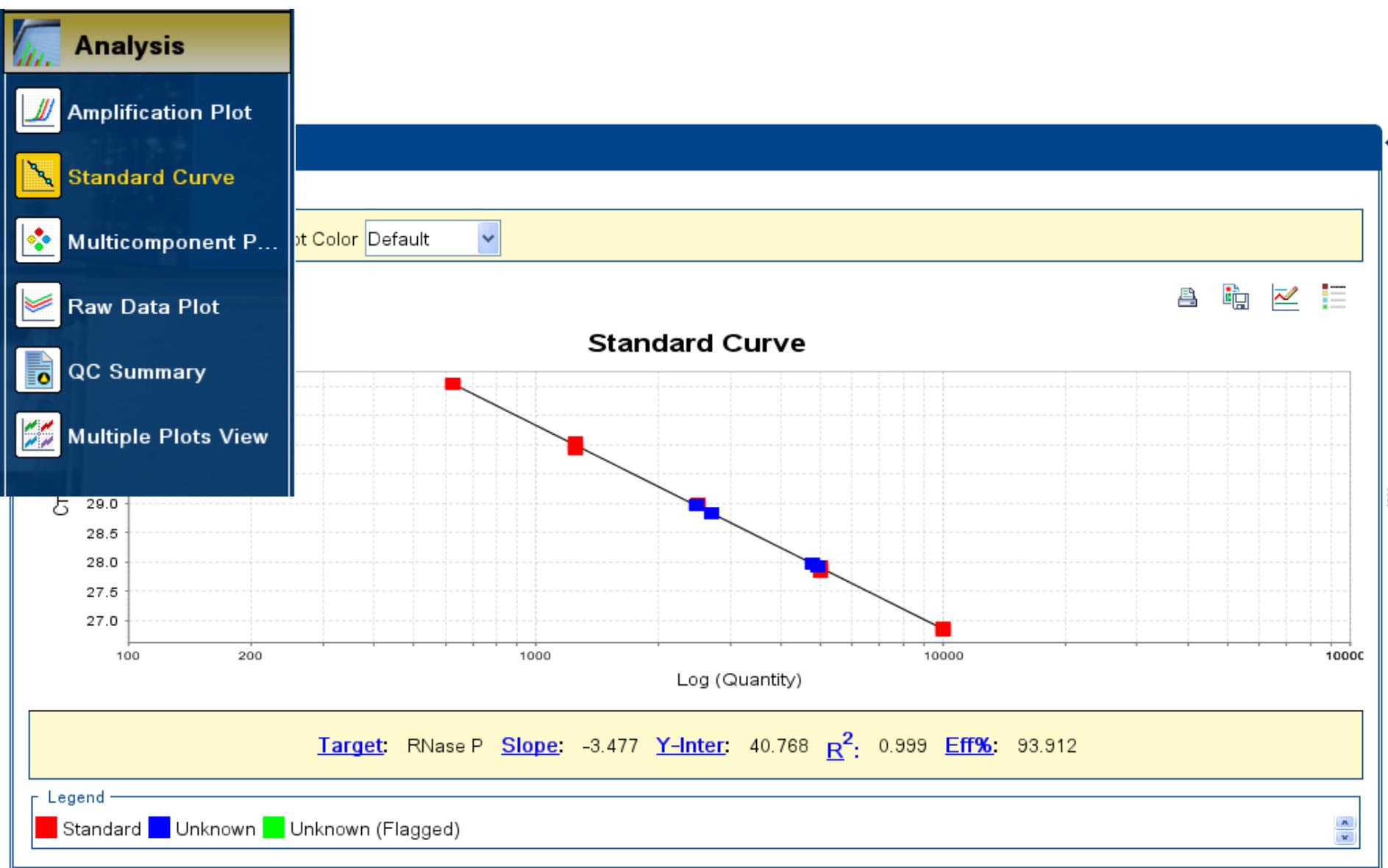


Replicate Results Table

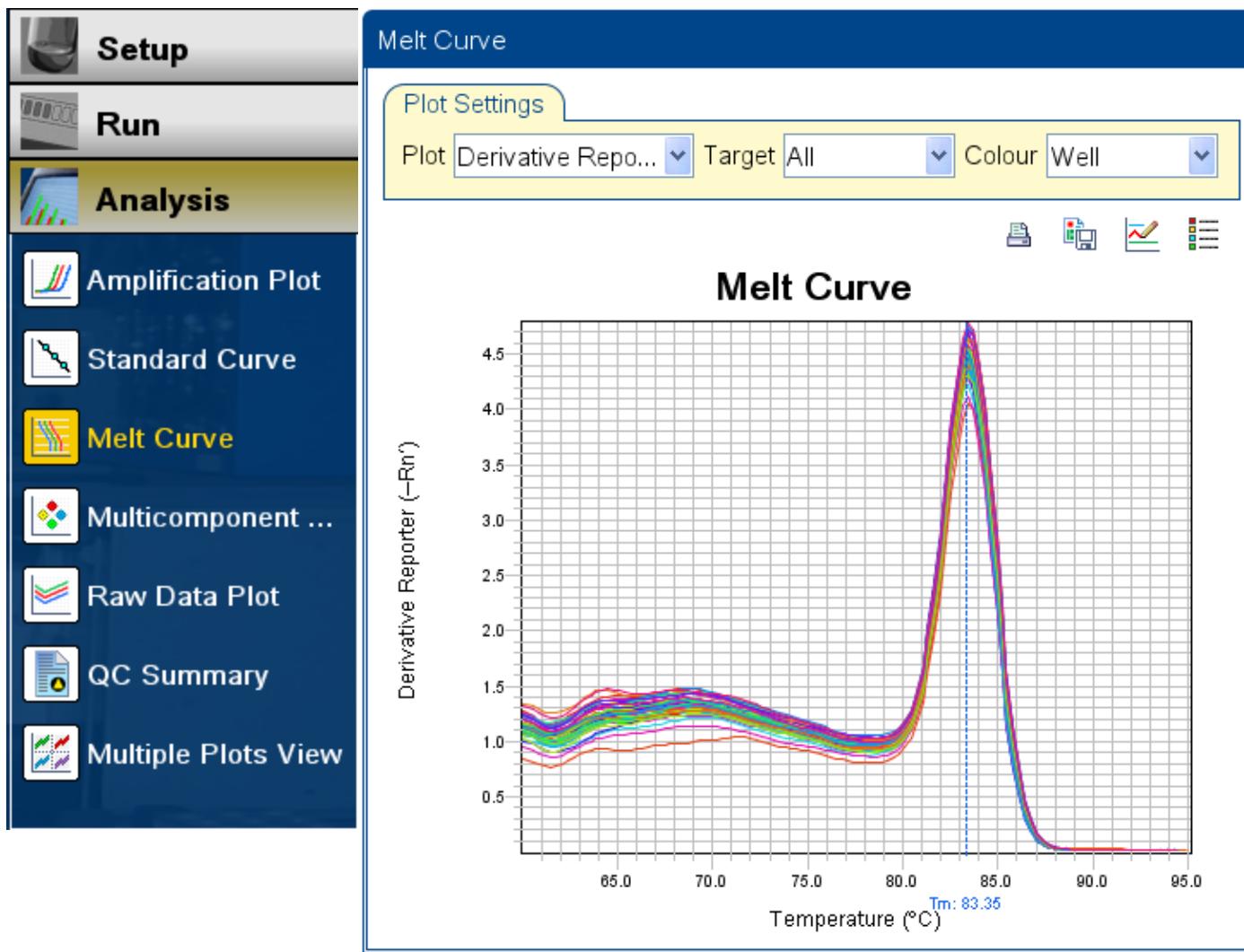
Show In Table ▾

#	Omit	Sample	Target	Ct Mean	ΔCt Mean	ΔCt SD	ΔCt SE	ΔΔCt	RQ	RQ Min	RQ Max
1	<input type="checkbox"/>	Liver	TP53	30.69	6.015	0.03	0.017	-1.47	2.77	2.68	2.863
2	<input type="checkbox"/>	Kidney	TP53	30.696	5.807	0.073	0.042	-1.677	3.198	2.948	3.469
3	<input type="checkbox"/>	Brain	TP53	30.912	7.484	0.083	0.048	0	1	0.912	1.097

# Analysis: Standard Curve



# Analysis: Melting Curve (SYBR Green)



# Analysis: QC Summary Helps with Troubleshooting

QC Summary

Flag Summary

Total Wells: 96 | Processed Wel... 65 | Manually Omitted Wel... 0 | Targets Used: 5  
Wells Set ... 65 | Flagged Wells: 21 | Analysis Omitted Wells: 0 | Samples Us... 4

Flag Details

Flag:	Name	Frequen...	Wells
AMPNC	Amplification in negative control	2	F1, F3
BADROX	Bad passive reference signal	0	
OFFSCALE	Fluorescence is offscale	0	
HIGHSD	High standard deviation in replic...	6	C7, C8, C9, C1...
NOAMP	No amplification	0	
NOISE	Noise higher than others in plate	0	
SPIKE	Noise spikes	0	
NOSIGNAL	No signal in well	0	
OUTLIER...	Outlier in replicate group	0	
EXPFAIL	Exponential algorithm failed	0	
RI FAIL	Baseline algorithm failed	0	

Flag: AMPNC—Amplification in negative control

Flag Detail: A sequence amplified in a negative control reaction.

Flag Criteria:  $\text{C}_T < 35.0$

Flagged Wells: F1, F3

[View AMPNC Troubleshooting Information](#)

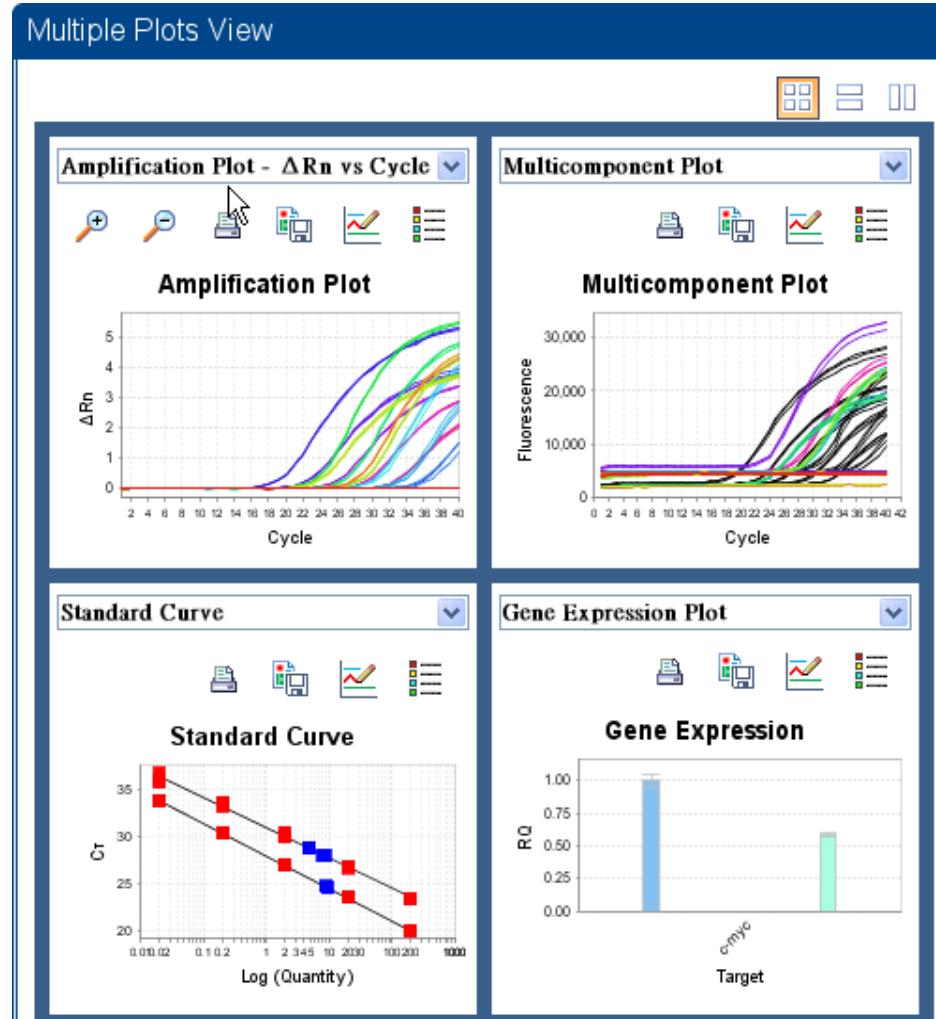
View Plate Layout | View We...

Select Wells Wit...

Show in Wells ▾

	1	2	3	4
A	1 U He... CT: ...	1 U He... CT: ...	NP U He... CT: ...	NPA U H... CT: ...
B	1 U He... CT: ...	NP U He... CT: ...	1 U He... CT: ...	NPA U He... CT: ...
C	NP U He... CT: ...	NP U He... CT: ...	1 U He... CT: ...	NPA U He... CT: ...
D	NP U Sa... CT: ...	NP U Sa... CT: ...	NP U Sa... CT: ...	NPA U Sa... CT: ...
E	NP U Qa... CT: ...	NP U Qa... CT: ...	NP U Qa... CT: ...	NPA U Qa... CT: ...
F	2 N He... CT: ...	N He... CT: ...	1 N He... CT: ...	N Sa... CT: ...
G				
H				

# Export to Excel, PowerPoint or save as jpeg



# Real-time PCR 中文線上講座

<http://www.thermofisher.com/tw/en/home/taiwan/real-time-pcr-webinars/real-time-pcr-experimental-configuration.html>

The screenshot shows the Thermo Fisher website's header with links for Life Sciences, Applied Sciences, Clinical, Shop All Products, Services & Support, About Us, and Cloud. A search bar and a shopping cart icon are also present. Below the header, a breadcrumb navigation shows 'Home > Real-Time PCR 實驗配置'. On the left, a sidebar lists various guides and protocols related to Real-Time PCR, such as '7500 相關耗材及實際儀器上機操作說明', '7500 2.0 軟體操作及結果分析說明', 'StepOne 2.1 軟體上機分析說明 Part I', 'StepOne 2.1 軟體上機分析說明 Part II', '7900 HT 相關使用耗材及儀器上機操作說明', 'Real-Time PCR 原理', 'Real-Time PCR 實驗設計', 'Primer & TaqMan Probe 設計方案', 'Primer Express v2.0 軟體操作流程介紹', and 'Primer Express v3.0 軟體操作流程介紹'. The main content area features a large image of a barcode and a gel electrophoresis pattern. The title 'Real-Time PCR 實驗配置說明' is displayed prominently. Below the title, a video player shows a thumbnail of a lab technician operating a PCR machine, with the text 'Real-Time PCR RT-PCR protocol' overlaid. To the right of the video, a text box states: '在這堂 15 分鐘的線上教學課程中，主講者將詳細介紹進行 Real-Time PCR 實驗配置，包含 Reverse Transcription 及 Real-Time PCR 反應配置流程。' A photo of the speaker, Wei-Hsing Han, is shown next to her name: '主講人 - 韓世芸，技術應用專家，萊富生命科技股份有限公司 (Life Technologies)'. A button at the bottom right says '現在就點選進入課程'.

**Thank You!**

技術服務E-mail: [Support.TW@lifetech.com](mailto:Support.TW@lifetech.com)

訂貨及維修服務專線: 0800-251-326