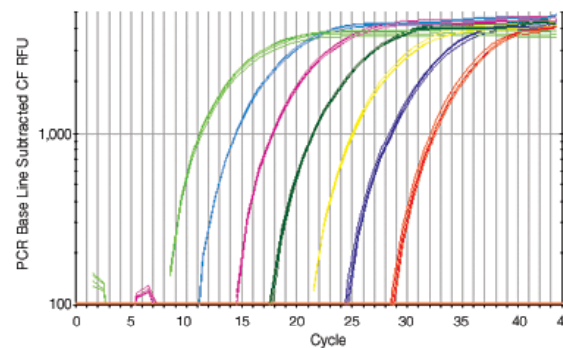




KIM FOREST ENTERPRISE CO.,LTD

聚合酶連鎖反應儀儀器操作(StepOnePlus) 暨 基因定量技術應用說明會 (qPCR 原理介紹與基本操作)



產品經理
鄒志成 博士 Ryan
金萬林企業股份有限公司



金萬林企業股份有限公司

1. Real-time PCR 原理暨技術應用介紹
2. 試劑配製注意事項Primer/ Probe 設計介紹
3. StepOnePlus 儀器介紹及使用注意事項
4. StepOnePlus 軟體簡介及結果分析演練

1. Real-time PCR 原理暨技術應用介紹

2. 試劑配製注意事項Primer/ Probe 設計介紹

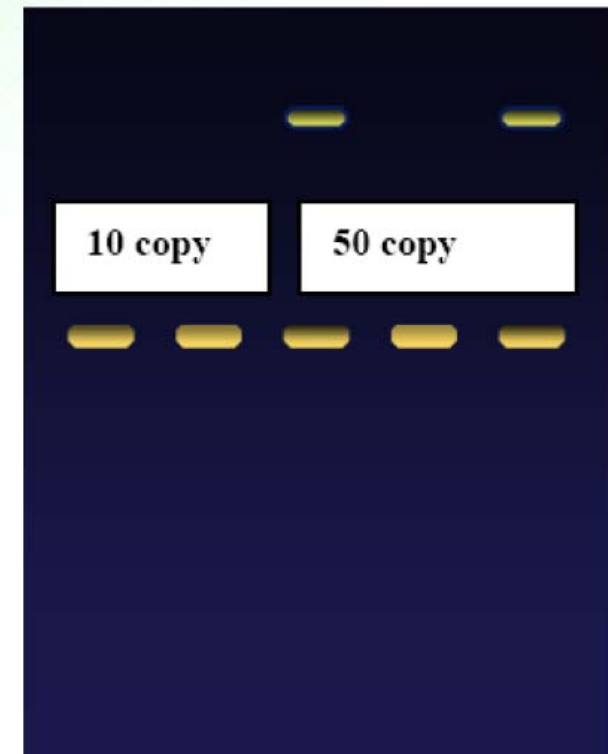
3. StepOnePlus 儀器介紹及使用注意事項

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Limitation of End point PCR



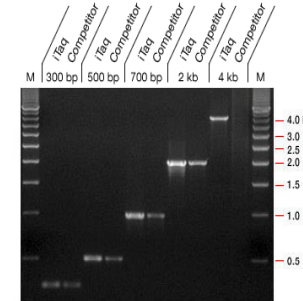
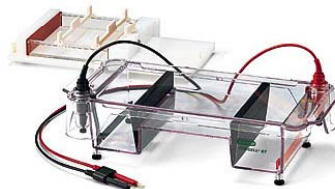
- 精確性差
- 靈敏度低
- 解析度低
- 無法自動化
- 只能藉由產物大小不同來分辨
- 結果無法以數字方式呈現
- 以EtBr染色無法達到定量效果
- PCR結束後仍需許多步驟



傳統PCR v.s qPCR



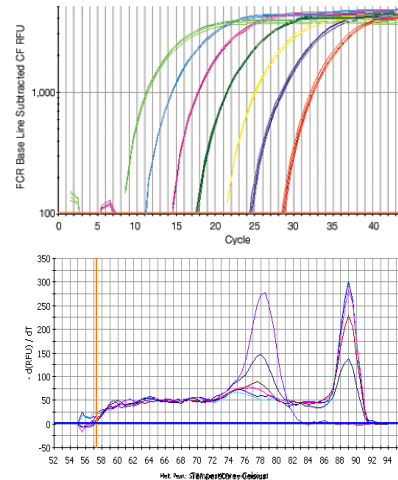
+Taq
+dNTP
+MgCl₂
+H₂O
+Buffer
+Template
+Primer
(On ice)



- End point
- Fuzzy data
- Labor intensive

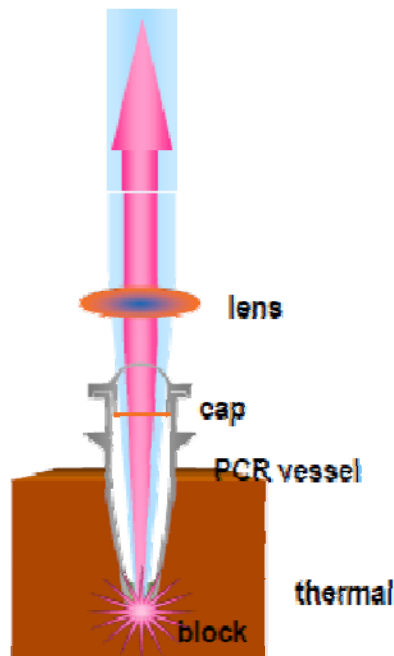
> 3 hrs(1.5 hrs labor)

+2x Master Mix
+H₂O
+Template
+Primer
(Room Temp)



- Online monitoring
- More sensitive
- Automated

<1 hrs (20 min labor)



1. 利用螢光物質與DNA片段結合的特性，來達到偵測的目的
2. 隨著反應進行，螢光強度也將隨之增加
3. 觀察螢光的強度，即可得到欲偵測物的起始濃度差異
4. 依據此原理，可進行多種實驗應用

TaqMan® Probe



Specificity

- Highly specific
- Probe Hybridization

Sensitivity

- Very High

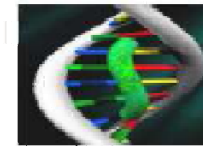
Flexibility

- Multiplex PCR
- SNP detection
- +/- application

Optimization

- Universal guideline
- Optimized 20x probe/primer mix

SYBR® Green 1 Dye



- Non-specific

- Very High

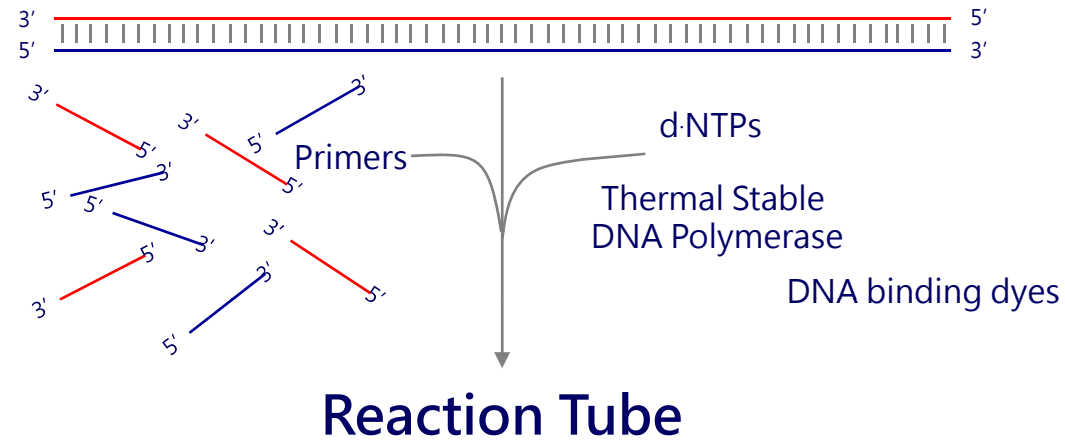
- No Probe is required
- Screening tool

- Universal Guideline
- Need to check primer-dimer information

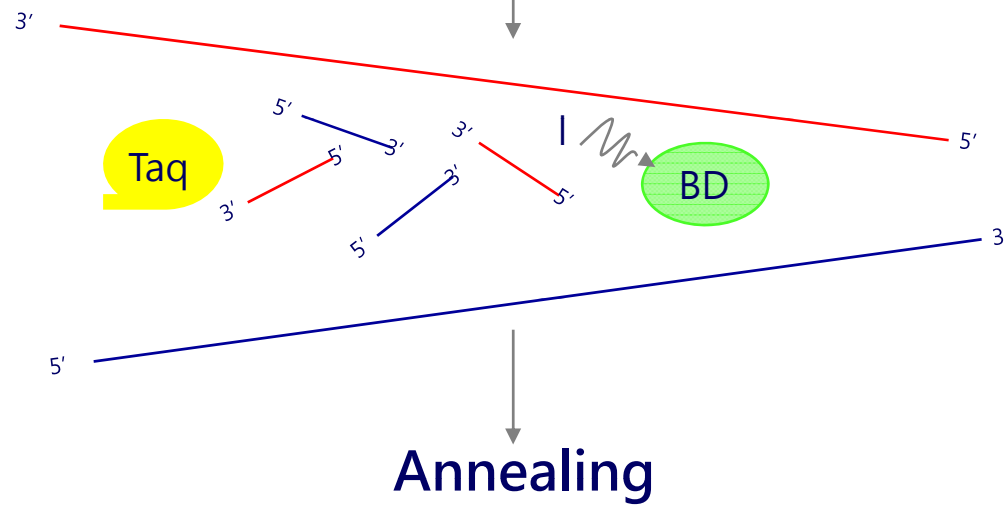
SYBR Green Dye



Add Master Mix
& Sample



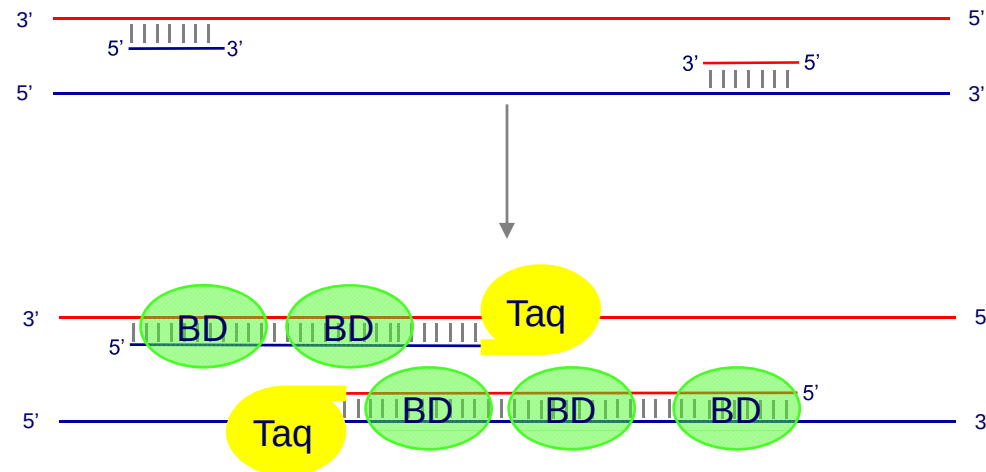
Denaturation



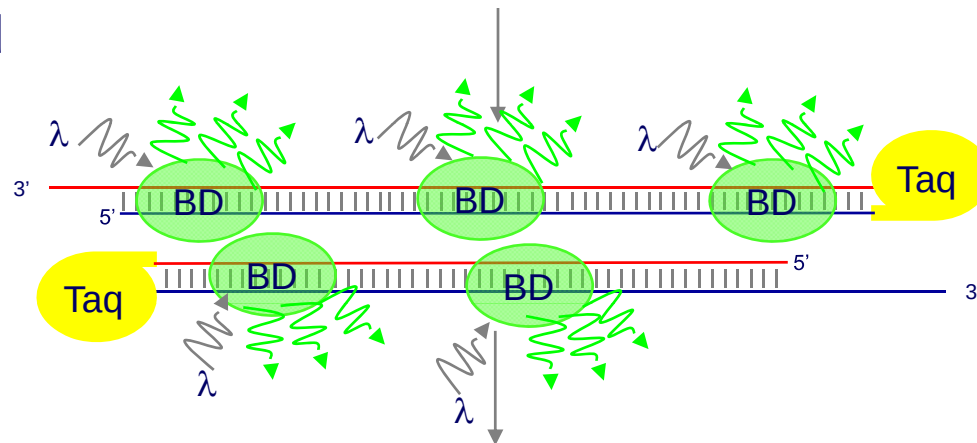
SYBR Green Dye



Extension



**Extension Continued
Apply Excitation
Wavelength**

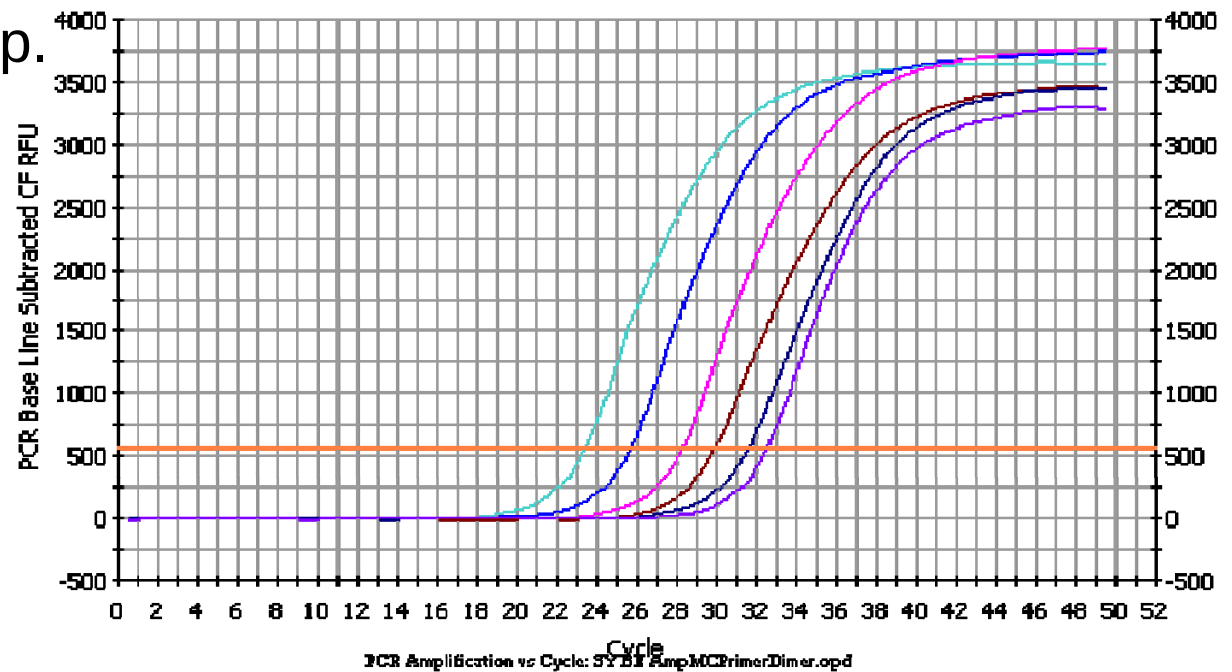


Repeat

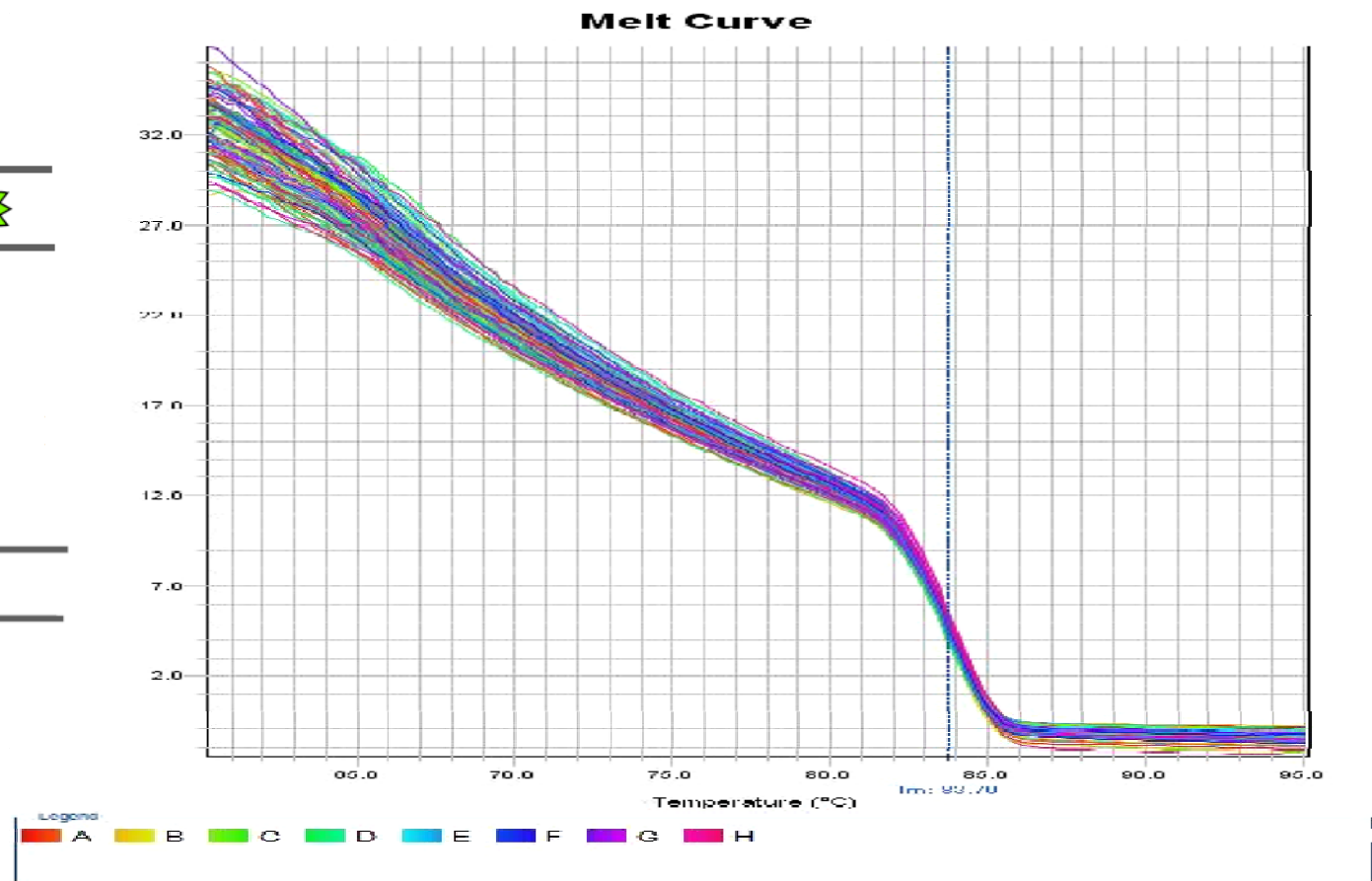
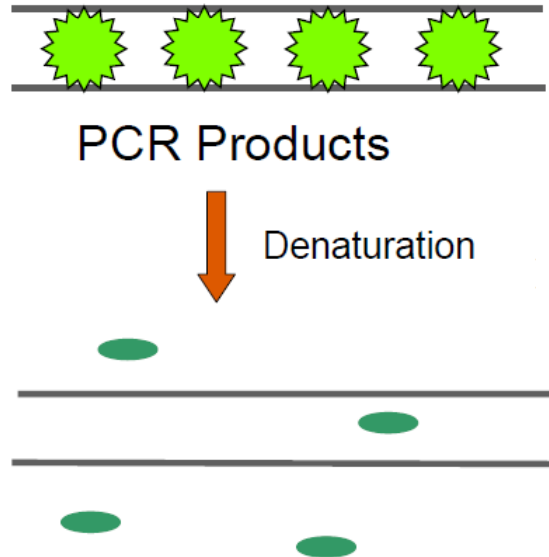
SYBR Green Dye



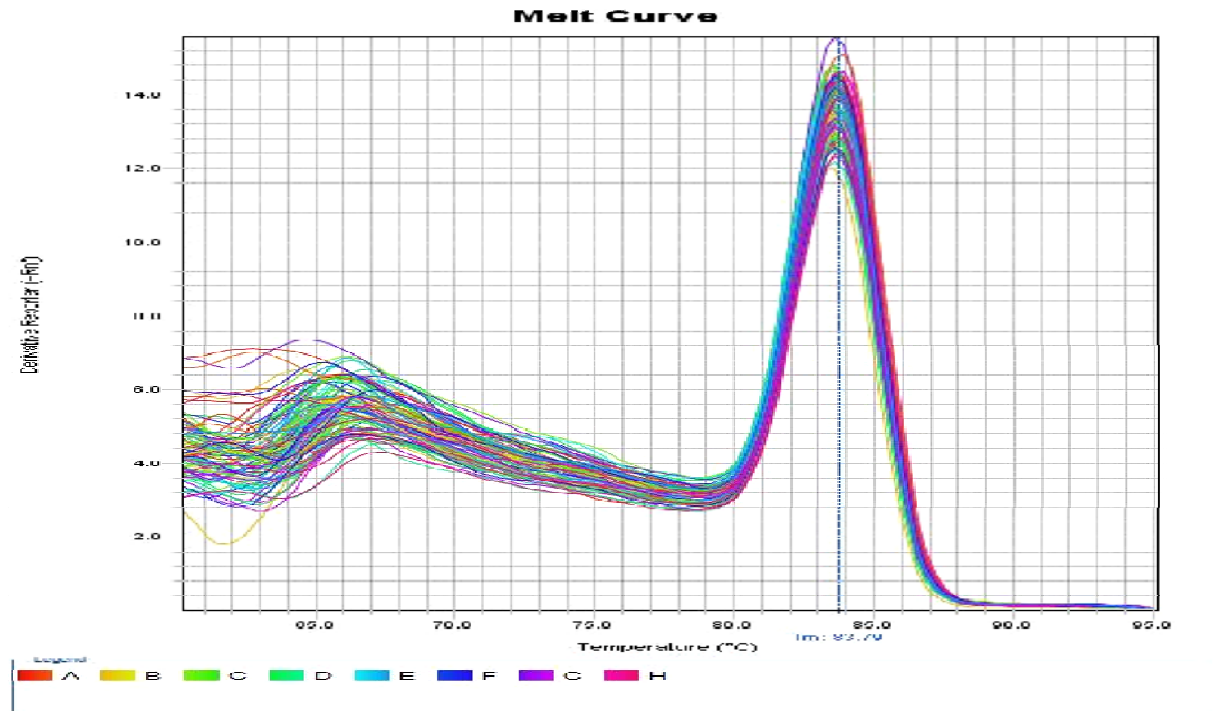
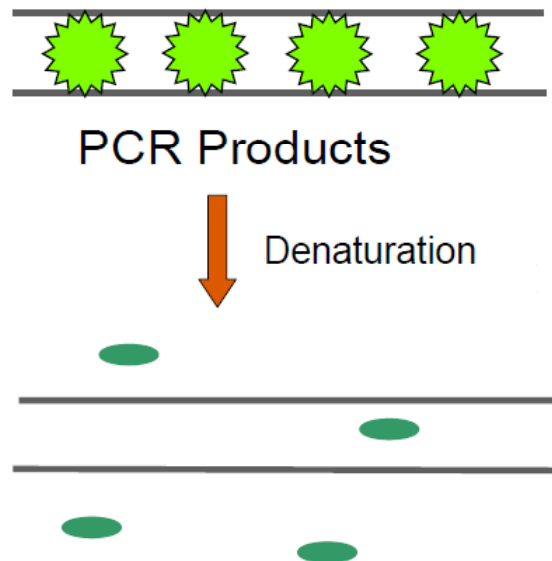
- Easy to start qPCR Exp.
- Inexpensive
- Accurate
- Large dynamic range
- Primer specificity
- Single target



Melt Curve Analysis (SYBR Green Dye)



Melt Curve Analysis (SYBR Green Dye)



- * Use **NTC** to check whether the nonspecific product is primer dimer
- * If the nonspecific product is primer dimer:
 1. try **optimization of the primer concentration**
 2. **Change Primer**

qPCR常用螢光偵測方法



TaqMan® Probe



Specificity

- Highly specific
- Probe Hybridization

Sensitivity

- Very High

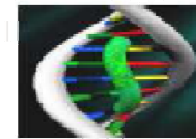
Flexibility

- Multiplex PCR
- SNP detection
- +/- application

Optimization

- Universal guideline
- Optimized 20x probe/primer mix

SYBR® Green 1 Dye



- Non-specific

- Very High

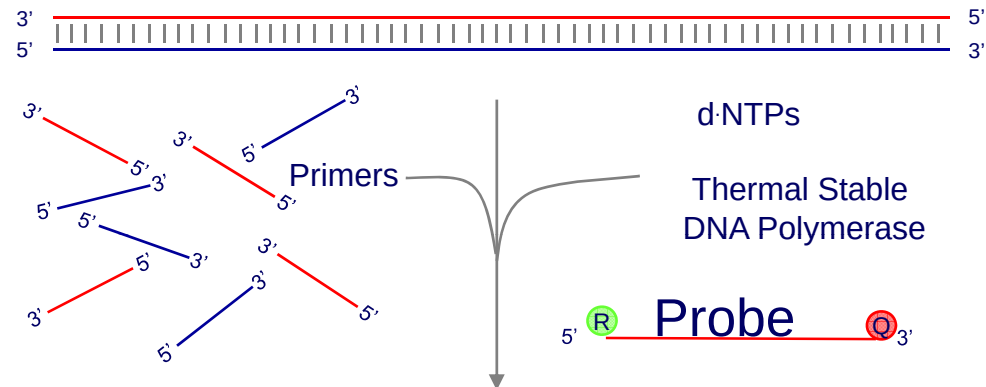
- No Probe is required
- Screening tool

- *Universal Guideline*
- Need to check primer-dimer information

Taqman® probe

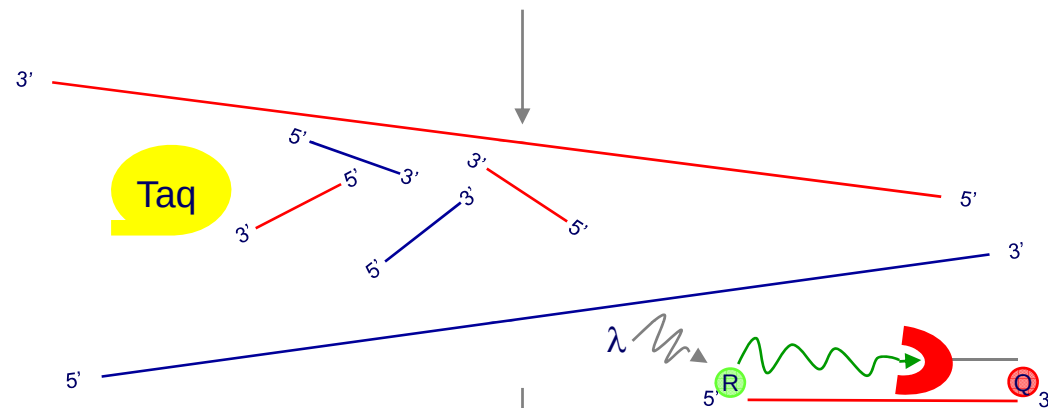


**Add Master Mix
& Sample**



Reaction Tube

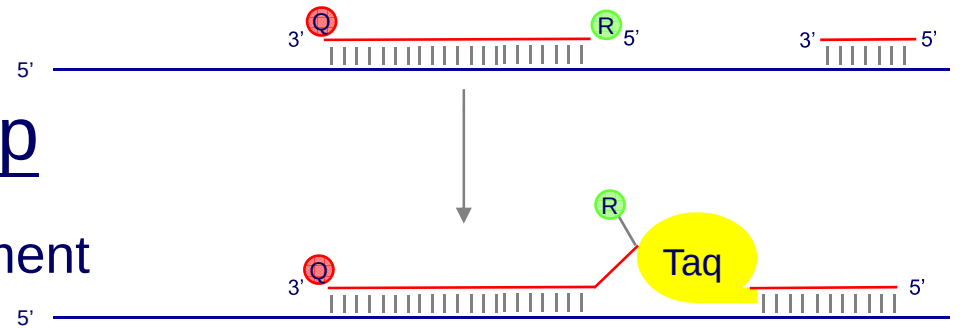
Denaturation



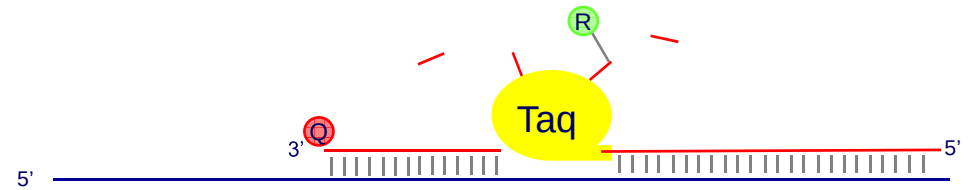
Annealing

Extension Step

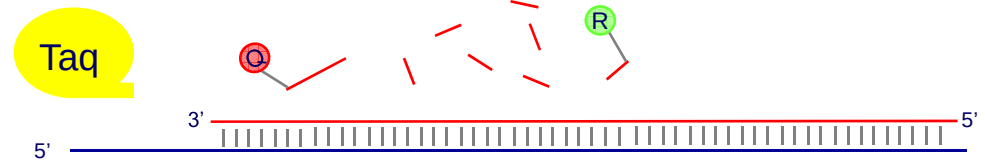
1. Strand Displacement



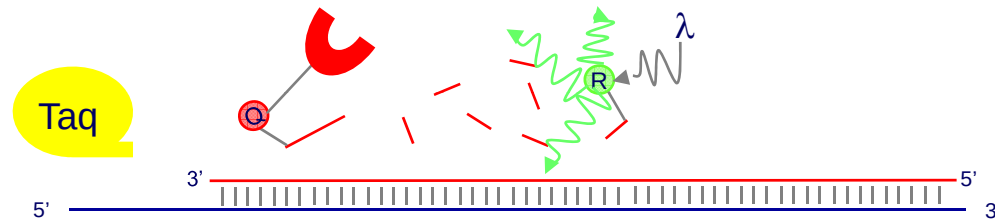
2. Cleavage



3. Polymerization Complete



4. Detection



TaqMan® MGB/NFQ Probes

Minor Groove Binder (MGB)

Small molecule that fits snugly into the minor groove of duplex DNA

Stabilizes probe annealing

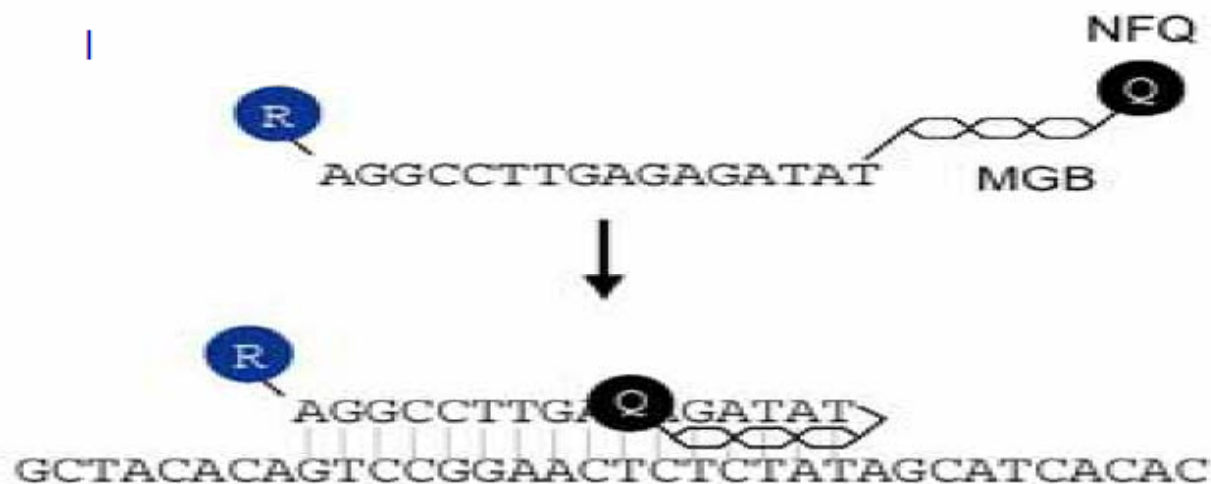
Non Fluorescent Quencher (NFQ)

“Dark” quencher

Acts as energy transfer acceptor that does not emit a detectable fluorescent signal

MGB probe design uses a special algorithm in Primer Express® Software.

All probes will be short (13-25-mers)





基因定量

- 檢測基因轉殖食品(GMO)
- 癌症基因及免疫基因的定量
- 病毒定量: HBV, HCV, HPV...
- miRNA 基因調控研究
- 基因套數變化(CNV)

定性研究

- 基因型研究
 - SNP與疾病關聯性
- 素食摻葷
- 病原菌偵測

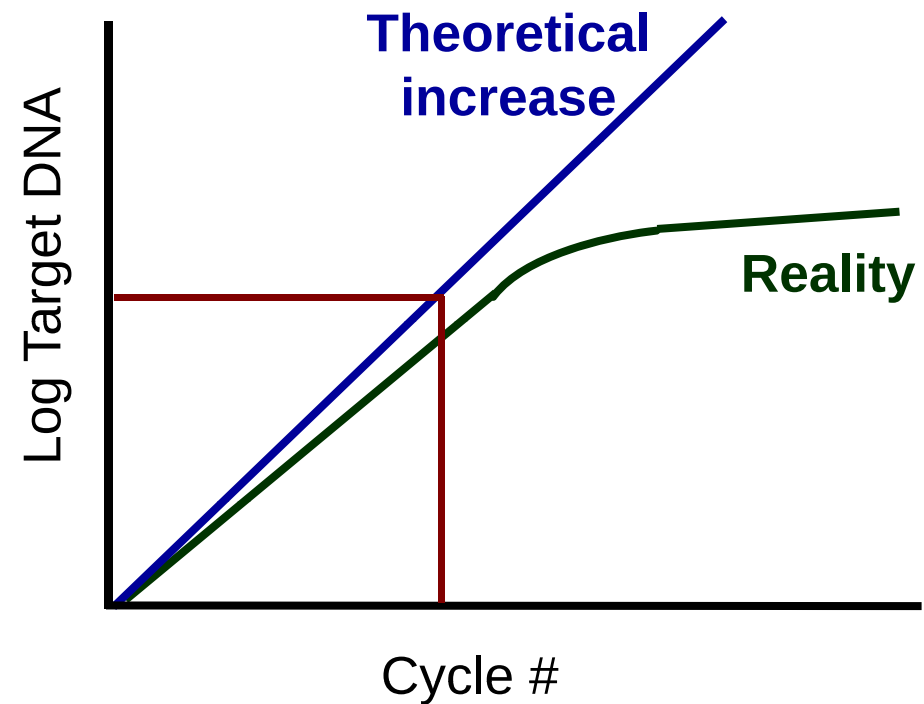
Protein detection

$$\text{產物量} = \text{起始濃度} \times 2^n$$

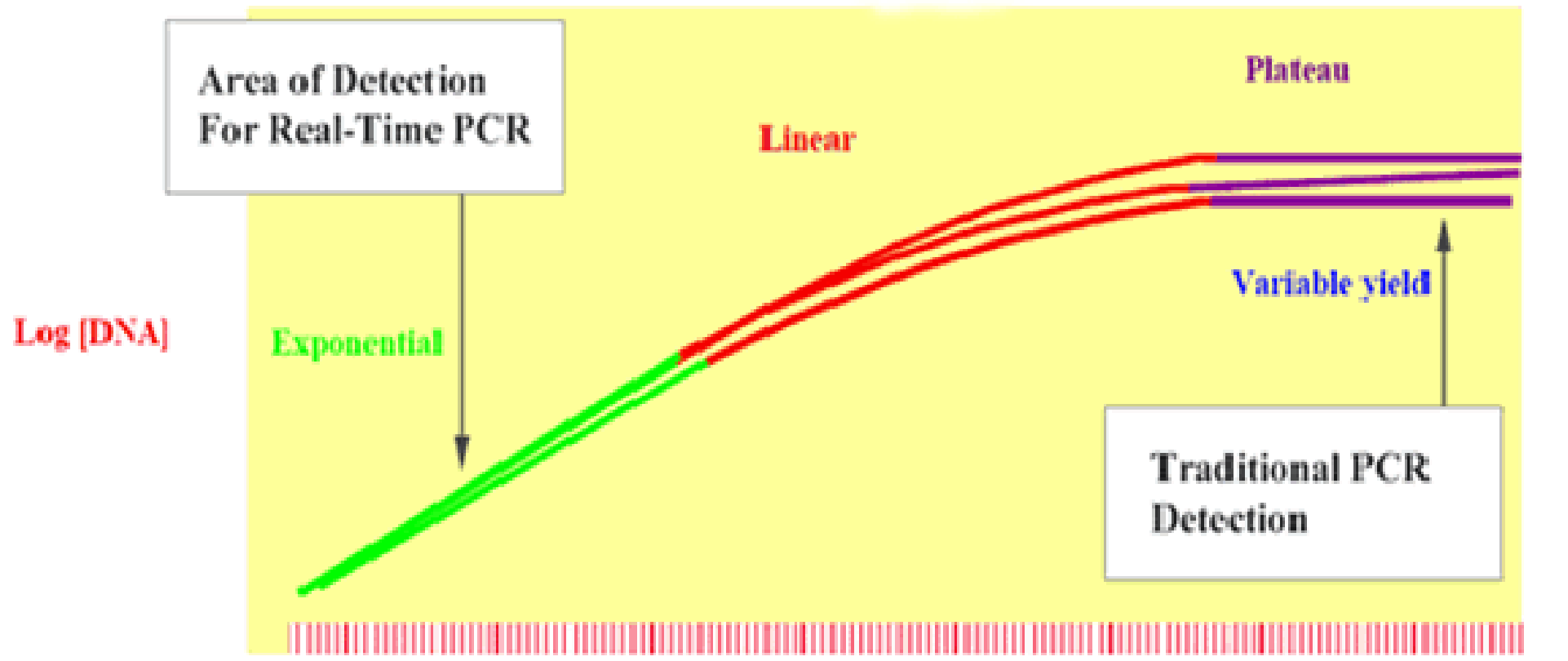


$$\text{起始濃度} = \frac{\text{產物量}}{2^n} = \text{產物量} \times 2^{-n}$$

- In theory, PCR reactions amplify exponentially, doubling every cycle and grow forever.
- In reality,



Amplification plot



- Exact doubling of product
- Reactions is very precise and specific

- Cycle #**
- The reaction components are becoming limited
 - The reaction efficiency is dropping

- The reaction has stopped
- No more products are being made

$$\text{產物量} = \text{起始濃度} \times 2^n$$

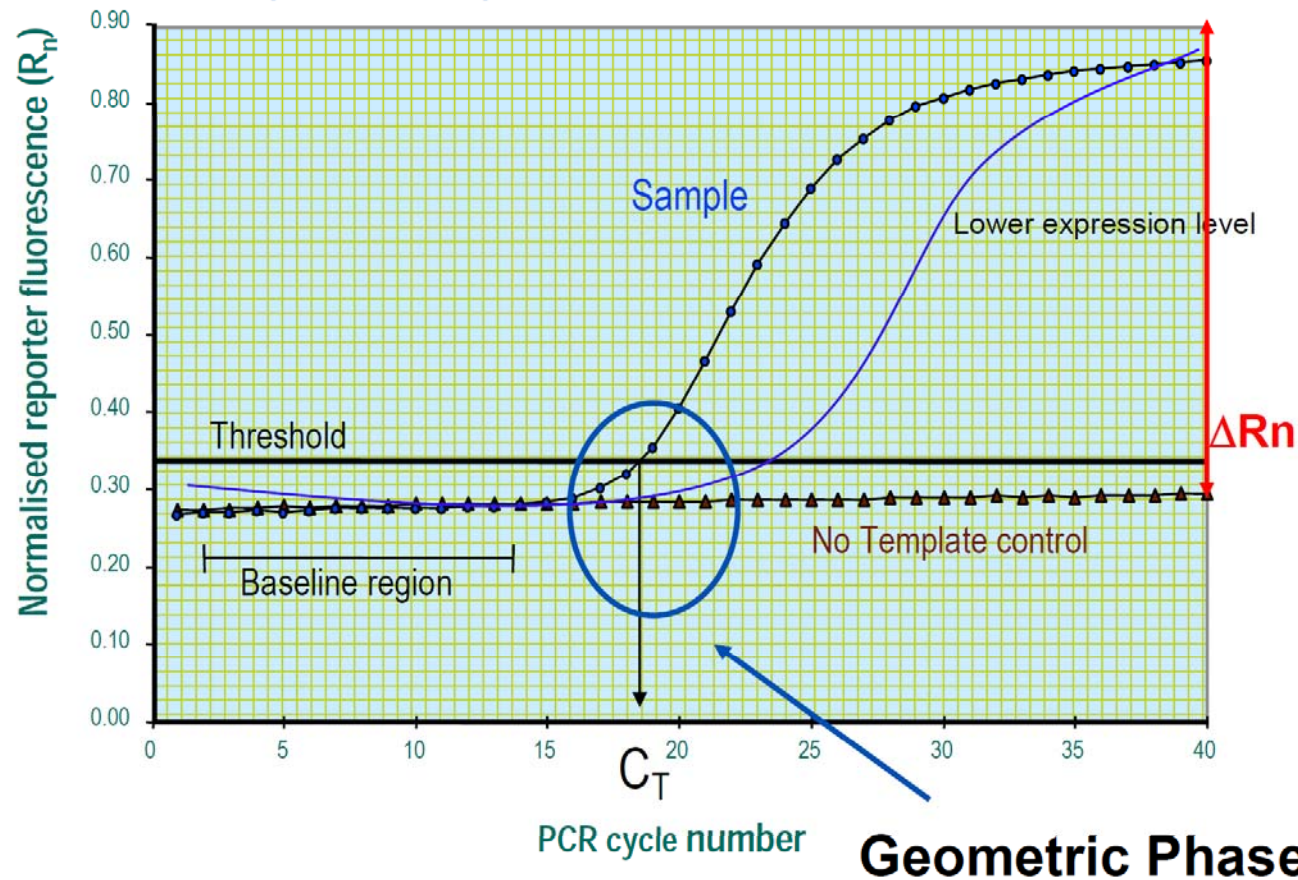


$$\text{起始濃度} = \frac{\text{產物量}}{2^n} = \text{產物量} \times 2^{-n}$$

$$\text{高起始濃度} = \frac{\text{產物量}}{2^n} = \text{產物量} \times 2^{-n}$$

$$\text{低起始濃度} = \frac{\text{產物量}}{2^n} = \text{產物量} \times 2^{-n}$$

The point at which the fluorescence rises appreciably above background



Threshold Cycle, C_t

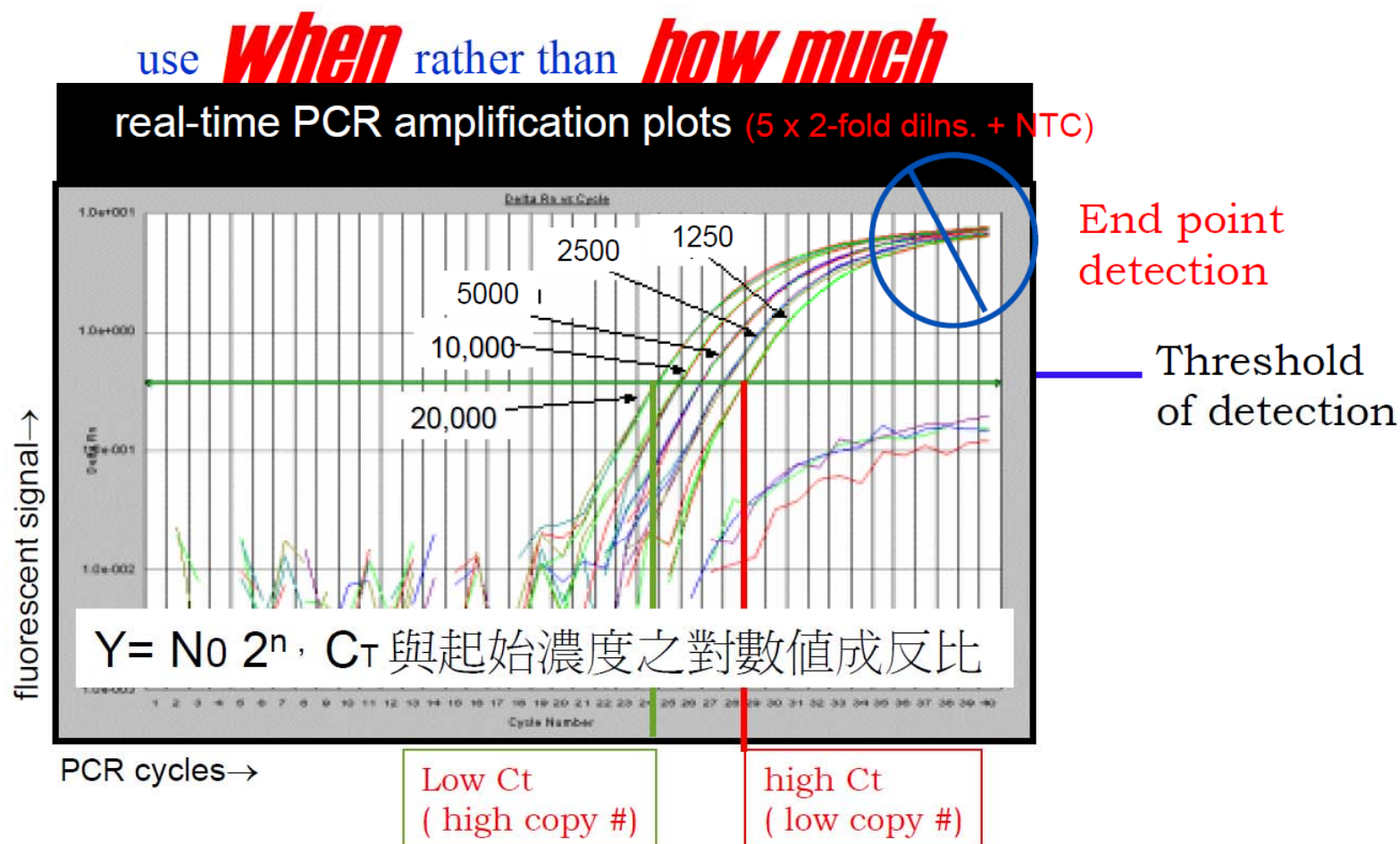
$Y = N_0 2^n$, C_T 與起始濃度之對數值成反比

高 — 少 →

低 — 多 →

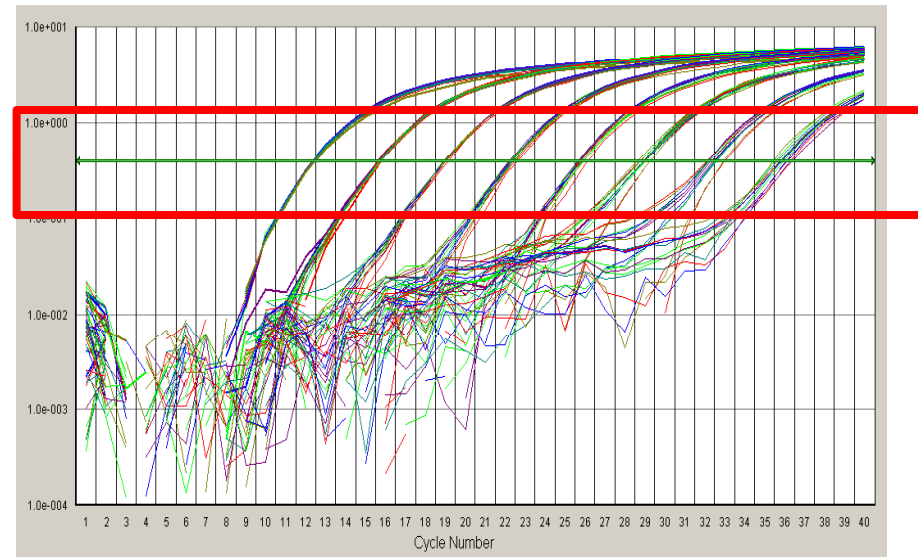
固定量

Principle of quantitative real-time PCR

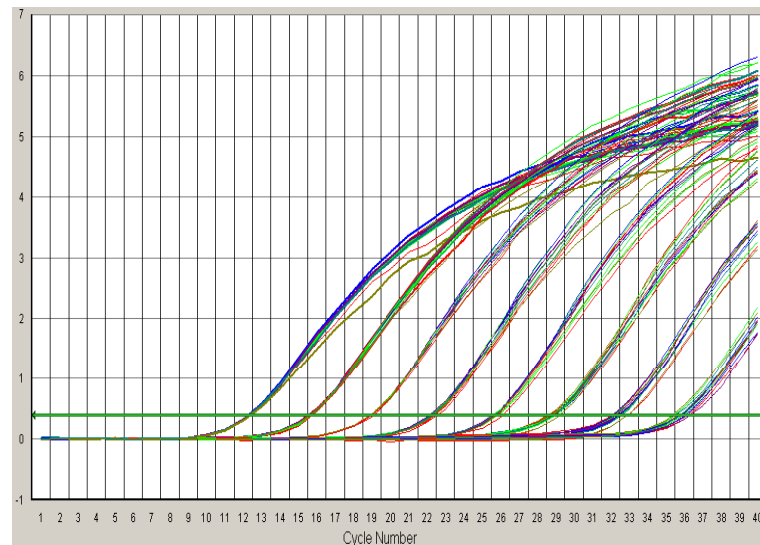


How to find Threshold ?

Y: log 對數圖譜



Y: linear 線性圖譜



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如何設計Real Time PCR的Primers/ Probe

The Three Solutions for Real time PCR



- 1. TaqMan Gene Expression Assays,
TaqMan SNP Genotyping Assays**
- 2. Custom TaqMan Assays service**
- 3. Design primer or Probe by software**

1. TaqMan Gene Expression Assays, TaqMan SNP Genotyping Assays



Gene Expression Analysis Using TaqMan® Assays

Real-Time PCR Assays

Gene Expression Analysis Using TaqMan® Assays

- Single Tube TaqMan® Gene Expression Assays
- Single Tube Custom TaqMan® Gene Expression Assays
- TaqMan® Endogenous Controls
- 96-Well TaqMan® Gene Expression Assays
- 384-Well Microfluidic Card TaqMan® Gene Expression Assays
- TaqMan® OpenArray® Real-Time PCR Plates
- TaqMan® Gene Expression Assays & Arrays Selection Guide
- Popular Gene Expression Assays
- Quantitative Gene Expression for PSCs Using TaqMan® Assays



TaqMan® Gene Expression Assays

TaqMan® Gene Expression Assays consist of a pair of unlabeled PCR primers and a TaqMan® probe with a FAM™ or VIC® dye label on the 5' end and minor groove binder (MGB) and nonfluorescent quencher (NFQ) on the 3' end. Over 1.3 million predesigned TaqMan® Gene Expression Assays covering 23 species are available in a single tube, 96-well plate, 384-well microfluidic card, and OpenArray® formats.

Use our **Search Tool** below to find the single tube assays you need.

New: More than 36,000 new TaqMan® gene expression assays for cynomolgus monkey

Assay search tool - find & buy your single tube TaqMan® Assays:

What type of experiment are you conducting?

Gene Expression

SNP Genotyping

Copy Number

siRNA

MicroRNA

Mutation Detection

Antibodies

What type of assay do you want?

All Gene Expression Assays

Noncoding RNA Only

Markers & Reporters

Controls

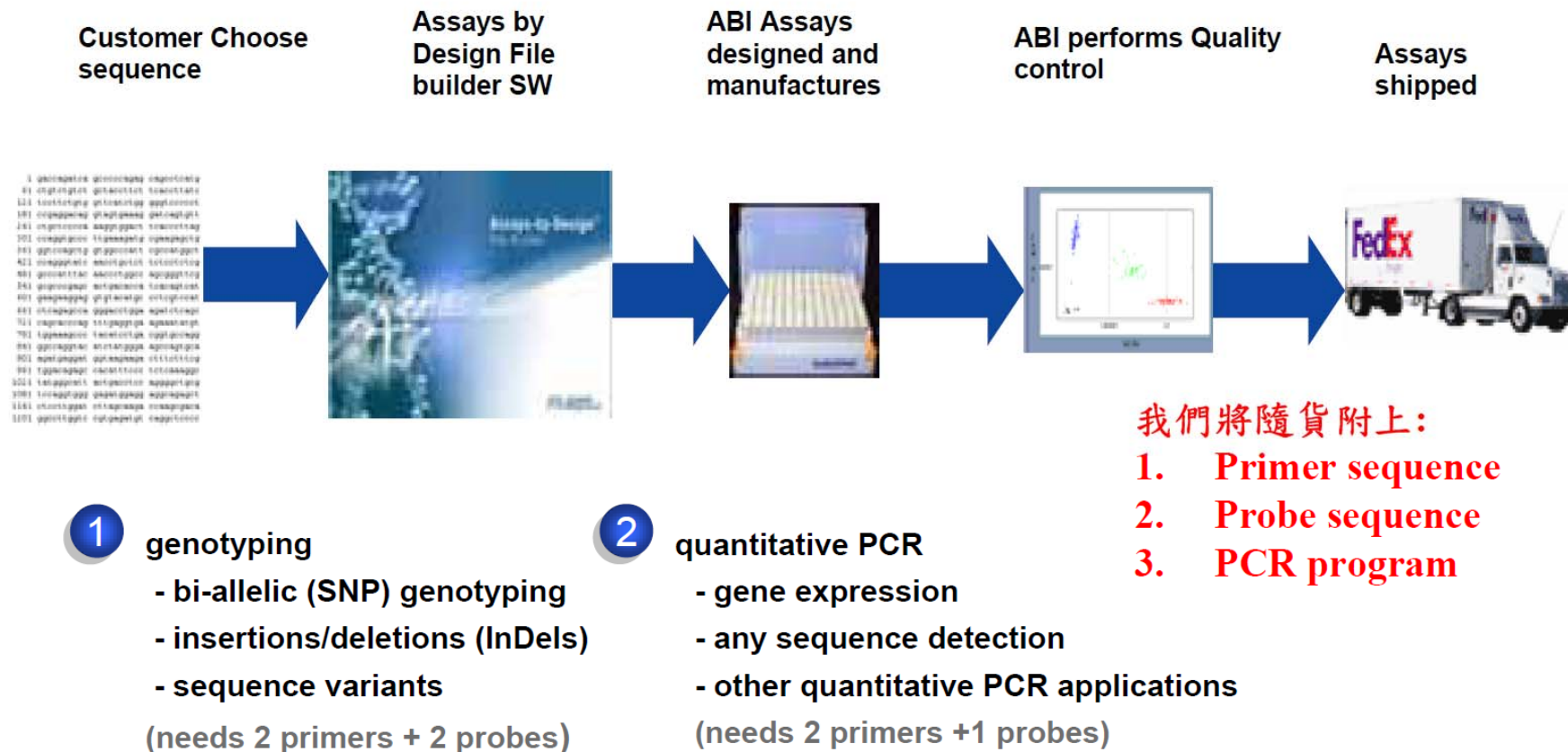
Fusion

- Ready made assays
- 網路選購
- 提供所有相關生物資訊
- Updated Human Genome information

2. Custom TaqMan Assays service



The Assays-by-Design Process



代客設計與測試

2. Custom TaqMan Assays service



fisher.com/tw/zt/home/life-science/pcr/real-time-pcr/real-time-pcr-assays/taqman-gene-expression/custom

lifetechnologies.com 現在正式更名為 thermofisher.com 瞭解更多 x

life technologies

訂購支持 | Sign In Quick Order 0

生命科學 應用科學 臨床 所有產品訂購 服務與支持 關於我們 Cloud

Home > Life Sciences > PCR > Real-Time PCR (qPCR) > Real-Time PCR Assays > Gene Expression Analysis Using TaqMan® Assays > Single Tube Custom TaqMan® Gene Expression Assays

Single Tube Custom TaqMan® Gene Expression Assays

Gene Expression Analysis Using TaqMan® Assays

Single Tube TaqMan® Gene Expression Assays

Single Tube Custom TaqMan® Gene Expression Assays

TaqMan® Endogenous Controls

96-Well TaqMan® Gene Expression Assays

384-Well Microfluidic Card TaqMan® Gene Expression Assays

TaqMan® OpenArray® Real-Time PCR Plates

TaqMan® Gene Expression Assays & Arrays Selection Guide

Popular Gene Expression

Custom TaqMan® Expression Assays are the custom version of our predesigned real-time PCR 5' nuclease TaqMan® Assays for relative quantification of RNA expression. Simply provide us with your target sequence of interest, or submit primer/probe sequences of your own design, and soon you'll be ready to perform RNA expression studies on any coding or non-coding RNA in any organism.

How to Order

1. Review our [Design and Ordering Guide \(PDF\)](#) for best results.
2. Use the **Custom TaqMan® Assay Design Tool** to input sequences, submit for design, and order Custom Assays.

Each custom assay is a mix of forward primer, reverse primer, and FAM™ dye-labeled TaqMan® MGB probe. Designed to run under universal oligo concentration and thermal cycling conditions for two-step RT-PCR, these assays are simple to use. Just add TaqMan® Master Mix and your cDNA sample to generate sensitive, reproducible, and truly quantitative RNA expression data.

[Custom TaqMan® Assay Design Tool](#)

Comparison of Custom and Custom Plus TaqMan® Gene Expression Assay products

There are two custom assay options to meet your RNA research needs: Custom TaqMan® Gene Expression Assays and Custom Plus TaqMan® RNA Assays. Both allow you to submit a target sequence to our TaqMan® Assay design pipeline, which uses your input sequence to design custom primer and probe sequences. See the key differences between the two options below:

3. Design primer or Probe by software

清楚明確的 TaqMan Probe & 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 ^b	
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') ^a	
避免 probe 的中間區域含有 2 個或以上的 CC di-nucleotides ^a	

200 bp amplicon

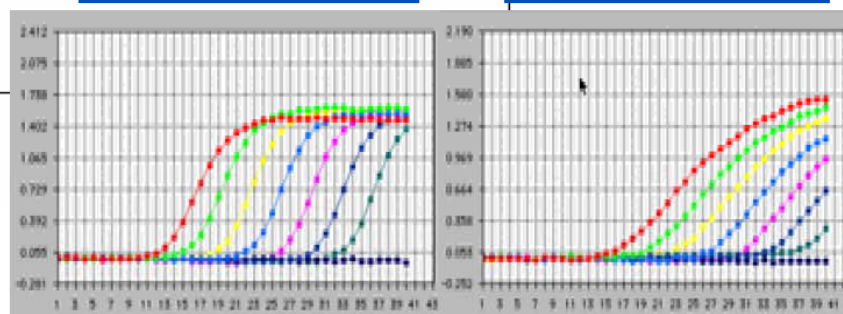


a: 針對 TaqMan MGB probe

b: 參數可選擇設定

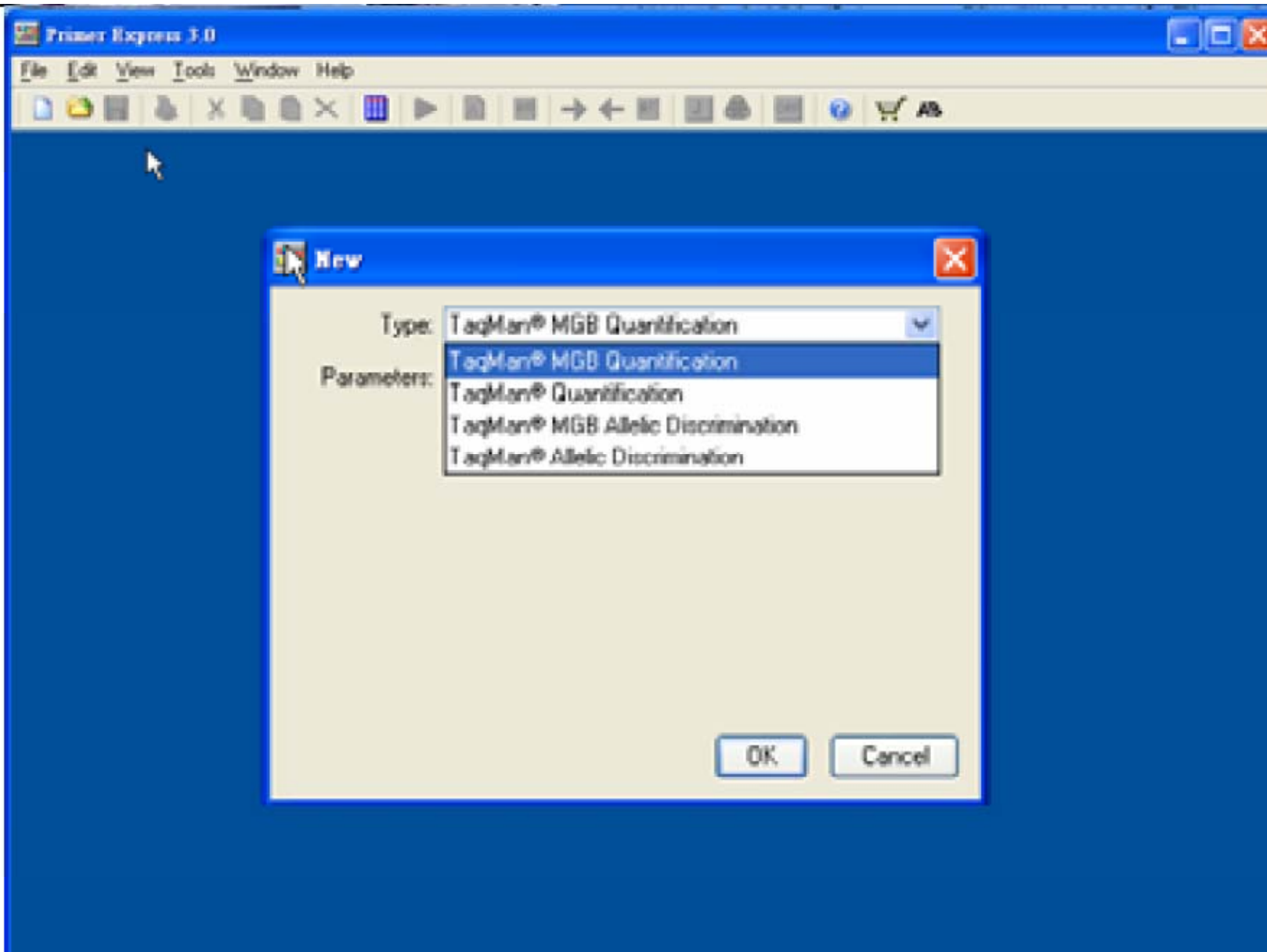
200 bp amplicon

500 bp amplicon



Primer Express 3.0 Operation





2. Find Primer/Probe

1. Add DNA file or Copy & Paste

To find Primers & Probes, click the "Find Primers/Probes" button.



Primer Express 3.0.1

File Edit View Tools Window Help

TaqMan® MGB Quantification # 1

Sequence Parameters Primers / Probes Order

Parameter	Value
☐ Primer Tm	
Min Primer Tm	58
Max Primer Tm	60
Max Difference in Tm of Two Primers	2
☐ Primer GC Content	
Min Primer %GC Content	30
Max Primer %GC Content	80
Max Primer 3' GC's	2
Primer 3' End Length	5
Primer 3' GC Clamp Residues	0
☐ Primer Length	
Min Primer Length	9
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

Factory Defaults

[illegible]

如何製備Real Time PCR的反應

Reverse Transcription : High Capacity cDNA archive Kit

Component
10x Reverse Transcription Buffer
25x dNTP
10x Random Primer
RT Enzyme 50U/uL
RNA (0.1~10 ug)
H2O



Step Type	Time	Temperature
HOLD	10 min	25 °C
HOLD	120 min	37 °C

Real Time PCR:

TaqMan Chemistry

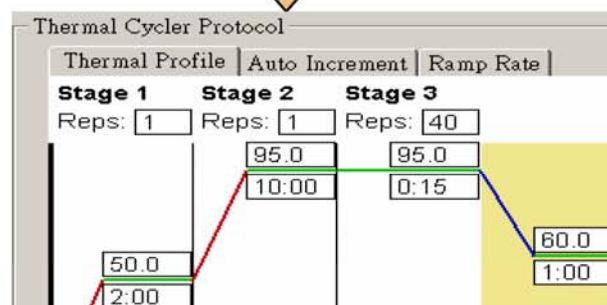
2x TaqMan Master Mix	1x	10 ul
20x Probe/primer Assay Mix		1 ul
Water		NA
cDNA	10-100 ng	5-10 ul

20 ul

SYBR Chemistry

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

20 ul



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StepOnePlus

儀器介紹





The StepOne Real-Time PCR system

- 48 well or 96 well 0.1 ml tube
- Single LED excitation
- 3 or 4color System (FAM / SYBR Green, VIC / JOE, ROX, TAMRA)

Features at a Glance

	StepOne™ System	StepOnePlus™ System
Throughput/Wells	48	96
FAM™/SYBR® Green dyes	✓	✓
VIC®/JOE™ dyes	✓	✓
ROX™ dye	✓	✓
NED/TAMRA™ dyes		✓
VeriFlex™ Block		✓



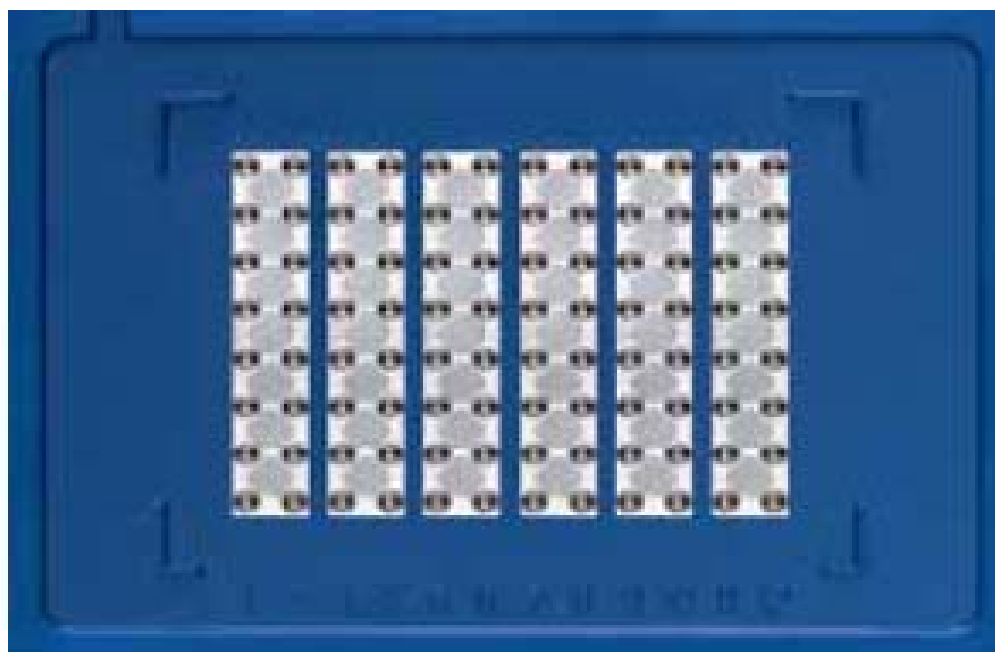
- 輕鬆擁有配備齊全的首選機器!
- 全機一體成型!
- 精確、穩定的PCR控溫系統!
- 彩色觸控式螢幕!
- USB插槽存取data!



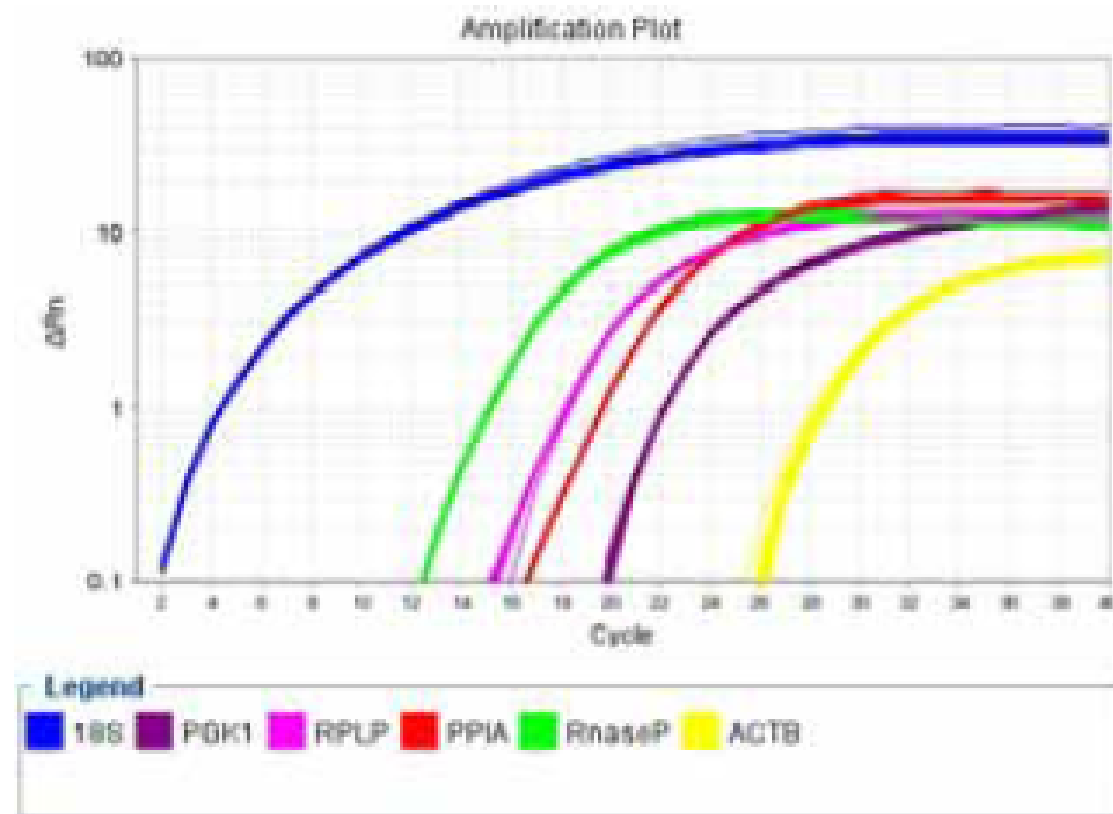
- 材質:
高科技鎳鉻合金
- 基座升降溫速度:
4.6 °C/sec
- 樣品升降溫速度:
Fast mode: ± 2.2 °C/sec
Std mode: ± 1.6 °C/sec
- 樣品反應管選擇: 0.1 ml
96 孔盤、8連排管、單管
- 反應體積: 10-30 μ l
- 精確、穩定的PCR控溫系統:
Peltier technology
- Veriflex: gradient

- Veriflex™ Block

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



Veriflex™ Block for customers who have legacy SYBR® Green primers



Zone	Target	Temp
1	18S	56
2	PPIA	57
3	ACTB	58
4	RNaseP	59
5	PGK1	60
6	RPLP	61

16 replicates for each target

單機操控



電腦操控



甚至是...

區域網路連線操控

LAN



1. Real-time PCR 原理暨技術應用介紹
2. 試劑配製注意事項Primer/ Probe 設計介紹
3. StepOnePlus 儀器介紹及使用注意事項
- 4. StepOnePlus 軟體簡介及結果分析演練**

StepOne 上機操作

Part 1: StepOne 單機操作



只需輕按觸控螢幕 不需電腦連線也能上機!!



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

Part 1: StepOne 單機操作



File View Help

Main Menu

Browse/New Experiments: New

Browse / New Experiments Settings Menu

TaqMan cDNA (Fast) TaqMan cDNA (Standard)

Create New Experiment Shortcut 6

2007-07-07 |

File View Help

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

Page 1 / 1

Selected: 0

Start Run **New** View/Edit Copy Delete

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

?

Part 1: StepOne 單機操作



File View Help

Experiment Parameters [X]

Reaction Volume: 20

Cover Temperature: 105.0

Experiment Name: SYBR_GREEN

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

File View Help

Sample: 31.3 °C **Stage 1** **Time Remaining:** 02:03:46

Experiment: SYBR_GREEN

Stage 1
1 of 1

Stage 2
X 40

Melt Curve
X 1

95.0° 10:00 0:15 60.0° 1:00 95.0° 0:15 60.0° 1:00 95.0° 0:15

PLEASE WAIT WHILE THE COVER IS HEATING

Current Run Temperature: 105.0°C

Required Run Temperature: 105.0°C

Add Melt Curve

2007-07-07 12:02:37 PM : Run started.

[?] [?]

Part 1: StepOne 單機操作

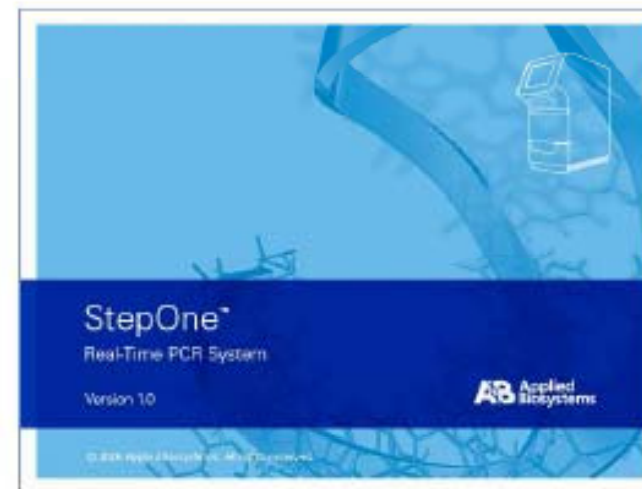


Part 2: StepOne 軟體上機步驟



一套軟體可以符合全方位的應用

- 絕對定量 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





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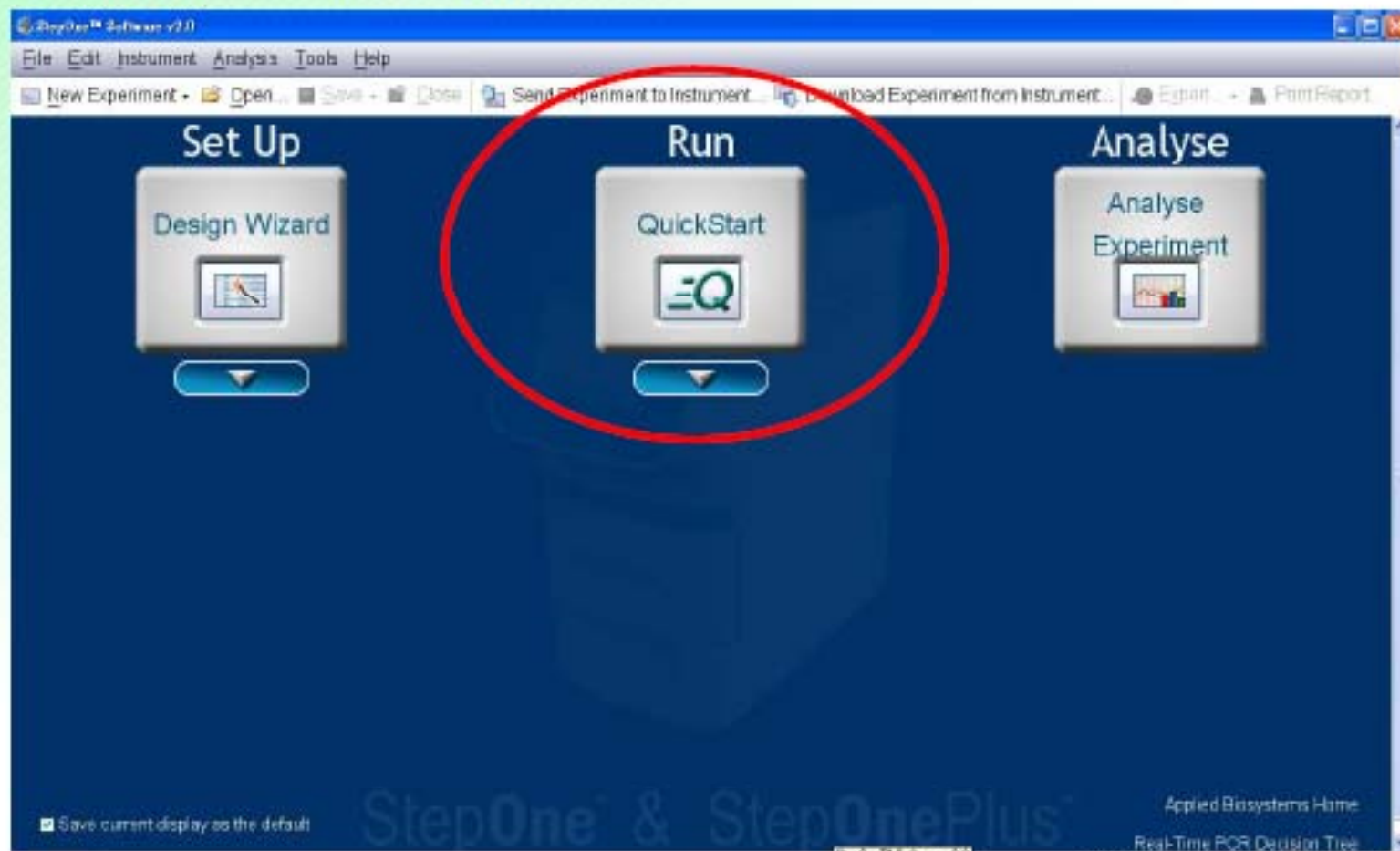
1.從桌面



StepOne Software v2.0
1212
2 KB

icon點2下，開啟軟體

2.按RUN，進入設定



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3.依照步驟一步一步設定實驗條件

EQ 1 Make selections and define the run method to run a new experiment with no plate setup information. Load the reaction plate into the instrument, then click "Start Run."

Experiment Properties Run Method

Enter Experiment Name and Location

*Enter Experiment Name: Untitled Location: D:\Applied Biosystems\StepOne System\experiments\Untitled.edb Browse

Select Experiment Type

☒ Quantitation - Standard Curve Quantitation - Relative Standard Curve Quantitation - Comparative Ct ($\Delta\Delta C_t$)

Melt Curve Genotyping Presence/Absence

Select Reagents

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

Other

Select Ramp Speed

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

Select Template

RNA (1-Step RT-PCR) ☒ cDNA (2-Step RT-PCR) or gDNA

☐ Save current settings as the default

Cancel **START RUN >**

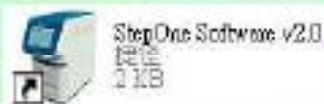
4. 設定好後，直接按 **START RUN >**

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StepOne 軟體分析操作步驟

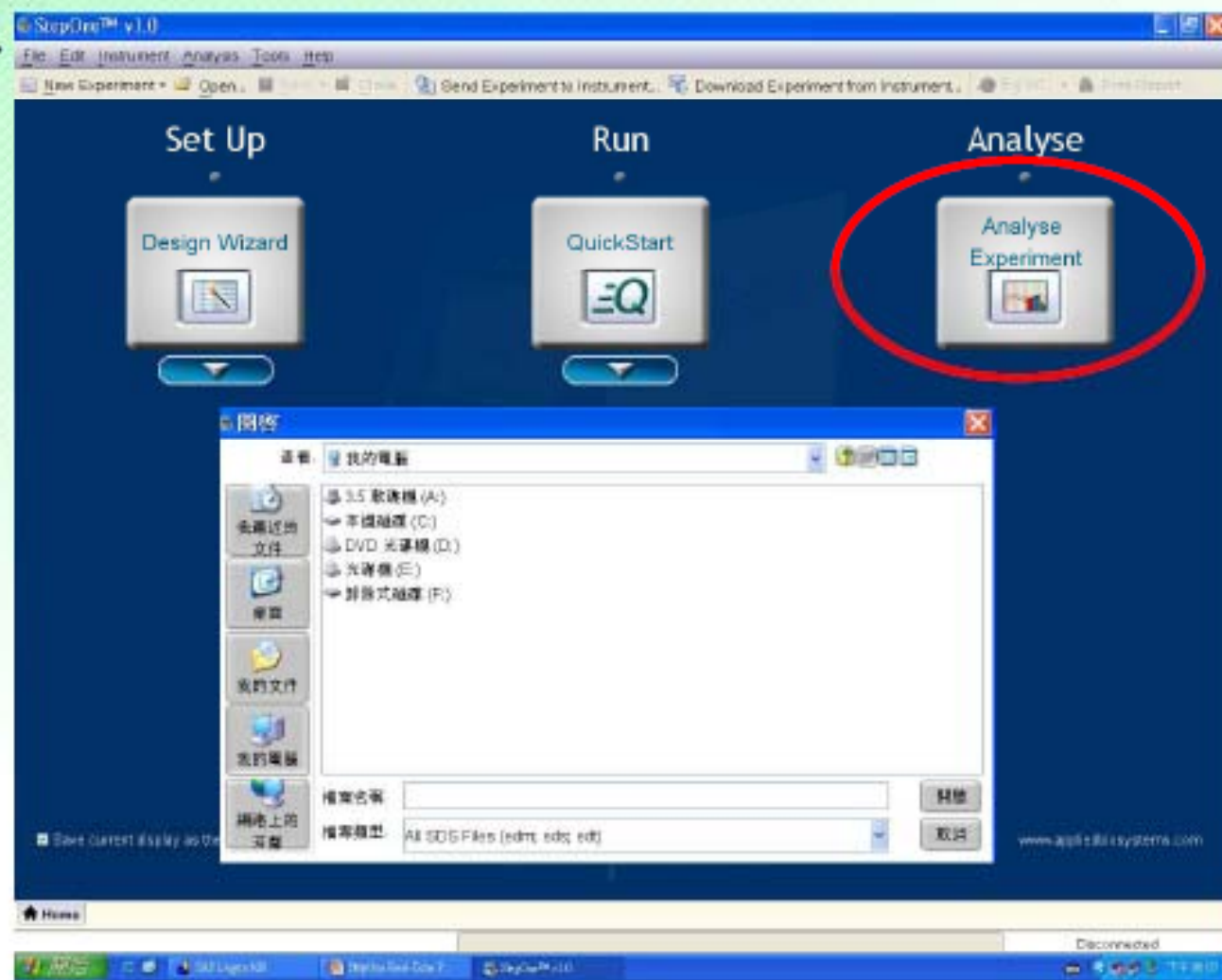


1. 先從桌面找到



icon，點2下開啟軟體。

2.

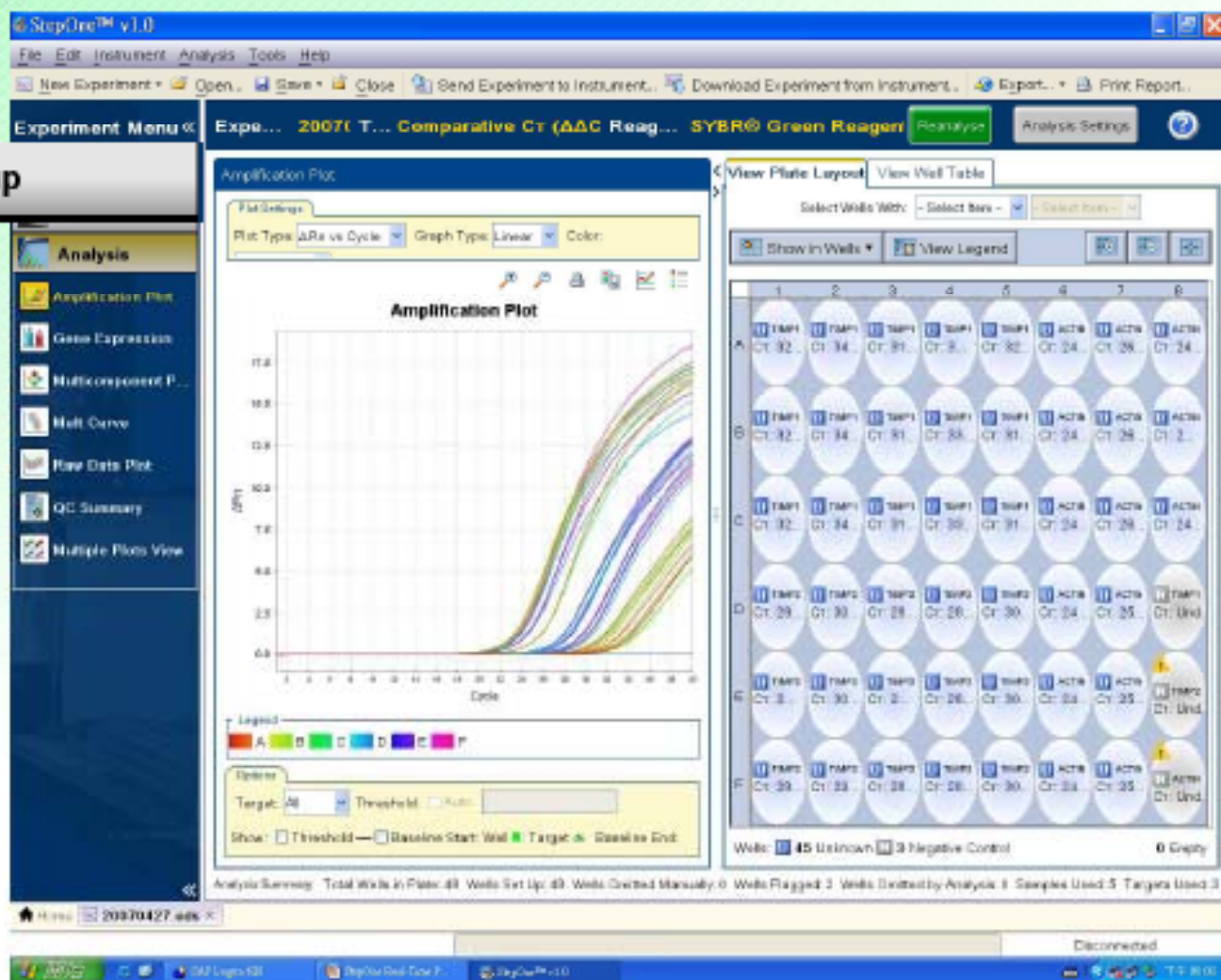




3.點選左側功能選項的 **Setup**，進行實驗的設定



Setup





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4.按照指示依步驟設定

StepOne™ v1.0

File Edit Instrument Analysis Tools Help

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

Experiment Menu

Setup

Experiment Properties

Enter an experiment name, select the type of experiment to set up, then select materials and methods for the PCR reactions and instrument run.

How do you want to identify this experiment?

Experiment Name: 20070427

Barcode (Optional):

User Name (Optional):

Comments (Optional):

What type of experiment do you want to set up?

Quantitation - Standard Curve Quantitation - Relative Standard Curve

Melt Curve Genotyping

✓ Quantitation - Comparative CT ($\Delta\Delta CT$)

Use a reference sample and an endogenous control to determine the relative quantity of target nucleic acid sequence in samples.

Which reagents do you want to use to detect the target sequence?

Standard Reagents ✓ SYBR® Green Reagents Other

The PCR reactions contain primers designed to amplify the target sequence and SYBR® Green dye to detect double-stranded DNA.

☒ Include Melt Curve

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

✓ Standard (~ 2 hours to complete a run) Fast (~ 45 minutes to complete a run)

For optimal results with the standard ramp speed, Applied Biosystems recommends using standard reagents for your PCR reactions.

Home 20070427.edb

Disconnected

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5.選擇左邊功能選項



Plate Setup

，設定sample name and gene name



Plate Setup

StepOne™ v1.0

File Edit Instrument Analysis Tools Help

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

Experiment Menu < Experiment... 2007042 Ty... Comparative Ct (ΔΔCt) Reage... SYBR® Green Reagents START ... ?

Setup Define Targets and Samples

Experiment Propo... Setup

Reaction Setup

Materials List

Run

Analysis

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	Repeats	Quencher	Col.
TMP1	SYBR	None	
TMP2	SYBR	None	
ACTB	SYBR	None	

Define Samples

Add New Sample Add Saved Sample Save Samples

Sample Name	Color
CD4-1	
CD8-1	
CD4+8-1	
CD4	
ATX control	

Assign Targets and Samples

Home 20070427.ads

Disconnected

20070427



6.再設定sample and gene的位置

StepOne™ v1.0

File Edit Instrument Analysis Tools Help

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

Experiment Menu << Setup Experiment Properties Plate Setup Run Method Reaction Setup Materials List Run Analysis

Experim... 2007042 Ty... Comparative Ct (ΔΔCt Reage... SYBR® Green Reagents START ... ?

Define Targets and Samples **Assign Targets and Samples**

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
☒	TIMP1	
☐	TIMP2	
☐	ACTIN	

☒ Mixed ☐ Unknown ☐ Negative Control

Assign sample(s) to the selected wells.

Assign	Sample
☒	CD4-1
☐	CD8-1
☐	CD4+8-1
☐	CC14

Select relative quantification settings.

Reference Sample: ATX control
Endogenous Control: ACTIN

Select the dye to use as the passive reference.

ROX

View Plate Layout View Well Table

Select Wells With: - Select Item - Select Sample

Show in Wells View Legend

	1	2	3	4	5	6	7	8
A	CD4-1 	CD8-1 	CD4+8-1 	CC14 	ATX control 	CD4-1 	CD8-1 	CD4+8-1
B	CD4-1 	CD8-1 	CD4+8-1 	CC14 	ATX control 	CD4-1 	CD8-1 	CD4+8-1
C	CD4-1 	CD8-1 	CD4+8-1 	CC14 	ATX control 	CD4-1 	CD8-1 	CD4+8-1
D	CD4-1 	CD8-1 	CD4+8-1 	CC14 	ATX control 	CD4-1 	CD8-1 	CD4+8-1
E	CD4-1 	CD8-1 	CD4+8-1 	CC14 	ATX control 	CD4-1 	CD8-1 	CD4+8-1
F	CD4-1 	CD8-1 	CD4+8-1 	CC14 	ATX control 	CD4-1 	CD8-1 	CD4+8-1

Wells: 45 Unknown 3 Negative Control 0 Empty

Home 20070427.edb

Disconnected

StepOne v1.0



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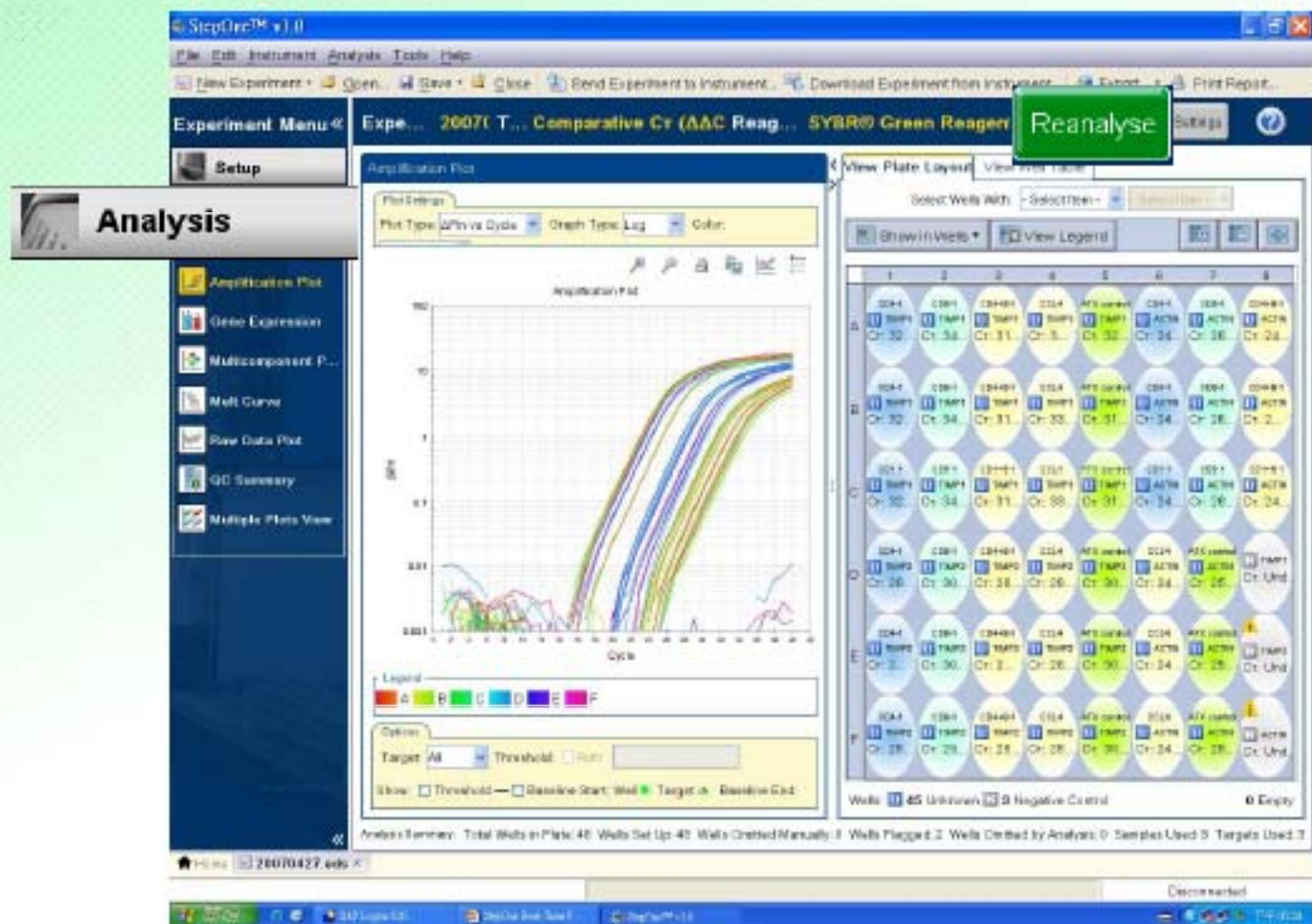
7.設定好所有實驗參數後，按左邊功能鍵

再按 **Reanalyse** 重新分析data



Analysis

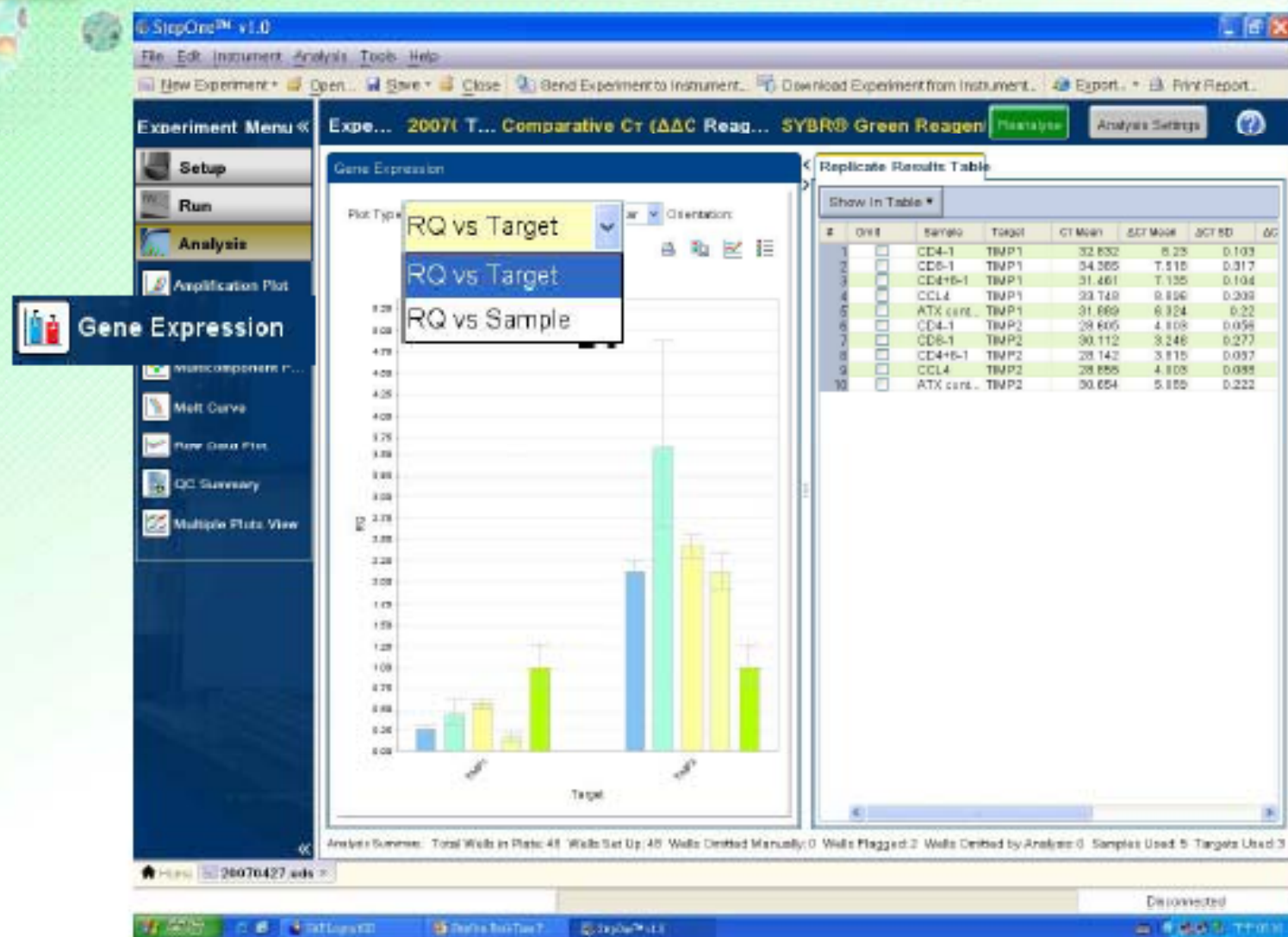
,



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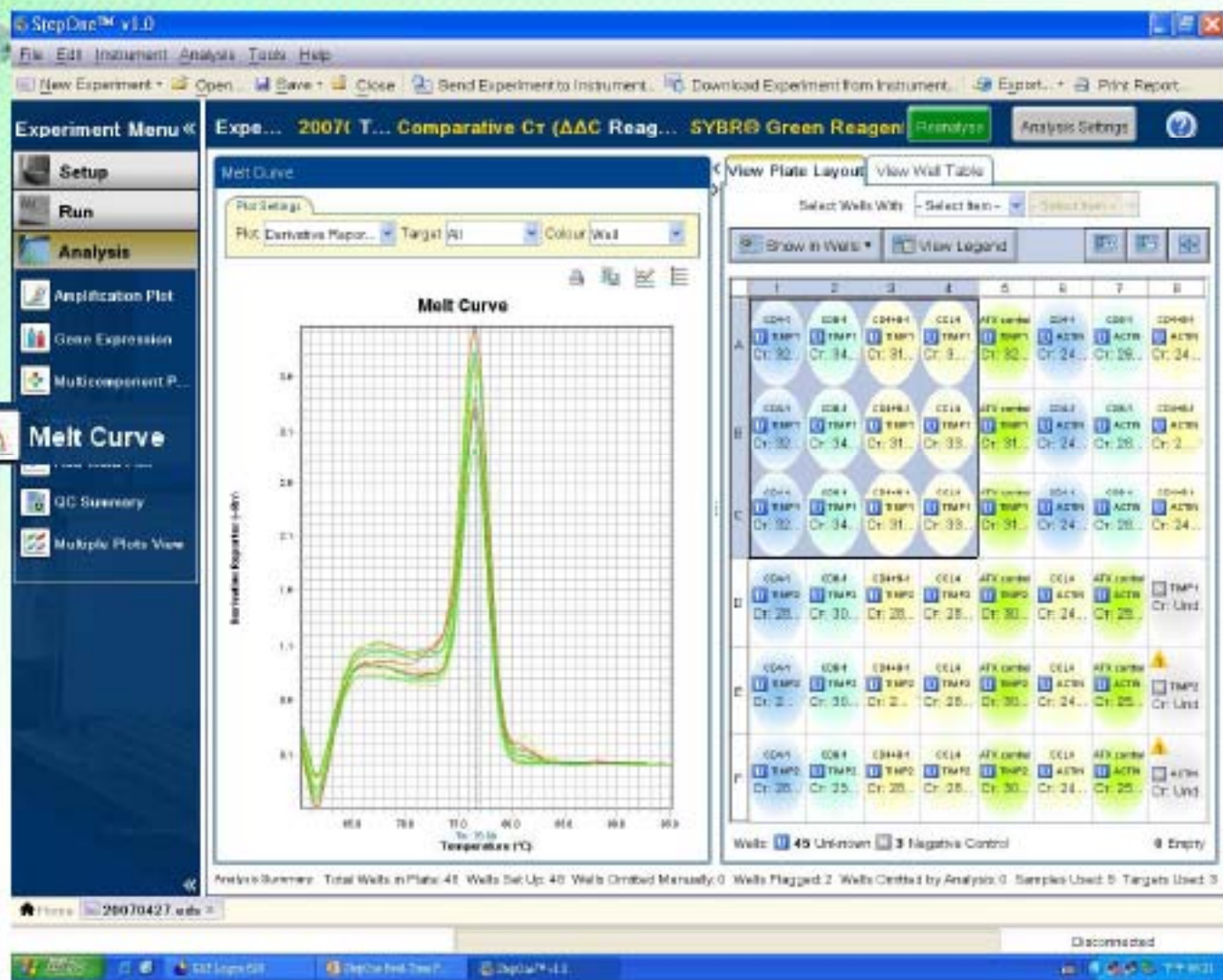
8.可利用左邊的功能鍵，觀看分析好的相對定量的data





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9. 利用 確認是否有 primer dimer or non-specific band

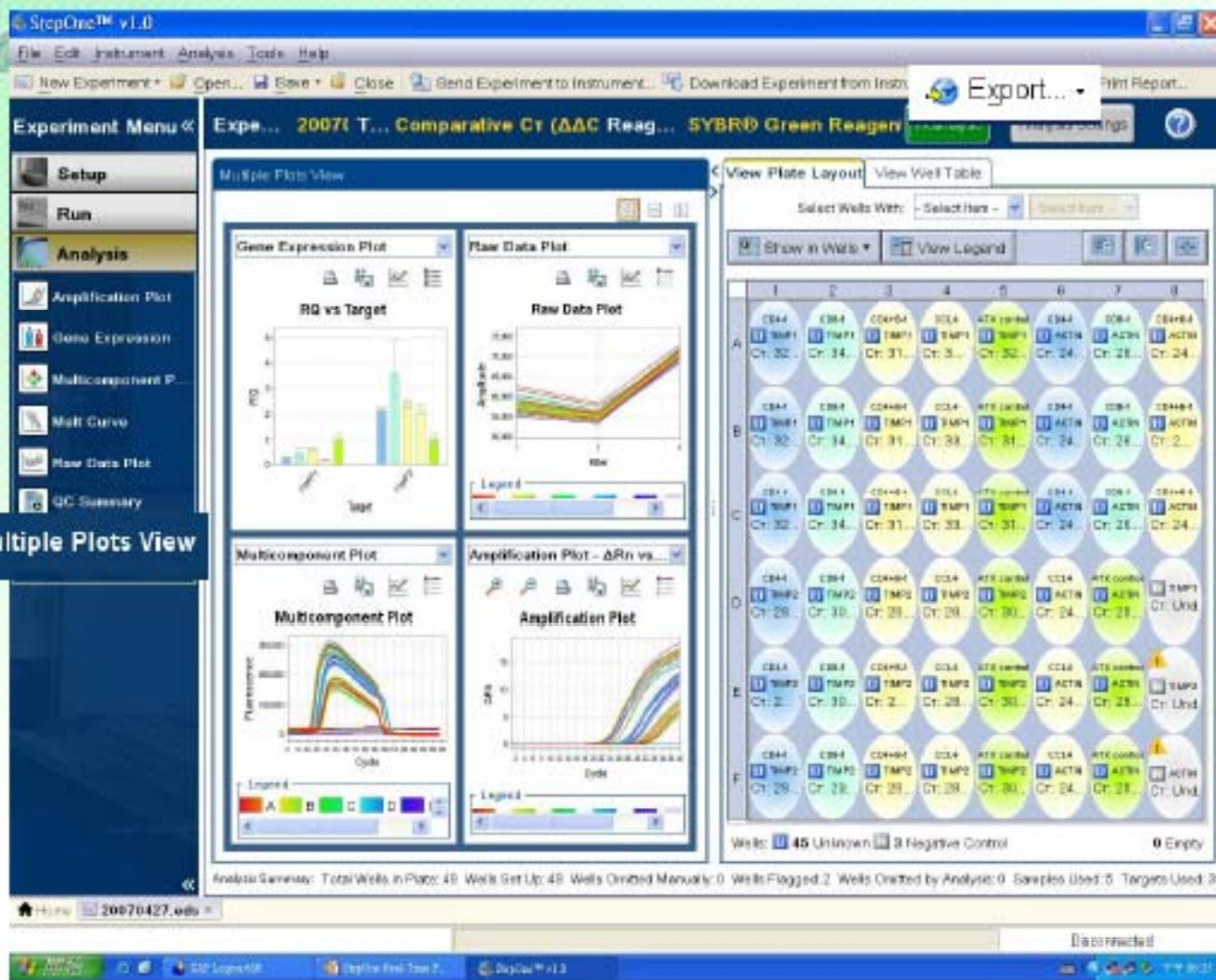


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10.也可以在同一視窗觀看4種不同的data圖形



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結果分析

絶対定量、相対定量

絕對定量

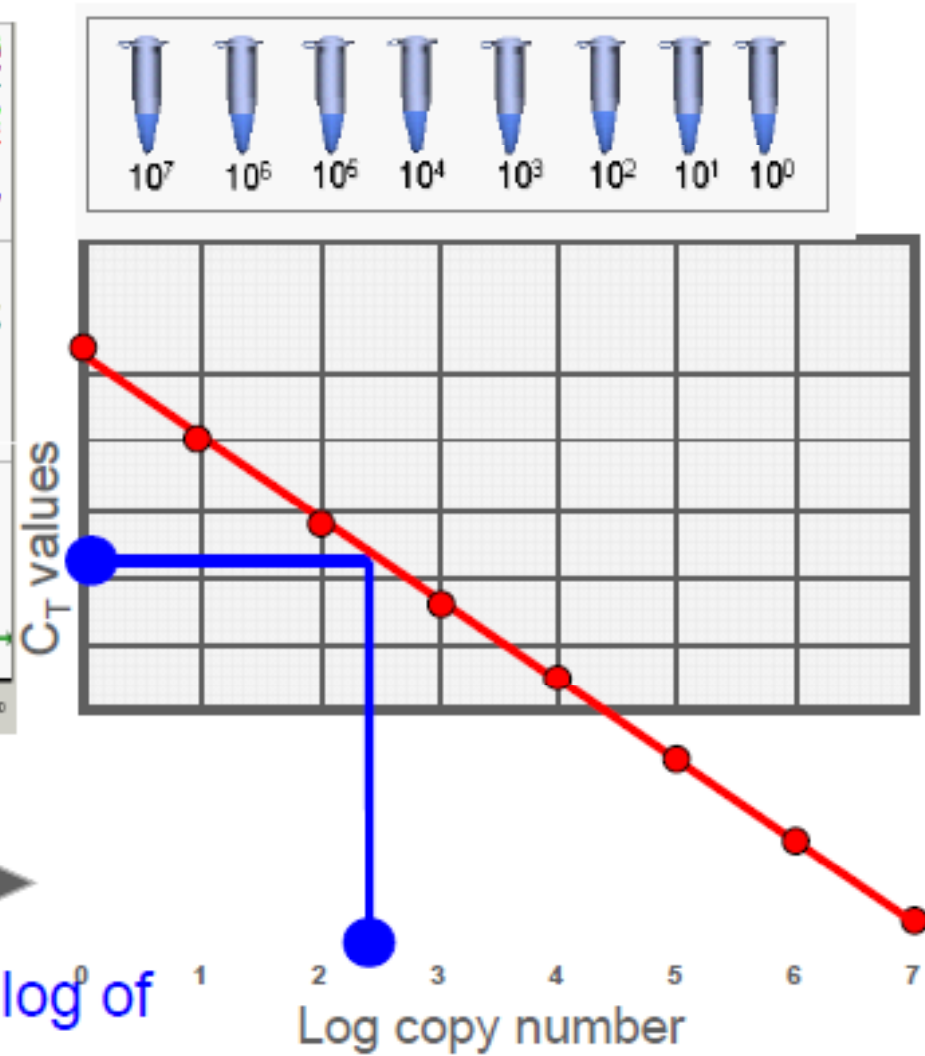
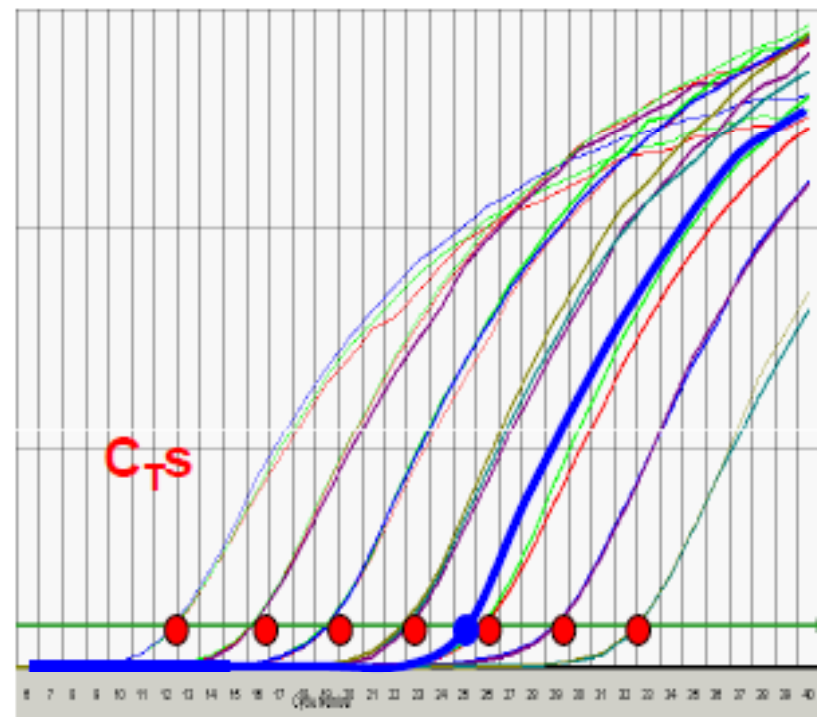


絕對定量 (Absolute Quantitation)

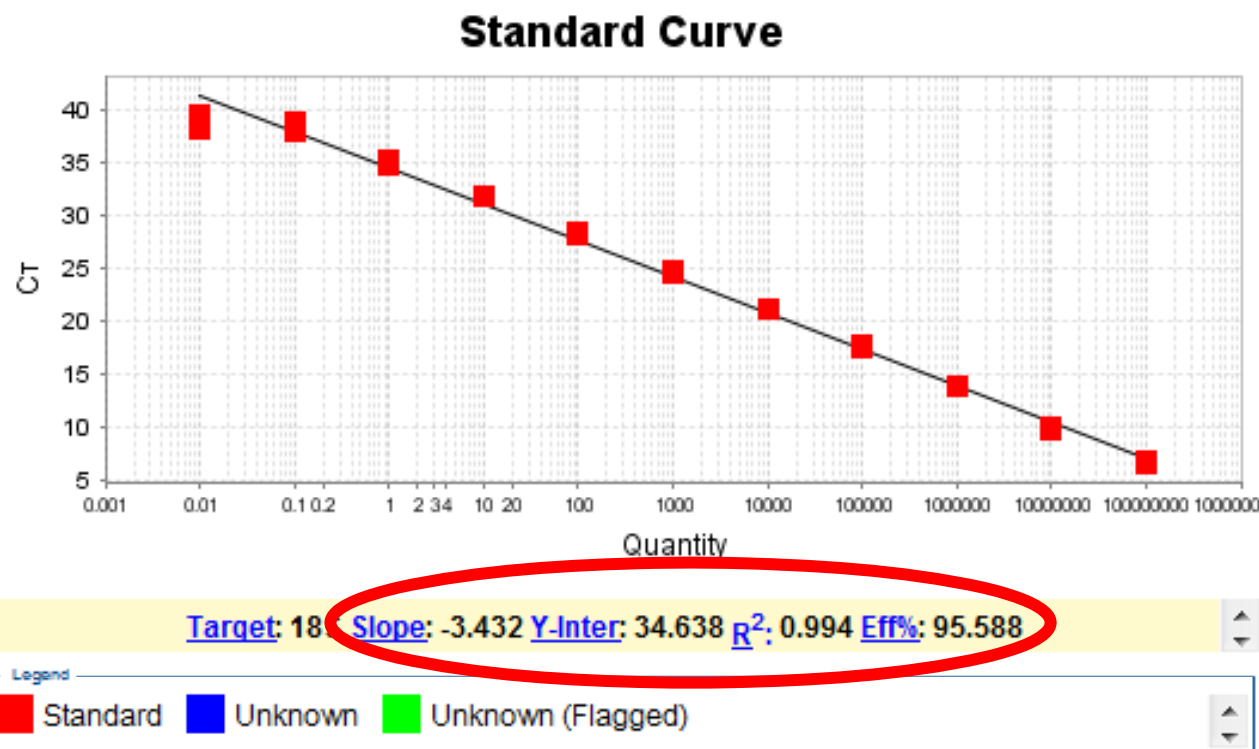
–主要應用於病毒量及病原菌偵測–

To determine the actual number of copies of a target nucleic acid within a sample with statistical confidence.

- needs a standard whose concentration is known absolutely
- identical amount of sample must be assayed for all unknowns
- unnecessary for most studies



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



The **slope** of the standard curve can be directly correlated to the **efficiency** of the reactions:

$$\text{Efficiency } (\eta) = [10^{(-1/\text{slope})}] - 1$$

when slope = -3.32, Efficiency = 100%

Points to Remember



- Threshold Cycle values (C_T) have a direct relationship to the amount of starting template
- 100% efficiency : $2^n = \text{fold dilution}$
- Efficiency of reactions between 90-110%
- R value should be ≥ 0.98

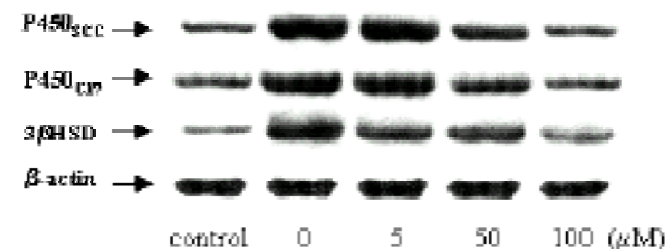
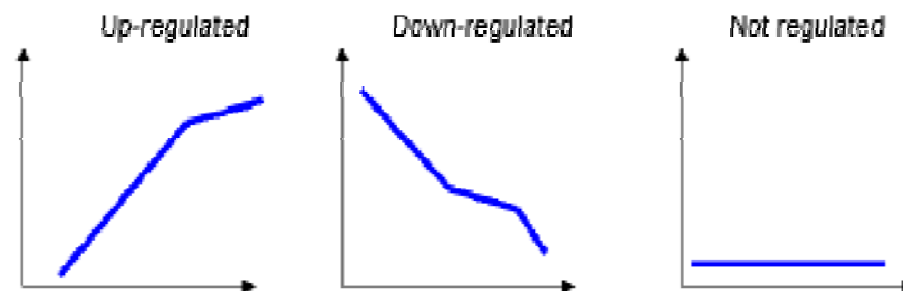
相對定量



相對定量 (Relative Quantitation)

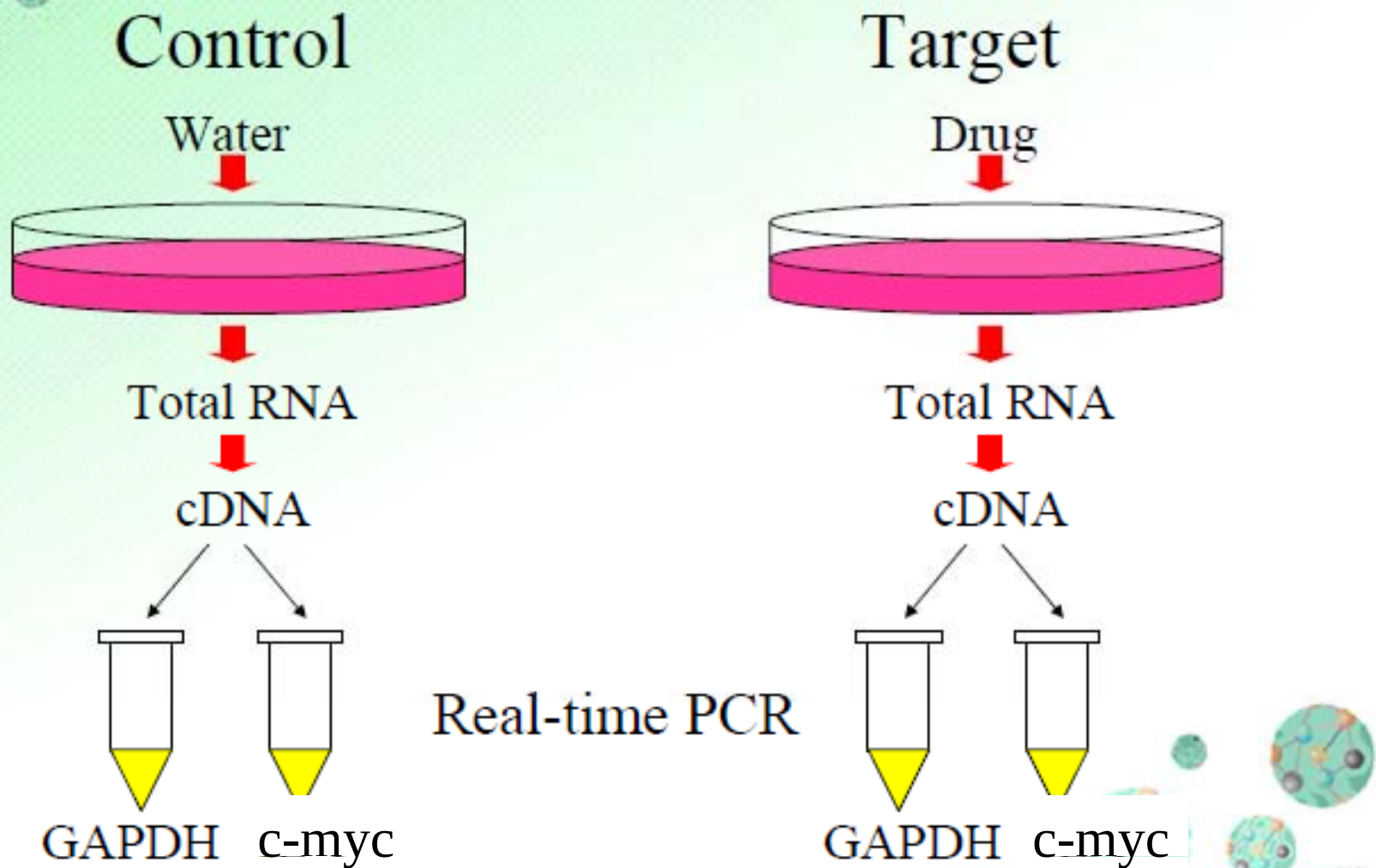
To determine **fold differences** of a target nucleic acid in a starting material with statistical confidence.

- 1. **$\Delta\Delta$ Ct analysis (most common)**
- 2. **Relative standard curve**
- ◆ Need endogenous gene normalizes the amount of sample added
Endogenous control (ex. 18S rRNA, GAPDH, β -actin.....)
- ◆ The most powerful and widely used method





Real-time PCR



$$\text{起始濃度} = \frac{\text{產物量}}{2^n}$$

$$\text{起始濃度} = \frac{\text{產物量(Threshold)}}{2^{\text{Ct}}}$$

$$\text{起始濃度 c-myc} = \frac{\text{產物量(Threshold)}}{2^{\text{Ct (c-myc)}}}$$

$$\text{起始濃度 GAPDH} = \frac{\text{產物量(Threshold)}}{2^{\text{Ct (GAPDH)}}}$$

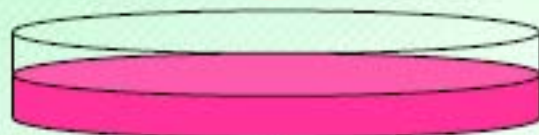
$$\begin{aligned} \frac{\text{c-myc}}{\text{GAPDH}} &= \frac{2^{\text{Ct (GAPDH)}}}{2^{\text{Ct (c-myc)}}} = 2^{\text{Ct (GAPDH)} - \text{Ct (c-myc)}} \\ &= 2^{- (\text{Ct (c-myc)} - \text{Ct (GAPDH)})} \\ &= 2^{- \Delta \text{Ct}} \end{aligned}$$



Real-time PCR

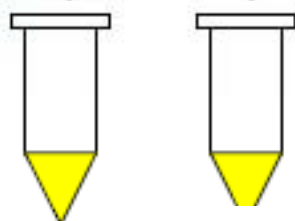
Control

Water



Total RNA

cDNA



GAPDH c-myc

$$2^{-\Delta Ct1}$$

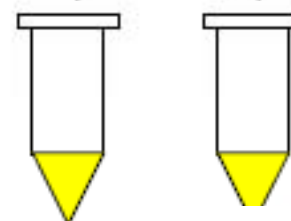
Target

Drug



Total RNA

cDNA



GAPDH c-myc

$$2^{-\Delta Ct2}$$

Real-time PCR

$$\begin{aligned}
 \frac{\text{加藥c-myc}}{\text{c-myc}} &= \frac{2^{-\Delta\text{Ct2}}}{2^{-\Delta\text{Ct1}}} = 2^{-\Delta\text{Ct2} - (-\Delta\text{Ct1})} \\
 &= 2^{-(\Delta\text{Ct2} - \Delta\text{Ct1})} \\
 &= 2^{-\Delta\Delta\text{Ct}}
 \end{aligned}$$

Comparative Ct Method



step 1: Normalization to endogenous control

Sample: $Ct \text{ c-myc} - Ct \text{ GAPDH} = \Delta Ct \text{ sample}$

Calibrator: $Ct \text{ c-myc} - Ct \text{ GAPDH} = \Delta Ct \text{ calibrator}$

step 2: Normalization to calibrator sample

$\Delta Ct \text{ Sample} - \Delta Ct \text{ Calibrator} = \Delta\Delta Ct$

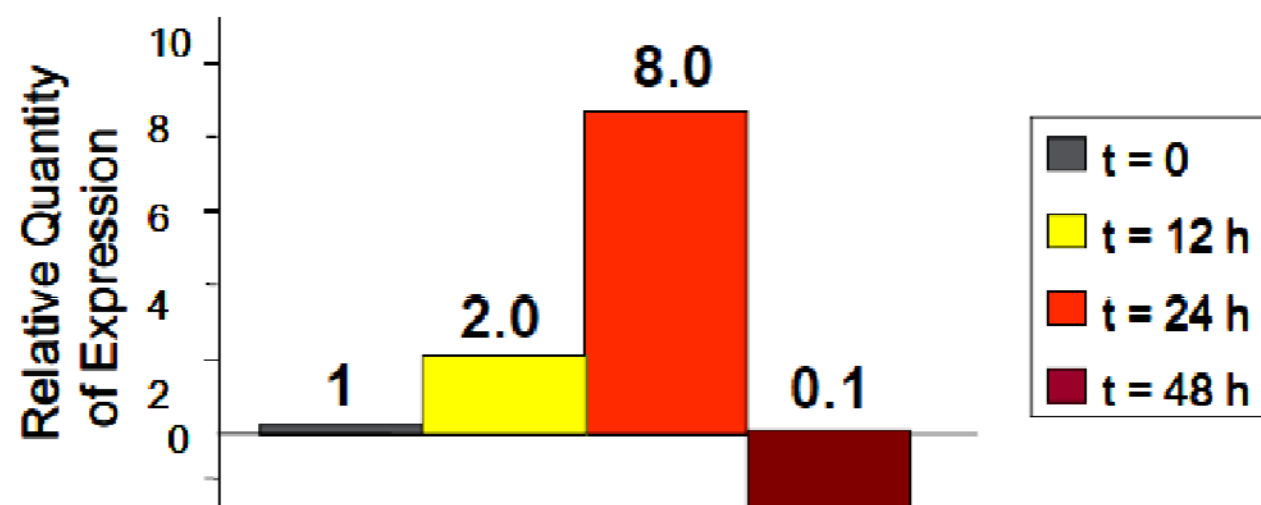
step 3: use the formula

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

A calibrator sample is a sample to which unknown samples are compared (ex. untreated sample or control).

$\Delta\Delta C_t$ Calculations (Comparative C_t)

	c-Myc	GAPDH	ΔC_t	$\Delta\Delta C_t$	$2^{-\Delta\Delta C_t}$
T=0 (calibrator)	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





StepOne & StepOnePlus

簡易維護保養與注意事項

1. 可使用 **0.1 mL** (96 well optical plate、八連排(8 tubes/strip) optical tubes 或單一的 optical tube)，配合適當的 precision plate holder 都可直接上機。
2. 管子及蓋子上請勿標記任何記號，避免墨色脫落沾到 PCR block 上增加背景值。
3. 請定期以棉花棒沾二次水清潔 PCR block 增加偵測靈敏度。



StepOne™ Real-time PCR System 簡易故障排除卡

Diagnostic Number/ Message	發生原因	故障排除
"java.lang.IllegalArgumentException:One of the raw spectra is null"	內建校正螢光讀值未寫入	打開檔案略過錯誤訊息，點選上方工具列 analysis>override calibration>usesystem calibration或是use calibration from another file (選擇RNase P檔案)
檔名後面出現副檔名(.eds~)，或是用隨身碟自儀器存取，存到兩個檔案，一個副檔名(.eds)正確，檔案約10bytes，一個附檔名(.eds~)檔案大小正常	實驗未正常結束或是隨身碟有問題	將檔案大小正確的檔案副檔名後"~"去除即可讀取
無法自儀器存取隨身碟	隨身碟格式不相容	確認原有隨身碟格式是否為NTFS並將之格式化成為FAT32或更換隨身碟
實驗結束後樣品槽無法開啟	操作不當造成卡盤	請立即聯絡工程師
電源無法開啟	插座未接妥或電源供應器損壞	確定電源插座或電源供應設備(不斷電系統，UPS)是否接妥或開啟，若仍無法開機請立即連絡工程師
電腦無法與儀器連結 Disconnected	連結線未接妥或IP設定錯誤	確認儀器與電腦連結線是否接妥，若仍無法連線至電腦 > 控制台>網路連線>區域網路>內容>Internet protocol(TCP/IP)>選擇自動取得IP

Thanks for Your Attendance!



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Tel: 0980-318-932
Email: Ryan@kimforest.com
Website: <http://www.kimforest.com>