

Applied Biosystems StepOne Plus Real-Time PCR 之操作與軟體介紹

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Field Application Scientist

同步定量PCR之應用



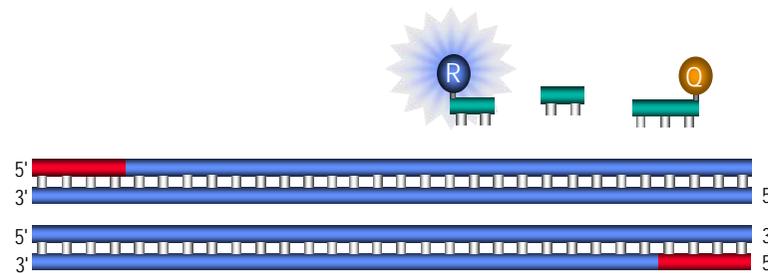
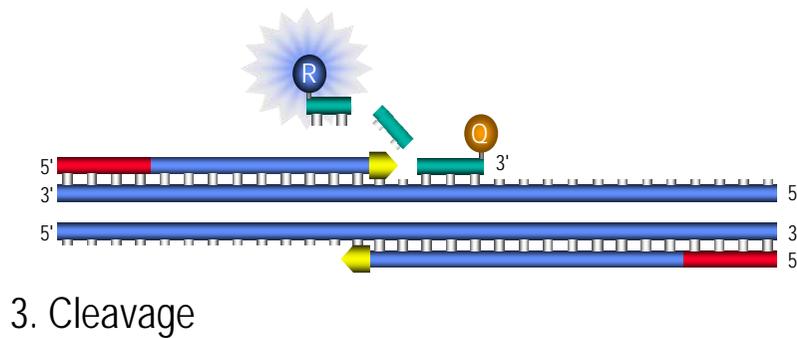
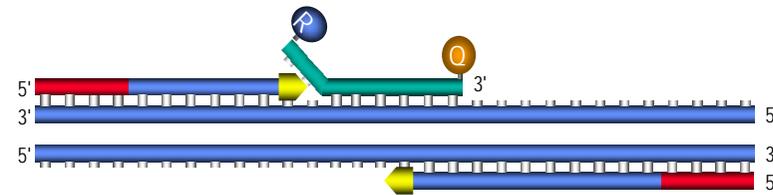
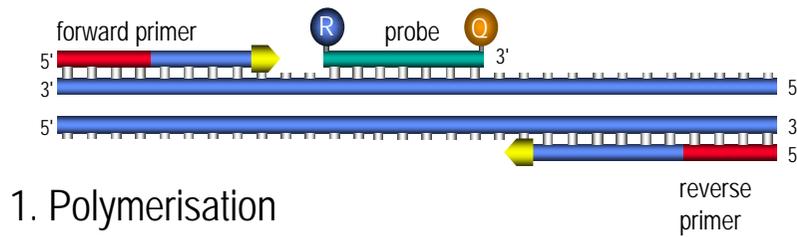
基因定量

- 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...
- 心臟血管疾病基因監測
- **miRNA** 基因調控研究
- 病原菌偵測
- Stem cell study
- KRAS/ BRAF

定性研究

- 基因型研究
 - **SNP**與疾病關聯性
 - **CNV**與疾病關聯性

TaqMan® probe chemistry: Fluorogenic 5' Nuclease assay



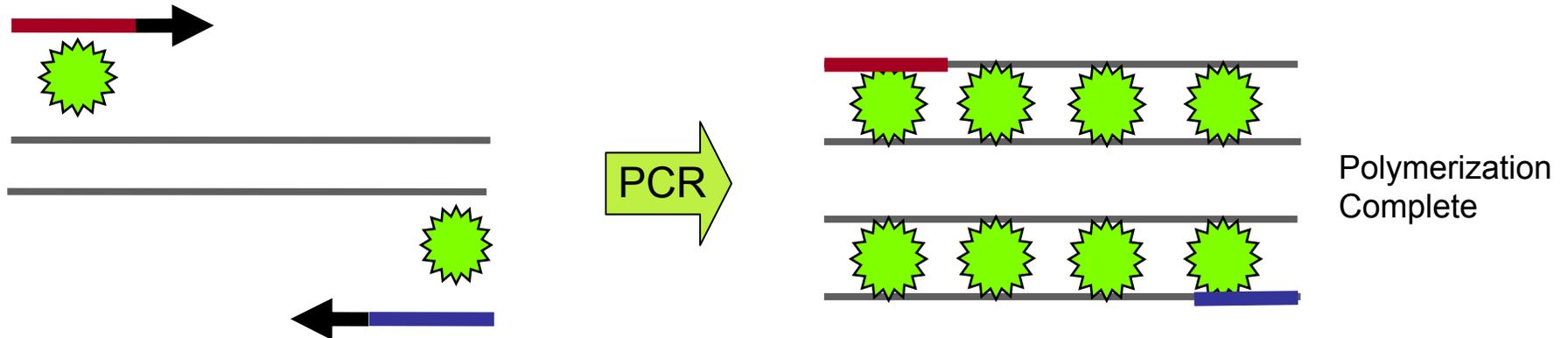
R = Reporter
Q = Quencher



4. Polymerisation completed

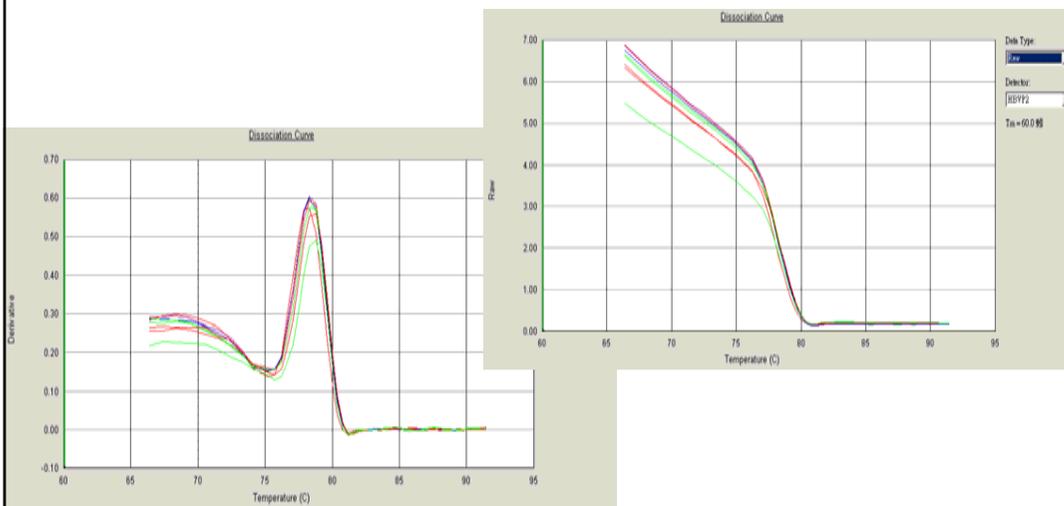
SYBR Green 1[®]

- dsDNA minor-groove binding dye



Polymerization

Denaturation



2011/07/05

TaqMan® Probe



Specificity

- Highly specific
- Probe Hybridization

Sensitivity

- Very High

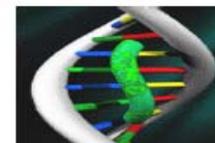
Flexibility

- Multiplex PCR
- SNP detection
- +/- application

Optimization

- Ready to use 20x primer/probe mix - no need to optimize
- Gold standard for MAQC
- PCR efficiency 100% ±10%

SYBR® Green 1 Dye



- Non-specific

- Very High

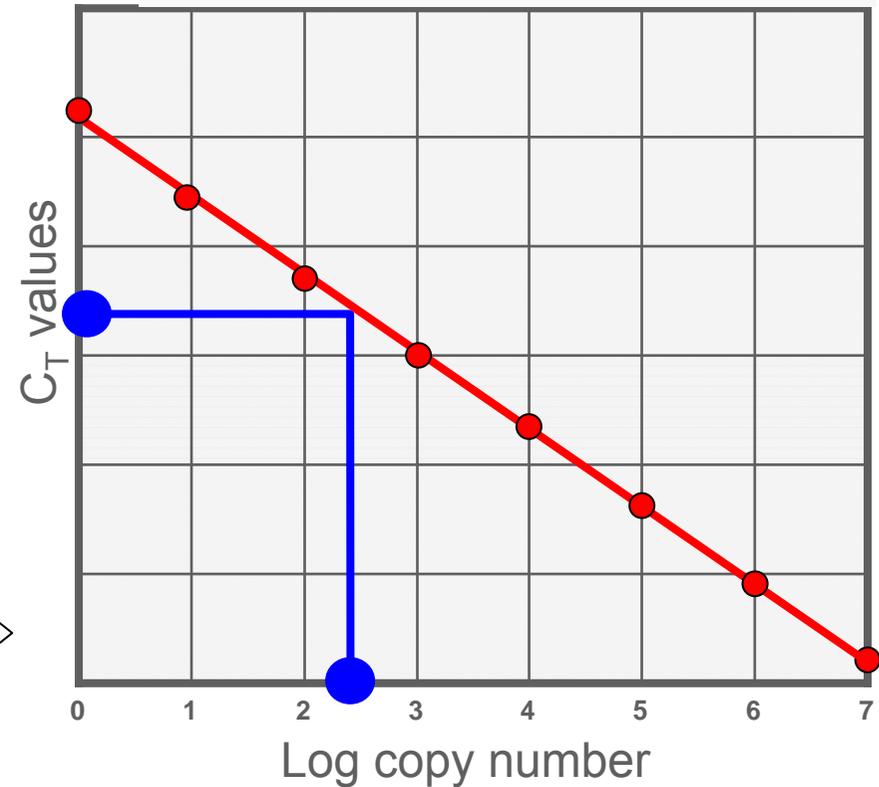
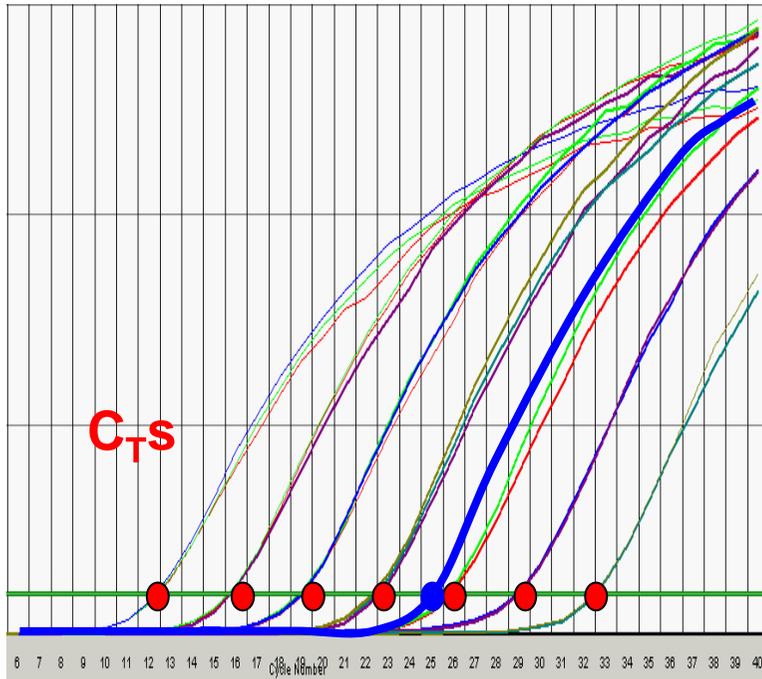
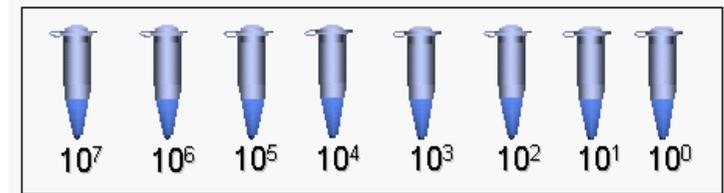
- No Probe is required
- Screening tool

- Need to optimize PCR program
- Need to check primer-dimer info
- Need to check PCR efficiency

絕對定量 (Absolute Quantitation)

- 主要應用於病毒量及病原菌偵測 -

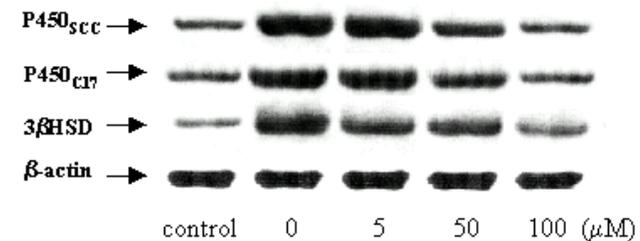
To determine the actual **number of copies** of a target nucleic acid within a sample with statistical confidence.



C_T is directly proportional to log of amount of input template

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

- 1. $\Delta\Delta$ Ct analysis (most common) -- UB2
- 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
- ◆ Check primer PCR efficiency first if using SYBR !!



step 1: Normalization to endogenous control

Sample: Ct Target gene – Ct Endogenous control = ΔCt sample

Reference: Ct Target gene – Ct Endogenous control = ΔCt reference

step 2: Normalization to calibrator sample

ΔCt Sample – ΔCt Reference = $\Delta\Delta\text{Ct}$

step 3: use the formula

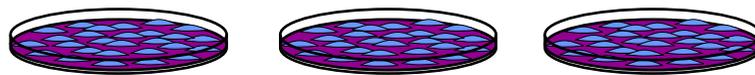
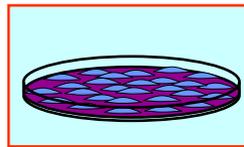
$$2^{-\Delta\Delta\text{Ct}}$$

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

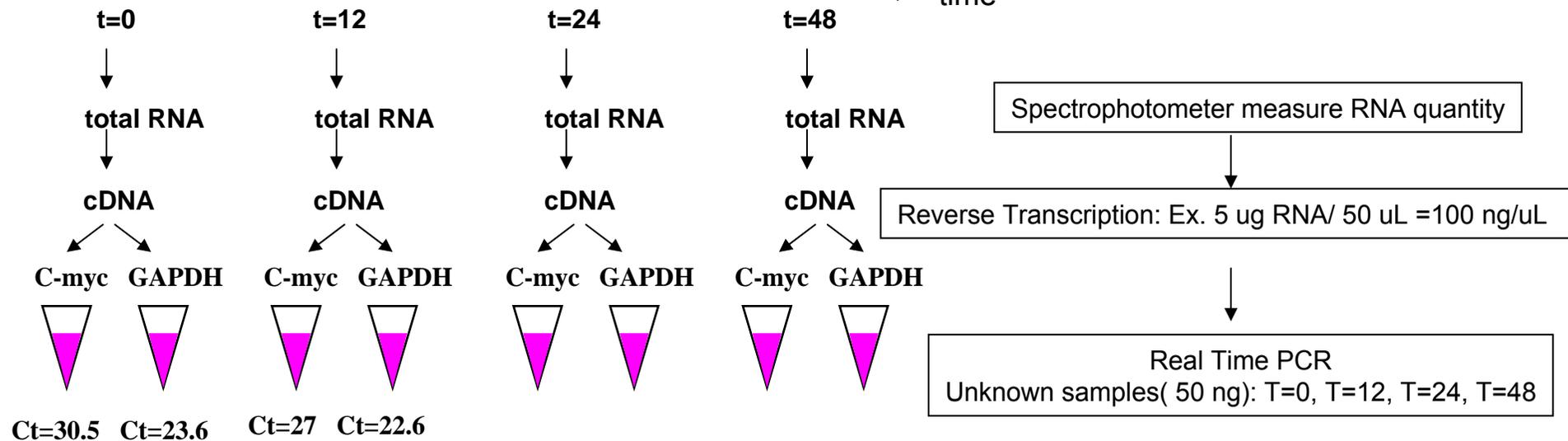
Comparative Ct Method

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

Calibrator

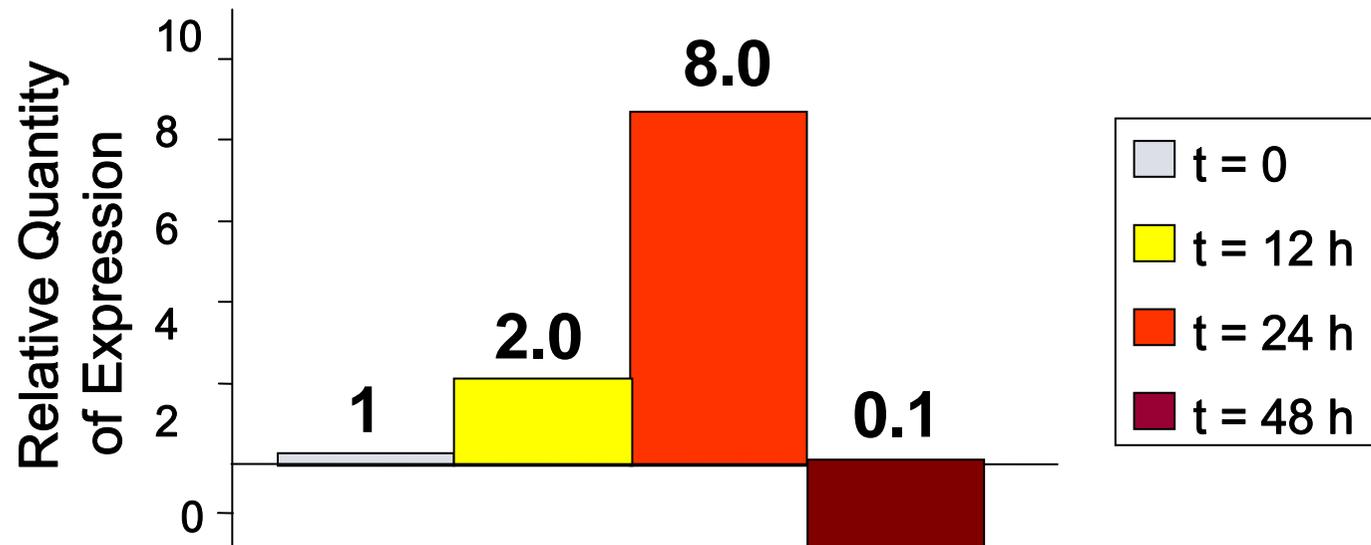


time

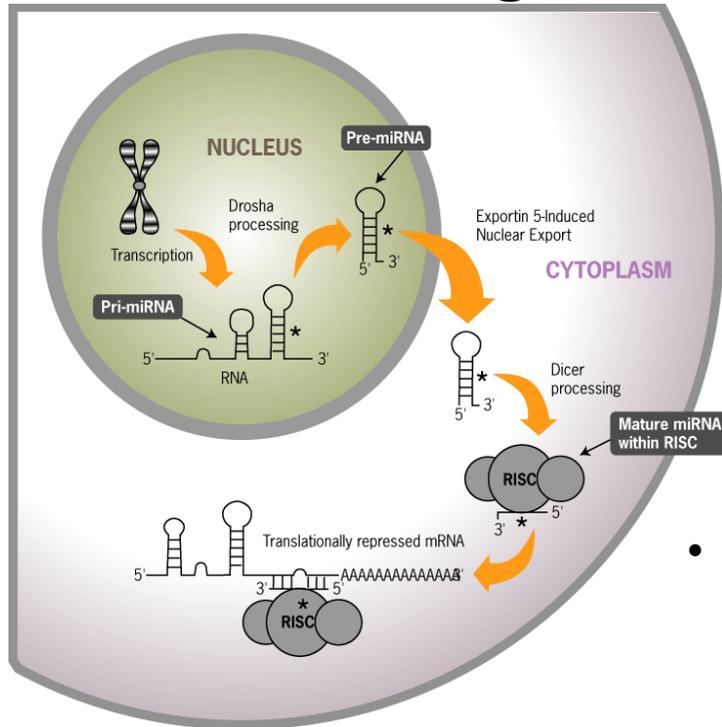


Δ Δ C_t Calculations (Comparative C_t)

	c-Myc	GAPDH	ΔC _t	ΔΔC _t	2 ^{-ΔΔC_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

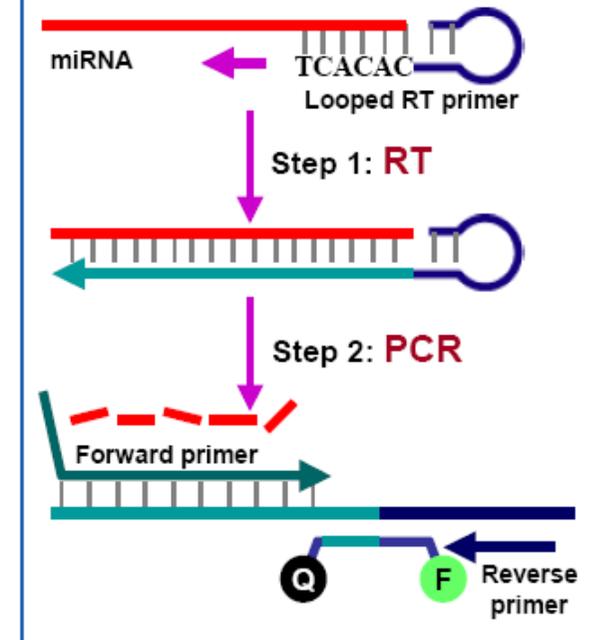


miRNA: a new gene expression regulator



Mature miRNA

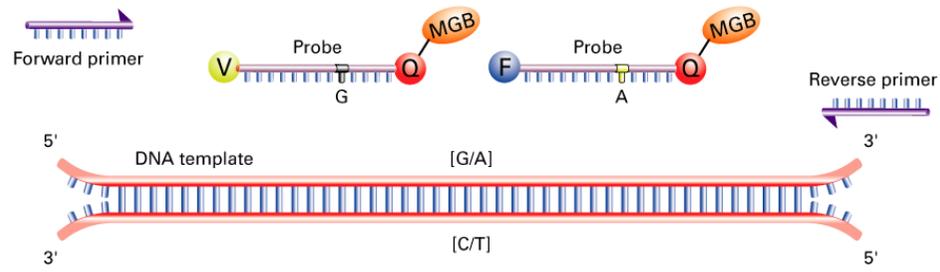
UGAUGAGCCCGUGUCCAUAU



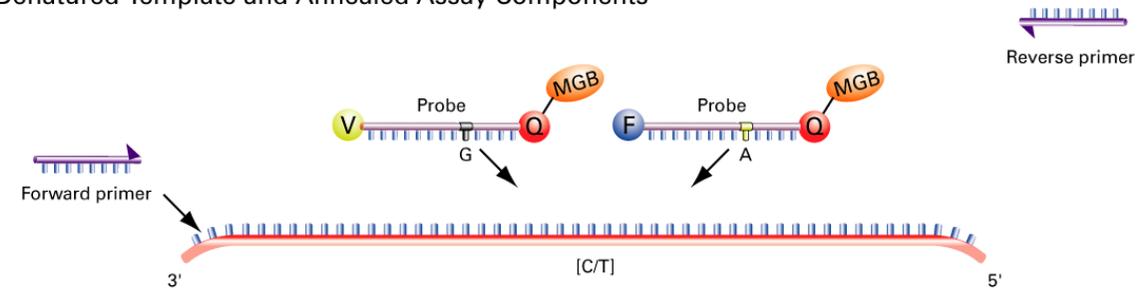
- Assay include: 812 Human;
583 Mouse;
349 Rat;
62 *Arabidopsis thaliana*
74 *Caenorhabditis elegans*
76 *Drosophila melanogaster*
- 46 endogenous controls (small nuclear RNAs)
U6, U19, U24, U38, U43, U44, U48, U49, U66, Z30.....
- Each assay contain: 1 RT primer, 1 TaqMan Assay
50x RT reaction and 150 real time reactions (20ul /rxn)

TaqMan® SNP Genotyping Assay Overview

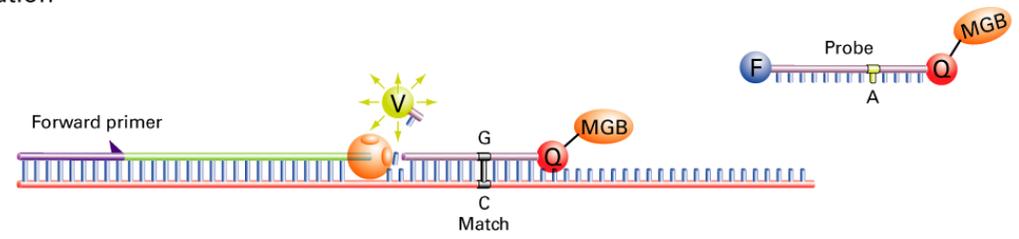
1. Assay Components and DNA Template



2. Denatured Template and Annealed Assay Components



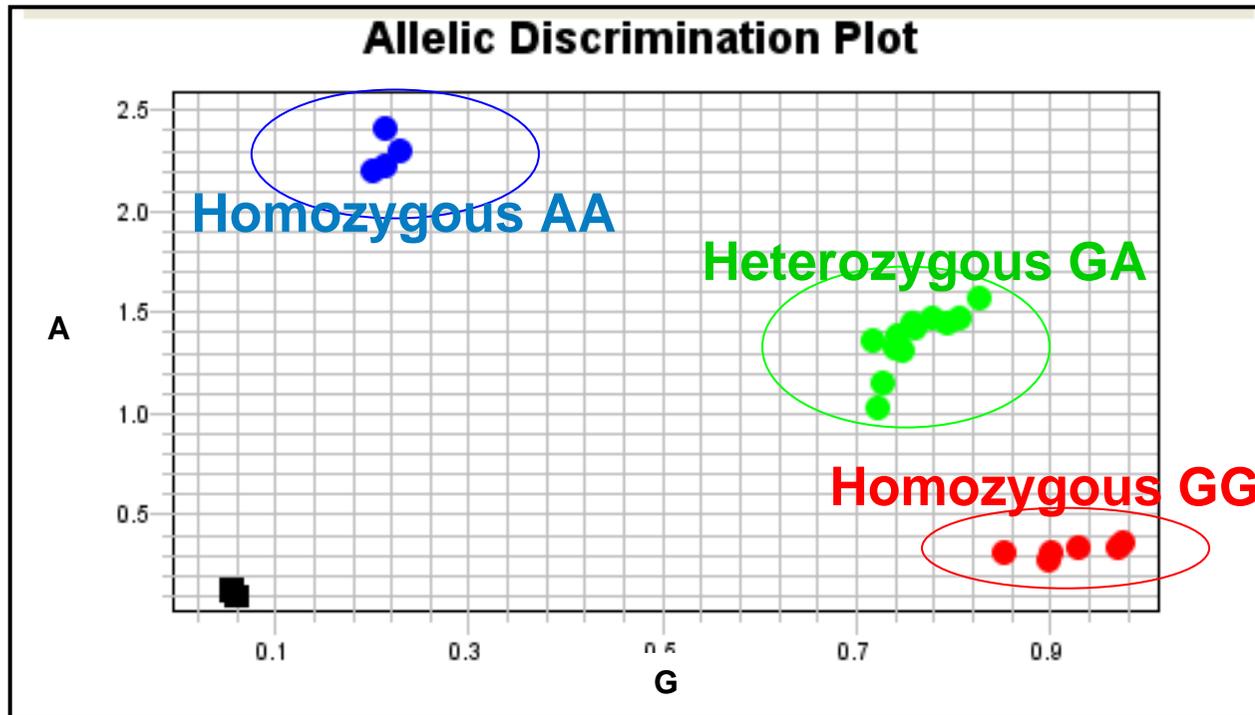
3. Signal Generation



LEGEND

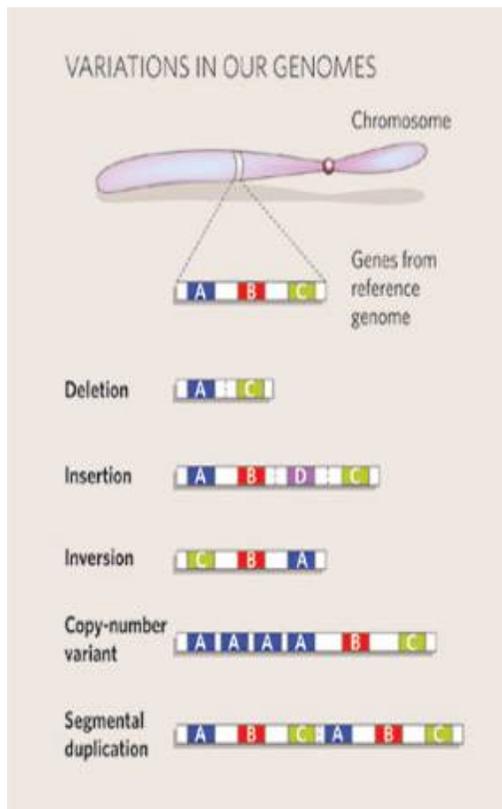
- V** VIC® dye
- F** FAM™ dye
- Q** Quencher
- MGB** Minor Groove Binder
- AmpliTaq Gold® DNA Polymerase
- Probe
- Primer
- Template
- Extended Primer

Allelic Discrimination (SNP) data



Sample	ΔF ₃₂₀	Pass	Ref	Call
Sample 15	0.152	3,617.946	■	Negative Control (NC)
Sample 16	0.186	3,784.869	■	Negative Control (NC)
Sample 17	0.334	4,068.745	●	Homozygous 1/1
Sample 18	2.282	3,774.144	●	Homozygous 2/2
Sample 19	0.392	4,004.767	●	Homozygous 1/1
Sample 2	1.5	3,991.875	●	Heterozygous 1/2
Sample 20	1.206	3,942.024	●	Heterozygous 1/2
Sample 3	1.624	3,956.087	●	Heterozygous 1/2
Sample 4	1.526	3,849.214	●	Heterozygous 1/2
Sample 5	1.478	3,793.905	●	Heterozygous 1/2
Sample 6	1.086	3,820.435	●	Heterozygous 1/2
Sample 7	1.371	3,945.303	●	Heterozygous 1/2
Sample 8	0.767	1.528	4,026.388	Heterozygous 1/2
Sample 9	0.748	1.478	3,793.905	Heterozygous 1/2
Sample 10	0.709	1.086	3,820.435	Heterozygous 1/2
Sample 11	0.736	1.371	3,945.303	Heterozygous 1/2
Sample 12	0.795	1.528	4,026.388	Heterozygous 1/2
Sample 13	0.84	0.364	3,737.053	Homozygous 1/1
Sample 14	0.888	0.37	4,099.657	Homozygous 1/1
Sample 15	0.189	2.251	3,643.652	Homozygous 2/2
Sample 16	0.963	0.421	3,826.976	Homozygous 1/1
Sample 17	0.705	1.418	3,982.397	Heterozygous 1/2
Sample 18	0.729	1.386	4,048.287	Heterozygous 1/2
Sample 19	0.916	0.393	4,015.545	Homozygous 1/1
Sample 20	0.73	1.437	3,797.601	Heterozygous 1/2

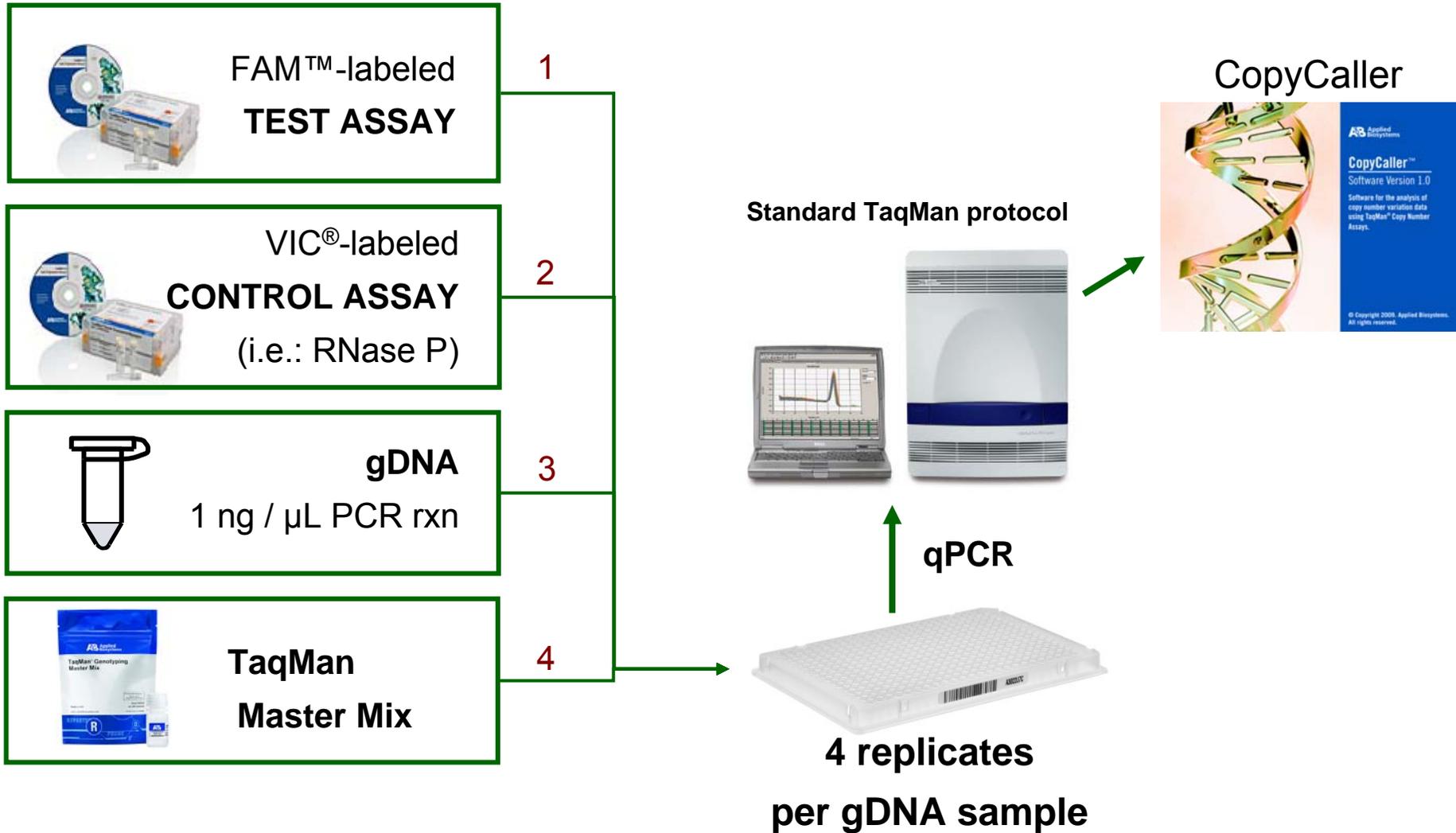
CNV Importance



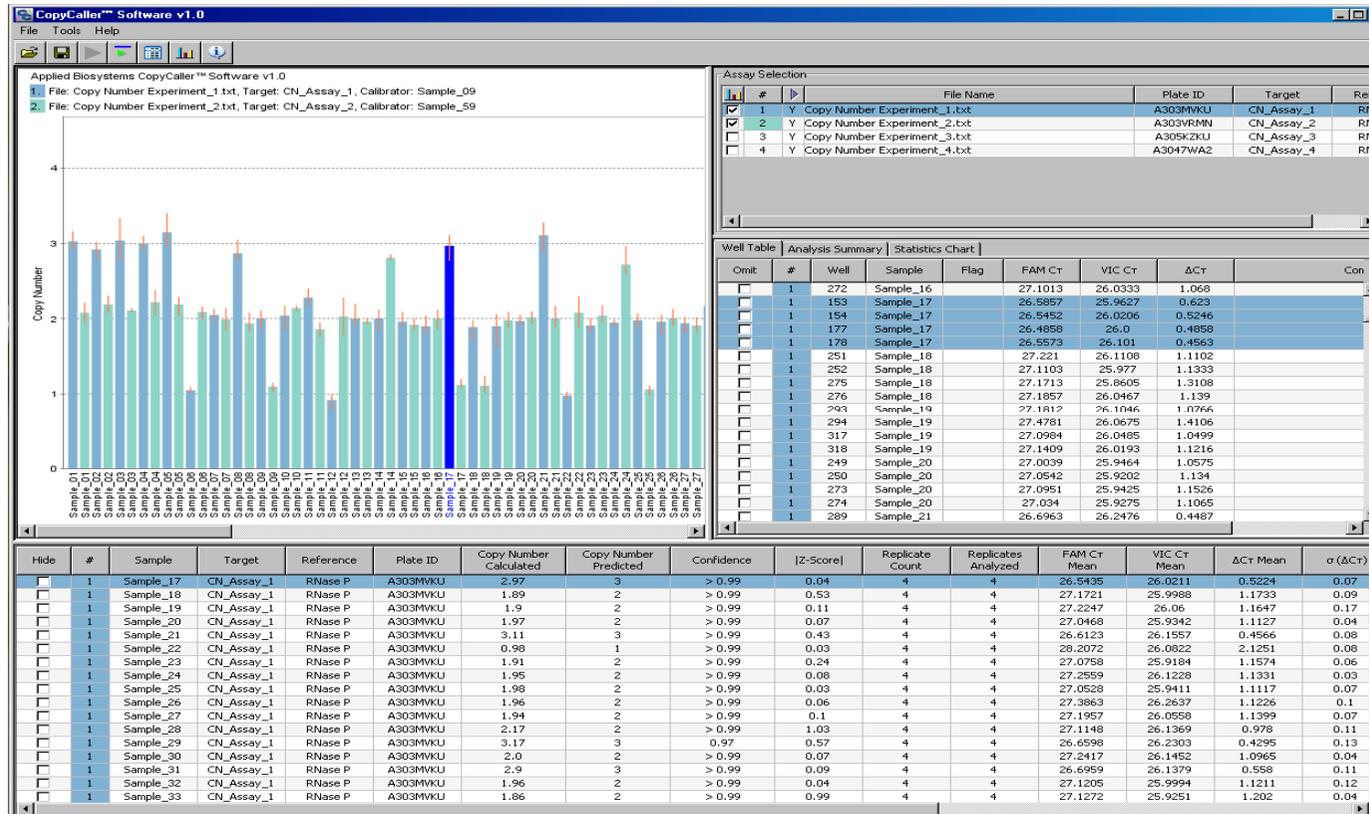
Source: "Global variation in copy number in the human genome." Richard Redon, et. al. *Nature* 444, 444-454 (23 November 2006)

- Redon et. al. defined copy number variation (CNV) as a deletion or duplication event involving >1 kb of DNA
 - An important polymorphism
 - ~20,000 identified CNVs
 - Corresponding to >6,000 unique regions/locus in human genome assembly
 - Associated with diseases or genomic disorders such as cancer, immune diseases, and neurological disorders, etc.
- Gene dosage effects can be phenotypic
 - CYP2D6 is associated with drug metabolizing phenotype
 - CCL3L1 affects the susceptibility to HIV/AIDS

Workflow of TaqMan® Copy Number Assays



★ > 1.6M Pre-Designed TaqMan Copy Number Assays available



- **Flexible** 不需要已知拷貝數的樣品當control
- **Free** 免費下載分析軟體
- **Easy to use** 幾分鐘內完成分析，搭配圖形化介面，輕鬆了解判讀結果
- **Results with confidence value** 軟體內建統計運算邏輯，提供值得信賴的結果

castPCR Technology

castPCR: Competitive Allele-Specific TaqMan PCR

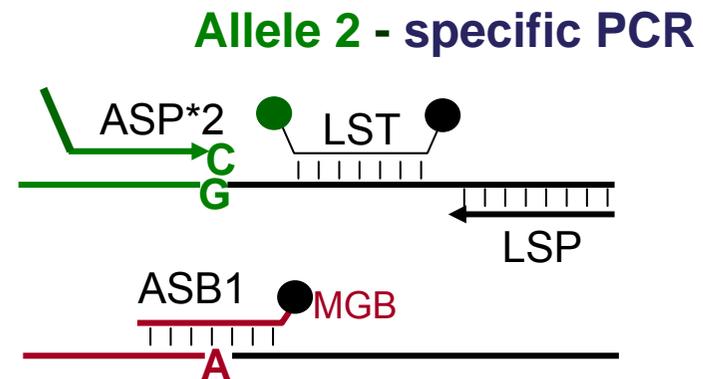
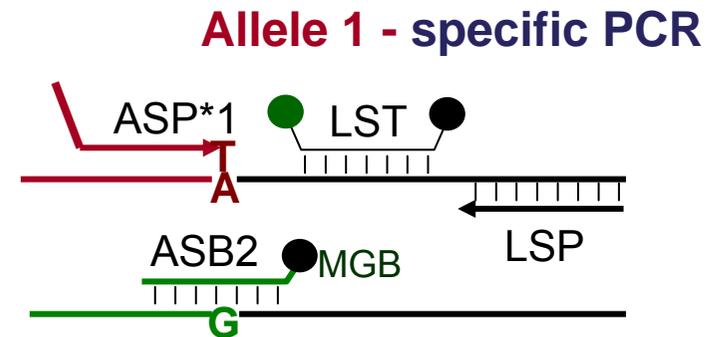
A pair of assays:

Allele-1 assay (wildtype)

- Allele-1 specific primer (ASP*1)
- Allele-2 Specific MGB blocker (ASB2)
- locus-specific TaqMan probe (LST)
- locus-specific PCR primer (LSP)

Allele-2 assay (mutant)

- Allele-2 specific primer (ASP*2)
- Allele-1 Specific MGB blocker (ASB1)
- locus-specific TaqMan probe (LST)
- locus-specific PCR primer (LSP)



Assay Specificity

Conventional AS-PCR vs. **castPCR**

Conventional AS-PCR

		Primer base at 3'		
		C	T	A
gDNA template	G	27.2	28.1	37.3
	A	28.5	28.1	37.1
	T	28.1	30.5	27.3

$$\text{Ave. } \Delta\text{Ct} (\text{Ct}_{\text{mm}} - \text{Ct}_{\text{pm}}) = 4.1$$

castPCR

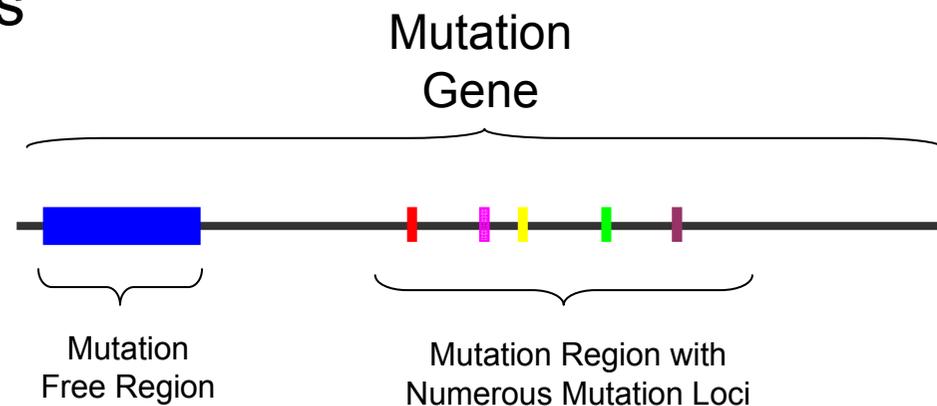
		Primer base at 3'		
		C	T	A
gDNA template	G	28.5	48.2	50.0
	A	48.5	28.5	50
	T	50	50	28.1

$$\text{Ave. } \Delta\text{Ct} (\text{Ct}_{\text{mm}} - \text{Ct}_{\text{pm}}) = 21.1$$

castPCR Is Much More Specific!

Fixed Set of Taqman Mutation Detection Assays

- A fixed set of RUO assays for detecting and quantifying the mutation status
- 68 Assays:
 - wild-type and mutant assays
 - 14 KRAS mutations: codon 12, 13, and 61
 - 1 BRAF mutation: codon V600
 - 29 EGFR mutations: Exon 18, 19, 20, and 21
 - One specialty assay detecting 19 deletions on EGFR Exon19
- Three Reference assays
 - EGFR_Ref
 - KRAS_Ref
 - BRAF_Ref
- IPC control kit



• www.appliedbiosystems.com/KRAS

針對基因定量與定性的解決方案

如何設計Real Time PCR的Primers/ Probe

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



TaqMan Gene Expression Assays

> 1,100,000 個已設計及測試過的
基因定量試劑組

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



- **TaqMan SNP Genotyping Assays/ CNV Assays**

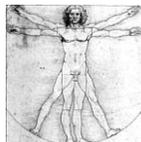
- Ready-to-Use Assays, single tube formulation
- 網路選購並提供所有相關生物資訊
- Updated Human Genome information



- **代客設計** for SNPs and Gene Expression
 - All-in One tube TaqMan-based Assay



- **Primer Express Software**



How to Search ABI TaqMan Gene Expression assay??

www.appliedbiosystems.com.tw

首頁

產品介紹

技術應用

產品服務

技術支援

活動新知

Catalog

- Cell Biology
- Chromatography
- Custom Primers & Probes
- DNA Sequencing by Capillary Electrophoresis
- DNA Sequencing by Ligation
- DNA Synthesis
- DNA/RNA Modification & Labeling
- DNA/RNA Purification
- Fluorescence Spectrophotometry
- Gene Expression
- Genotyping
- Human Identification & Forensic DNA
- Information Management
- Mass Spectrometry
- Microarrays
- MicroRNA Analysis
- Northern/Southern Blotting & Ribonuclease Protection Assays
- PCR/RT-PCR
- Peptide Synthesis
- Protein Sequencing
- Quality & Safety Testing
- Real-Time PCR
- siRNA/RNAi
- Services
- Transcription & Translation Systems

What's New

- New Products
- Special Offers

Assay Searches

- *Silencer*® siRNAs
- TaqMan® Custom Array
- TaqMan® Gene Expression Assays
- TaqMan® MicroRNA Assays
- TaqMan® SNP Genotyping Assays

All Categories

網頁最多人瀏覽...

- 中文線上科技講座
- 美商應用生命系統自7月1日起正式直營Ambion品牌在台業務銷售
- Stem Cell Research
- Total Solutions for gene expression with real-time PCR
- TaqMan® MicroRNA Assays

1 2 3 4 5

研究解決方案

產品選用指引

服務與支援

最新消息

TaqMan® Array Gene Signature Plates



Plate Guide

http://www3.appliedbiosystems.com/AB_Home/products/guides/PlateGuide/index.htm

Which TaqMan® Array Gene Signature 96-Well Plate is right for you? Simply select a species and disease or pathway below.

Find Plates:

By Species

- All Species
- Human
- Mouse
- Rat

By Disease/Pathway

- All Diseases/Pathways
- Apoptosis
- Biomarkers Related Pathway
- Cancer
- Cell Cycle Proliferation and Regulation
- Development and Stem Cells
- ECM Matrix and Adhesion
- Endogenous Controls
- Immune System and Inflammation
- Neurology
- Reproduction
- Signal Transduction
- Toxicology and Drug Metabolism

14-3-3 Induced Intracellular Signaling Plate, Human

Signal Transduction Pathways Plate, Human

Signaling in GAP Junctions Plate, Human

SMAD Signaling Network Plate, Human

Sperm Motility Plate, Human

Stem Cell Pluripotency Plate, Human

Stem Cell Pluripotency Plate, Mouse

T-Cell Receptor and CD3 Complex Plate, Human

Telomere Extension by Telomerase Plate, Human

Stem Cell Pluripotency Plate, Human

Diseases/Pathways: Biomarkers Related Pathway, Cancer, Cell Cycle Proliferation and Regulation, Development and Stem Cells, Neurology, Reproduction

Plate Description

The TaqMan® Array Human Stem Cell Pluripotency 96-well Plate contains 92 assays to stem cell associated genes and 4 assays to candidate endogenous control genes.

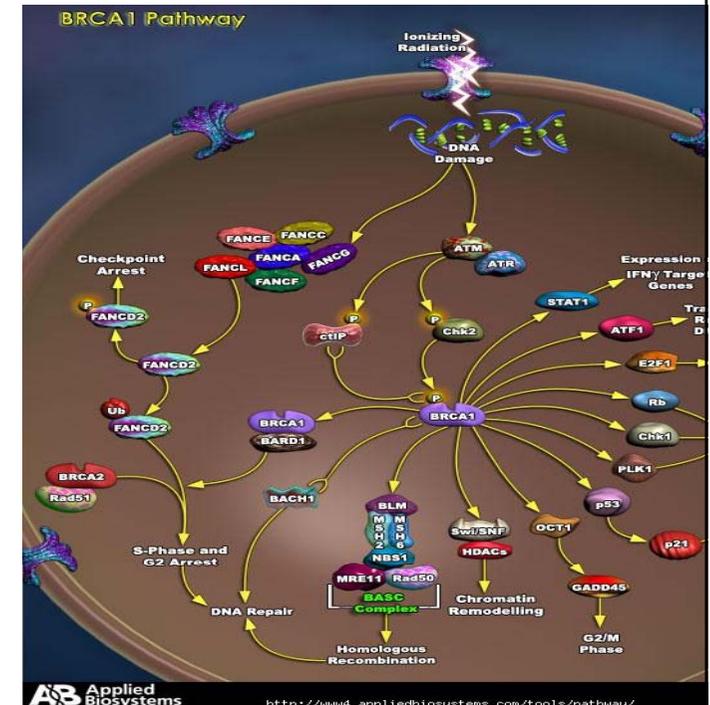
The panel of assays in this plate was selected in cooperation with the International Stem Cell Initiative (2007 Nature Biotechnology, 25:803-816). The assays target genes which are validated as markers for the characterization of human embryonic stem cell identity, and which assess variations between embryonic stem cell isolates. Genes are included based on the following criteria:

Assay ID	1	2	3	4	5	6	7	8
A	Hs99999901_s1	Hs99999905_m1	Hs99999909_m1	Hs99999908_m1	Hs00606316_m1	Hs00173490_m1	Hs00217848_m1	Hs00156373_m1
B	Hs00164004_m1	Hs00156568_m1	Hs00201350_m1	Hs00275636_m1	Hs00170025_m1	Hs00251859_m1	Hs00157258_m1	Hs00171876_m1
C	Hs00170454_m1	Hs00176573_m1	Hs00277509_m1	Hs00232764_m1	Hs00255287_s1	Hs00241459_m1	Hs00544355_m1	Hs00171403_m1
D	Hs00220998_m1	Hs00157674_m1	Hs00917999_g1	Hs00747223_g1	Hs00744391_s1	Hs00232128_m1	Hs00169095_m1	Hs00705137_m1
E	Hs00236830_m1	Hs00158126_m1	Hs00174029_m1	Hs00196158_m1	Hs00300550_m1	Hs00158620_m1	Hs00267056_m1	Hs00764128_m1
F	Hs02387400_g1	Hs00707120_s1	Hs00159598_m1	Hs00415443_m1	Hs00271352_s1	Hs00383230_g1	Hs00187067_m1	Hs00265966_m1
G	Hs00193638_m1	Hs00742896_s1	Hs00829813_s1	Hs00603586_g1	Hs00234119_m1	Hs00194498_m1	Hs00231692_m1	Hs00173810_m1
H	Hs00538143_m1	Hs00300531_m1	Hs00610080_m1	Hs00356930_m1	Hs02339499_g1	Hs00162669_m1	Hs00232708_m1	Hs00165941_m1

Gene Symbol	1	2	3	4	5	6	7	8
A	18S	GAPDH	HPRT1	GUSB	ACTC1	AFP	BXDC2	CD34
B	COL1A1	COL2A1	COMM3D3,BMI1	CRABP2	CTNNB1	DDX4	DES	DNMT3B
C	FGF5	FLT1	FN1	FOXA2	FOXD3	GABRB3	GAL	GATA4
D	GDF3	GFAP	GRB7	HBB	HBZ	MX1	IAPP	IFITM1
E	PDX1	ISL1	KIT	KRT1	LAMA1	LAMB1	LAMC1	LEFTY1
F	NANOG	NES	NEUROD1	NODAL	NOG	NPPA	NR5A2	NR6A1

Pathway Study (II): GeneAssist™ Pathway Atlas

- Provides >350 interactive, signal transduction, metabolic and disease state cell pathway maps
- Incorporates information from publications & bioinformatics
- When a protein is selected, additional gene information appears along with the recommended *Silencer®* select siRNAs and TaqMan® Gene Expression Assays



GeneAssist™ Pathway Atlas

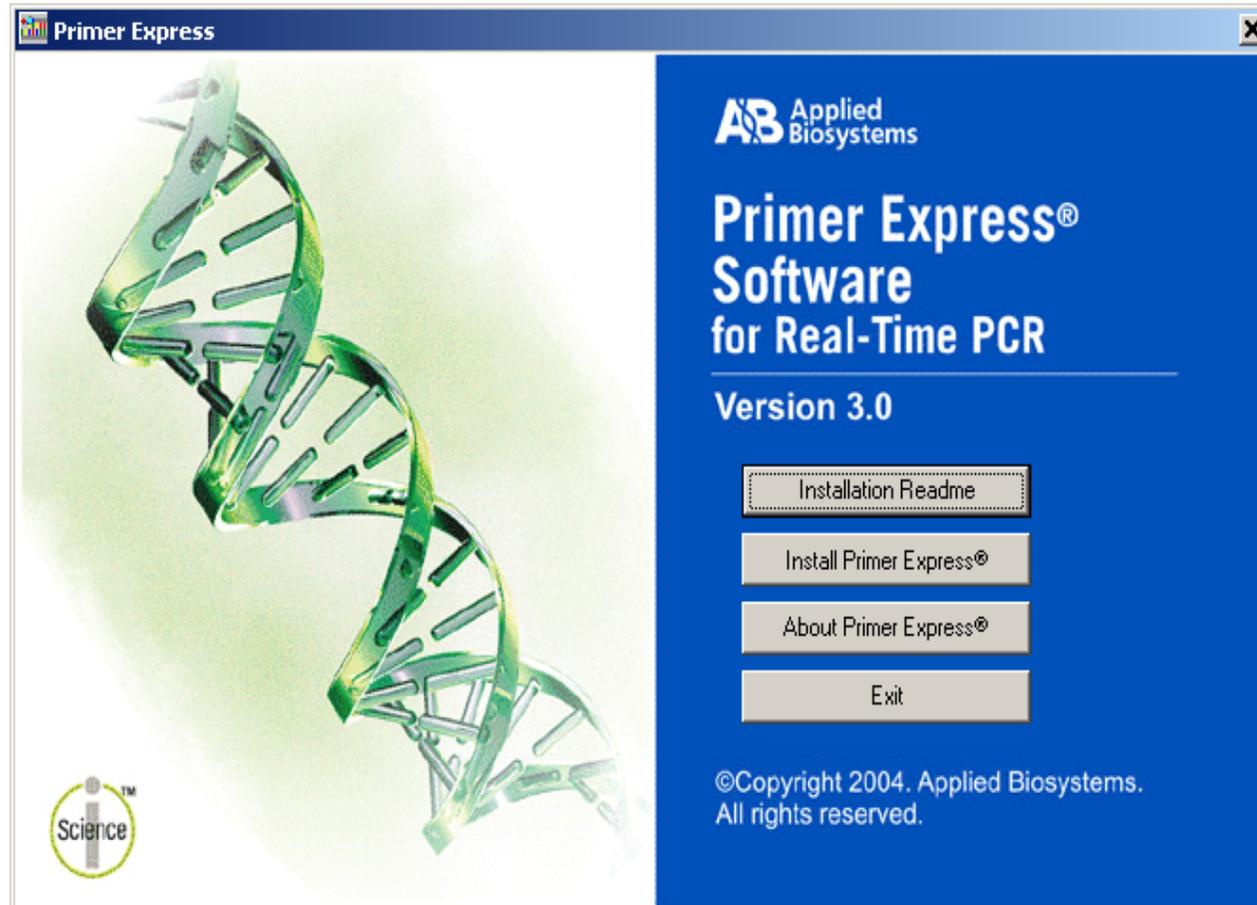
Close Window

Export Results

BRCA1 Pathway contains total of 64 proteins listed below.

Protein Name	Gene Id	Gene Symbol	Gene Name	Silencer® Select/ Silencer® siRNA	TaqMan®
ATF1	466	ATF1	activating transcription factor 1	s1697, s1698, s1696 / 41923, 42010, 115615	Hs00270896_m1
ATM	472	ATM	ataxia telangiectasia mutated	s1710, s1709, s1708 / 214707, 118231, 111194	Hs00175892_m1
ATR	545	ATR	ataxia telangiectasia and Rad3 related	s534, s535, s536 v / 82 v, 83 v, 103302	Hs00169878_m1
BACH1	571	BACH1	BTB and CNC homology 1, basic leucine zipper transcription factor 1	s1859, s1860, s1858 / 3268, 115188, 3176	Hs00895421_m1
BARD1	588	BARD1	BRCA1 associated RING domain protein 1	s1887, s1886, s1885 /	Hs00144427_m1

定量PCR Primers/ Probe設計軟體



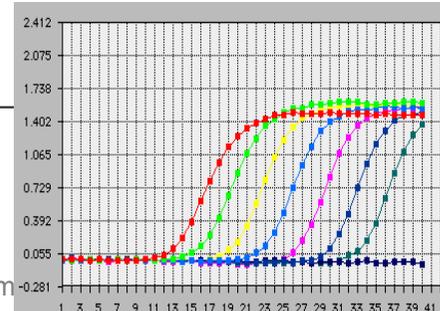
清楚明確的 TaqMan Probe & Primer 設計規範

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	

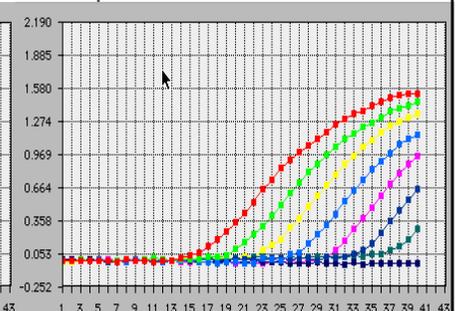
a: 針對 TaqMan MGB probe

b: 參數可選擇設定

200 bp amplicon



500 bp amplicon



SYBR Green experiment procedure

1. Primer conc. Optimization

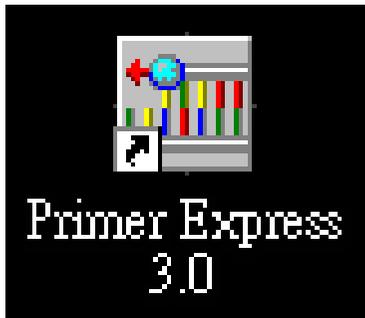
- Primer Final conc. 100-300 nM
- No primer dimer or non-specific product involved

2. PCR Primer Efficiency Validation

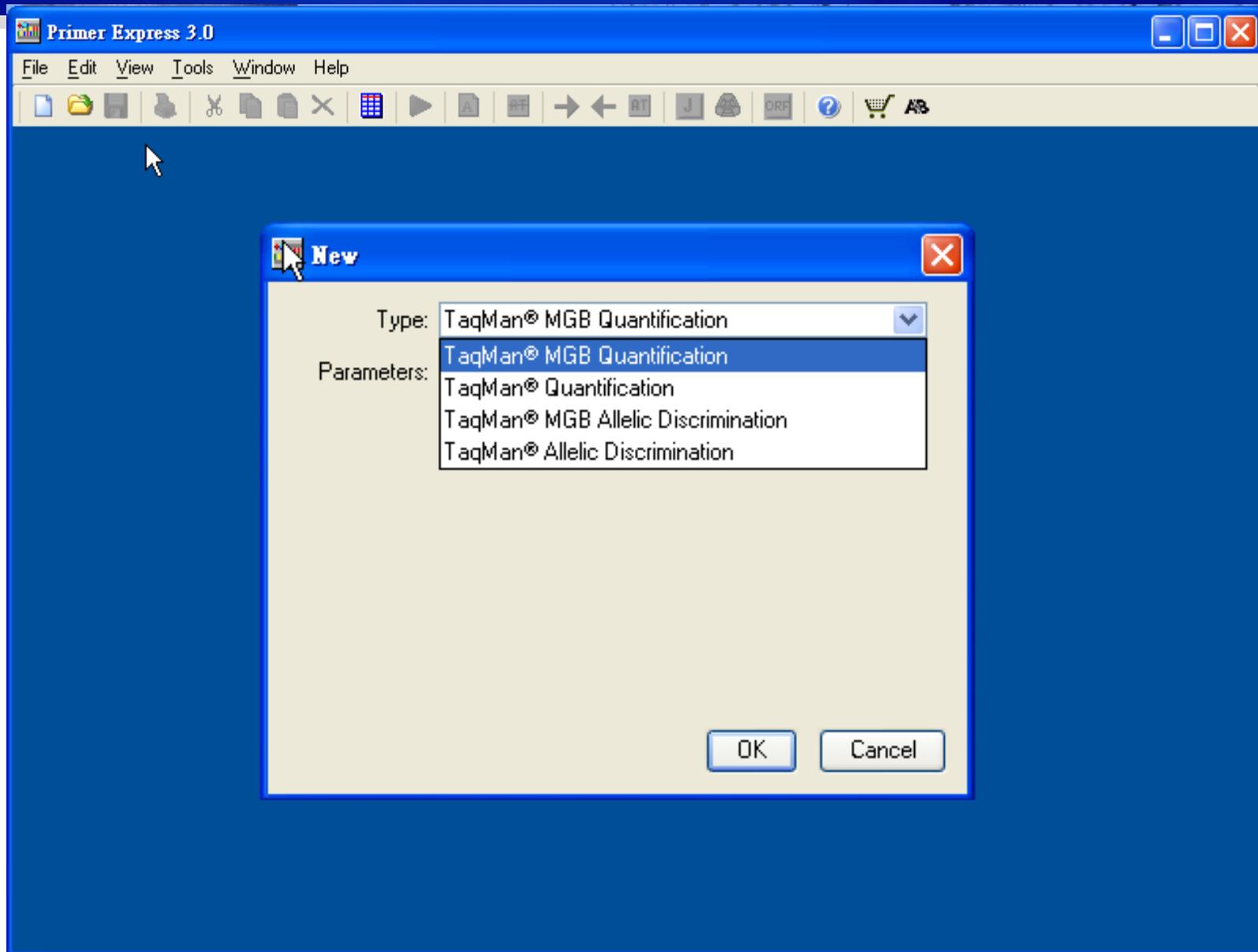
- Sample serial dilution to run standard curve for target gene and endogenous control gene

3. Real sample run for each gene

Primer Express 3.0 Operation



Primer Express 3.0 Operation



The screenshot shows the 'Primer Express 3.0' application window. The title bar includes 'File Edit View Tools Window Help' and a toolbar with various icons. A red circle highlights the 'Find Primers/Probes' button (a green play icon) in the toolbar. Another red circle highlights the 'File Name' input field, which contains 'FBXW3.txt'. A white box with the text '2. Find Primer/Probe' is positioned over the toolbar area. Below the input field, the text '1. Add DNA file or Copy & Paste' is displayed. The main window displays a DNA sequence with a vertical scale on the right side ranging from 50 to 383 bp. At the bottom, a message reads: 'To find Primers & Probes, click the "Find Primers/Probes" button'.

2. Find Primer/Probe

1. Add DNA file or Copy & Paste

File Name: FBXW3.txt

Length: 383 bp. Selection:

```

BWAGCCATCA CCCAGCCTT GTGTGCCGTG TGTCCCAGG GGCAAGGCGG 50
CAGTGTGTG CCTTCCTAC CAACCTGATA TCCTGGTGAC TGGTACCTAT 100
GACAAGAAGG TGACCATCTA TGATCCCAGA GGTGAGCCTT TAATCCCAGT 150
GCGTAGAAGG CAAAGGGAAG CAGATCTCTA AGTTCAAGAT CAGCATGGGC 200
TACATAGTAA ATTCTAGGCC AGCTAGGGCT ACACAGTAAG ATCCTGTCAC 250
AAAAAACTC AATAAACAAA ACACAACAAA AAACAAAAGA AAGGAAACAC 300
AACACAACAG AAAAGAGCAT GGGGGCAGGA TGCAGGGGCT GAAAAGATGG 350
CTCAGCAATT AAGAACGCTG GTTGCCCTTC CBW 383

```

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

Result

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



Secondary Structure

Oligo	Length
<input checked="" type="radio"/> Forward Primer	18
<input type="radio"/> Reverse Primer	26
<input type="radio"/> Probe	17
Forward Primer	
CGGCAGTGCTGTGCCTT	
Reverse Primer	
CACCTTCTTGTGCATAGGTACCAGTCA	
Probe	
CTACCAACCTGATATCC	

Hairpin Self Dimers Cross Dimers

Most Stable Structure Found

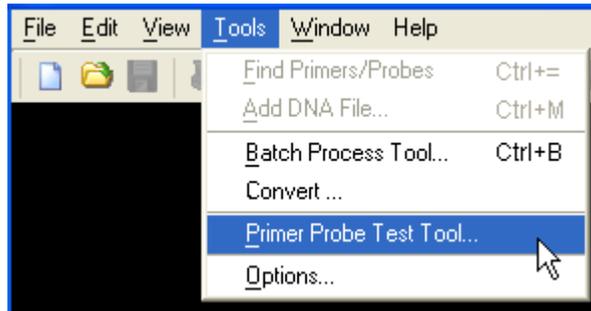
```

GTGACGGC 5'
  ||||
CTGTGCCTT 3'
    
```

Design Parameter

TaqMan® MGB Quantification # 1	
Parameter	Value
max primer length	40
Optimal Primer Length	20
<input type="checkbox"/> Primer Composition	
Max Primer G Repeats	3
Max Num Ambig Residues in Primer	0
<input type="checkbox"/> Primer Secondary Structure	
Max Primer Consec Base Pair	4
Max Primer Total Base Pair	8
<input type="checkbox"/> Primer Site Uniqueness	
Max % Match in Primer	75
Max Consec Match in Primer	9
Max 3' Consec Match in Primer	7
<input type="checkbox"/> Probe Tm	
Min Probe Tm	68
Max Probe Tm	70
<input type="checkbox"/> Probe GC Content	
Min Probe %GC Content	30
Max Probe %GC Content	80
<input type="checkbox"/> Probe Length	
Min Probe Length	13
Max Probe Length	25
<input type="checkbox"/> 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"/>
<input type="checkbox"/> Probe Secondary Structure	
Max Probe Consec Base Pair	4
Max Probe Total Base Pair	8
<input type="checkbox"/> Amplicon	
Min Amplified Region Tm	0
Max Amplified Region Tm	85
Min Amplified Region Length	50
Max Amplified Region Length	150
<input type="checkbox"/> General	

Check Tm of primers



The 'Primer Probe Test Tool' dialog box is shown. It has a 'Parameters' section with 'Document Type' set to 'TaqMan® MGB Quantification' and 'Parameter' set to 'Default'. The 'Primers and Probes' section contains input fields for 'Fwd Primer' (ACTGATCGATCAGCTACGCATC), 'Rev Primer' (TCGATCGATCGATCGATGC), 'Probe 1', and 'Probe 2'. A 'Trim' button is at the bottom left. To the right is a table with columns 'Tm', '%GC', and 'Length'. The first two rows have values: 58.1, 50, 22 and 59.2, 53, 19. The last two rows have 0.0, 0, 0. The first two rows of the table are circled in red.

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

Standalone (PC-Free) Operation 簡易三步驟!



The image shows a white and blue Applied Biosystems StepOne Plus PCR system. A white USB drive labeled 'StepOne Plus' and a small white square component are shown in an inset in the top left. Handwritten white text in circles is connected to the machine by lines: '1. Set-up' points to the touchscreen, '2. Run' points to the front panel, and '3. Analyze' points to the machine's base. A white box in the bottom left contains a numbered list of instructions.

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

StepOnePlus™ Real-Time PCR System

The Basics

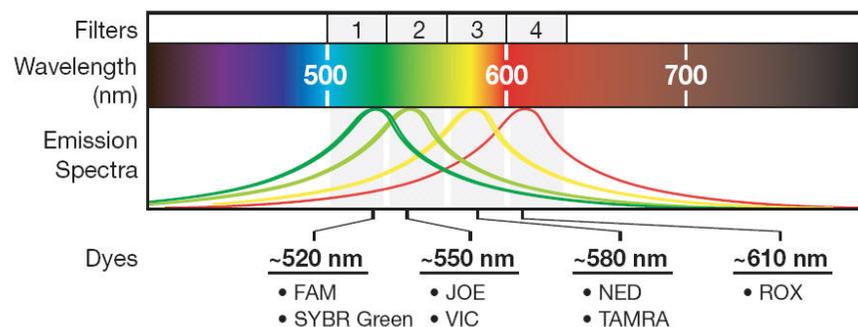
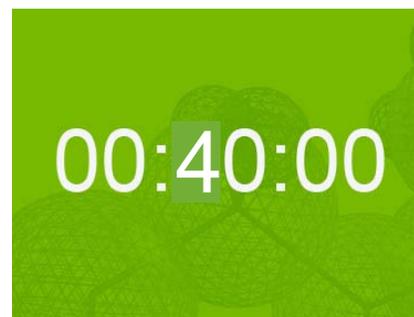
- **96-Well Block**

- One block, 2 speeds

- **Fast cycling:** 40 cycles in under 40 minutes

- **Standard cycling:** 40 cycles in under 2 hours

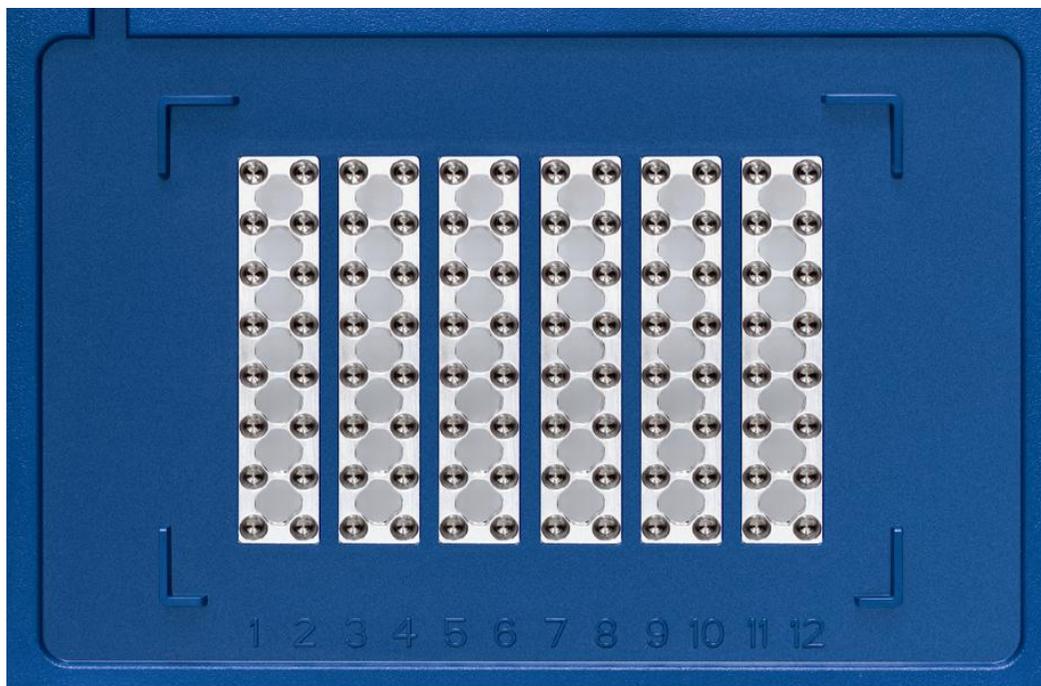
- 10-30 µl reaction volume



StepOnePlus™ Real-Time PCR System

The Basics

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



StepOnePlus™ Real-Time PCR System

The Basics

- Supported consumables:

- **P/N 4346907**

Fast 96-Well Reaction Plate (0.1 mL) - 10 plates

- **P/N 4360954**

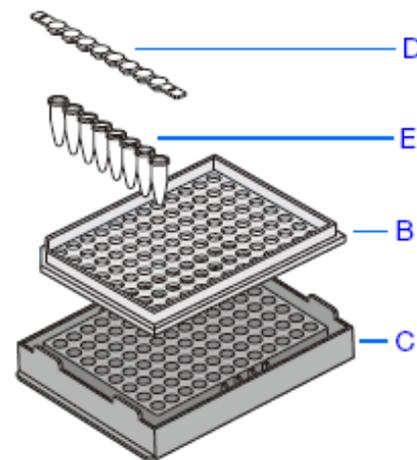
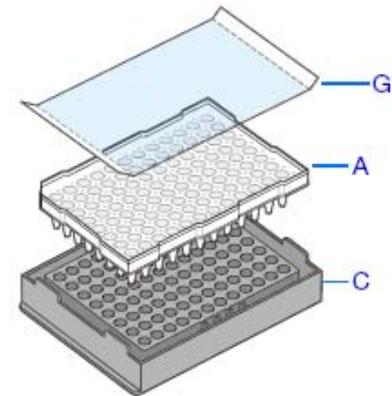
Optical Adhesive Film - 25 films

- **P/N 4358293**

Fast 8-Tube Strip (0.1 mL) - 125 strips

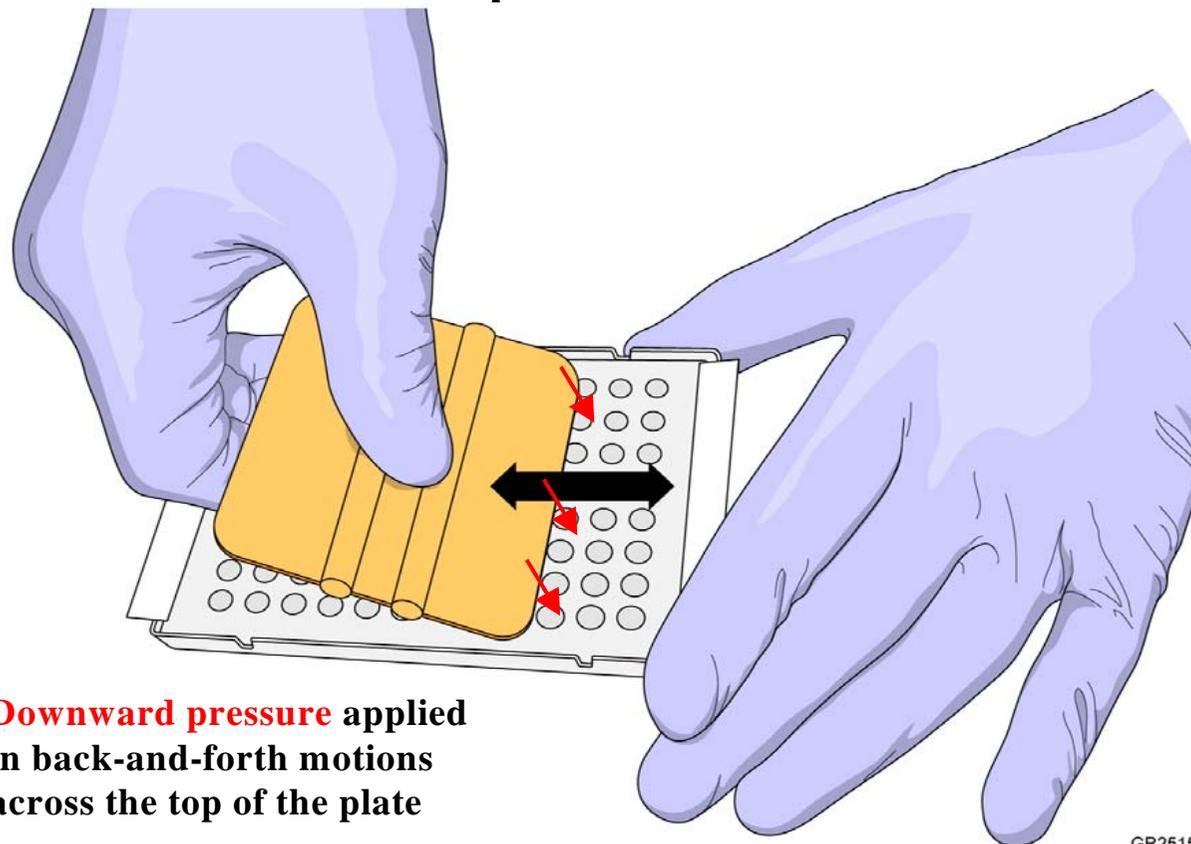
- **P/N 4323032**

Optical 8-Cap Strip - 300 strips



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

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

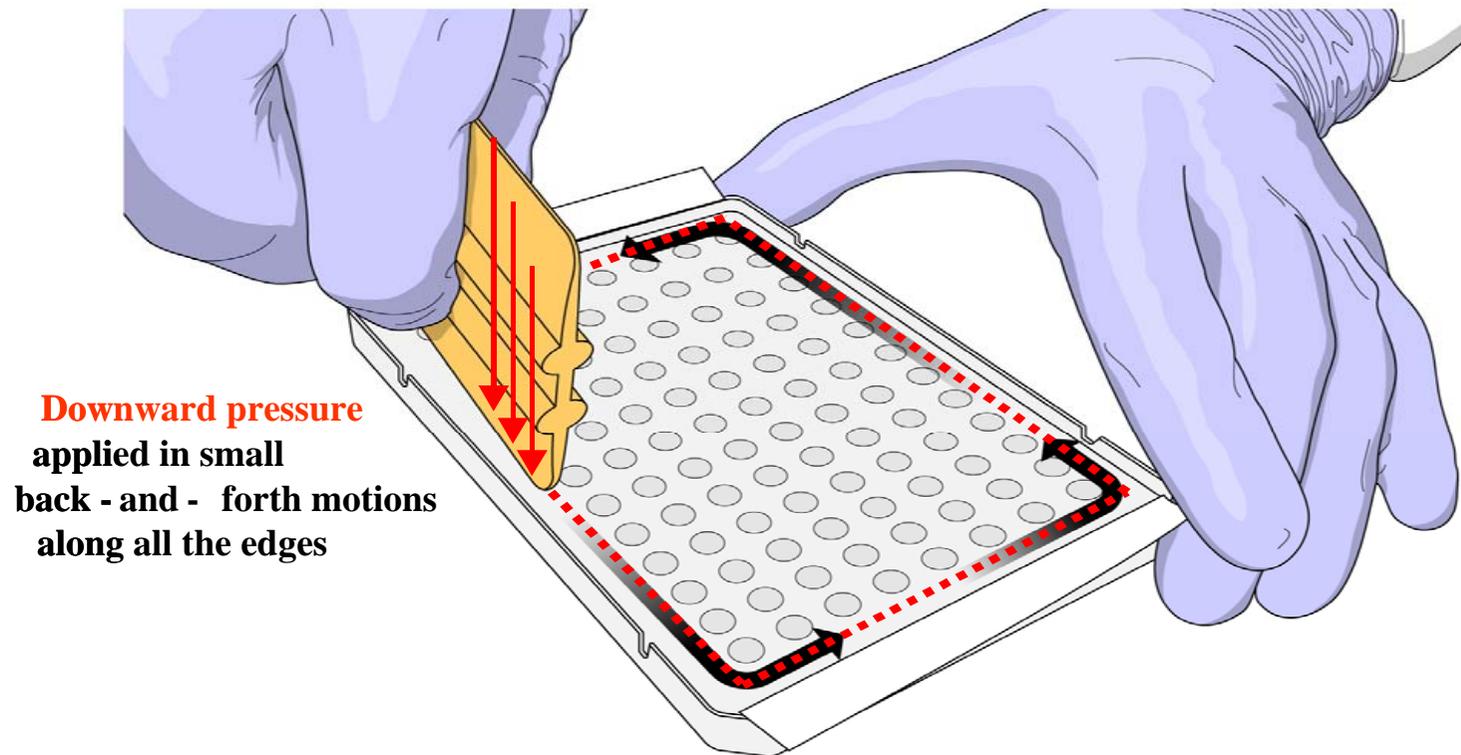


Downward pressure applied in back-and-forth motions across the top of the plate

GR2515

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

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



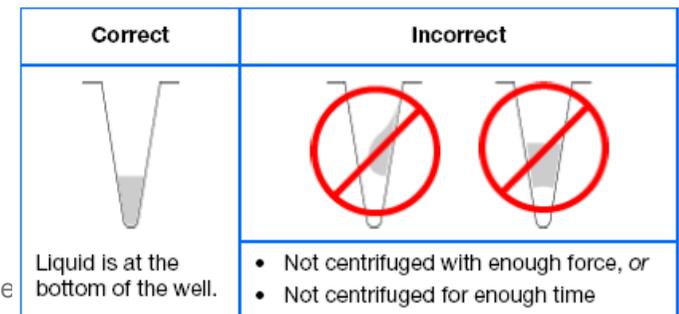
Downward pressure
applied in small
back - and - forth motions
along all the edges

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 the fast individual tubes or 8-tube stripes, load the tube with fast 96-well tray
- Save your data by a USB device after each run (standalone)
- Do not mark any labels on the consumables
 - ✓ This may increase the background signal
- Avoid bubbles when pipetting into each well
 - ✓ Centrifuge samples
- No Screen Saving during the run



Standalone Operation (單機操作)

- Soft power button on the LCD touchscreen



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	Page 1 / 1
20070401-LILY	AB	2007-04-19	
TaqMan_Fast	AB	2007-04-19	
TaqMan_Std	AB	2007-04-08	



Start Run



New



View/Edit



Copy



Delete

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.



Select Experiment : Save

File View Help



Create an Experiment



Template Experiment

Folder

Last Used

SYBR_GREEN*

GTYPE_FST*

PRES_ABS_FST*

1_STP_RT_PCR*

AB_RNASE_P*



Select

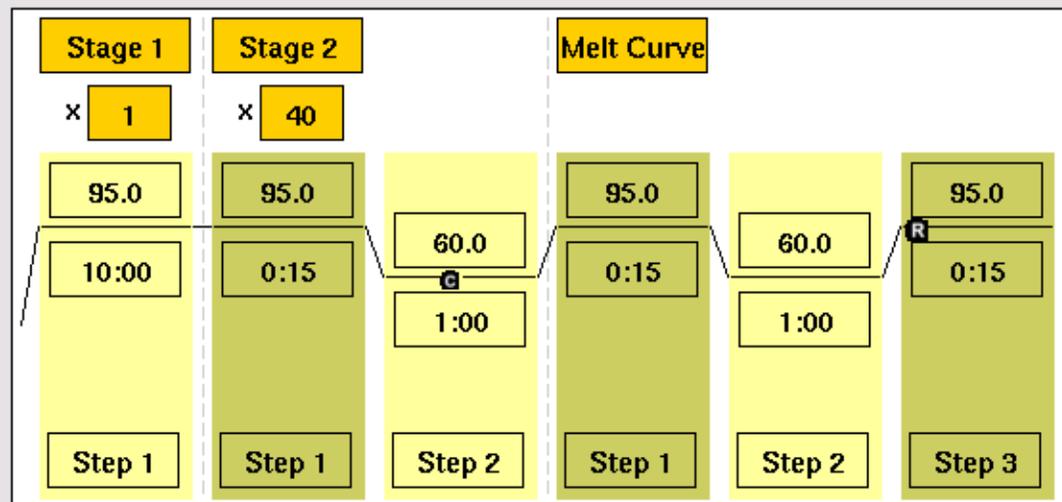
Touch a template experiment to
Select to make it the selected t

2011/07/05

File View Help



Edit Experiment: SYBR_GREEN



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.



File View Help



Save Experiment



Run Experiment:

SYBR_GREEN

Folder:

Default

Reaction Volume:

20

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

2011/07/05

File View Help



Browse Last Accessed Experiments (5)



Experiment	Folder	Last Used
SYBR_GREEN	lily	2007-07-07
TaqMan_Std		
20070401-LILY		
TaqMan_Fast		
TaqMan_Std		



Warning

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

Collect

Overwrite

Cancel

Page
1 / 1

Selected:
1

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.



File View Help



Experiment Parameters



Reaction Volume:

Cover Temperature:

Experiment Name:

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

File View Help

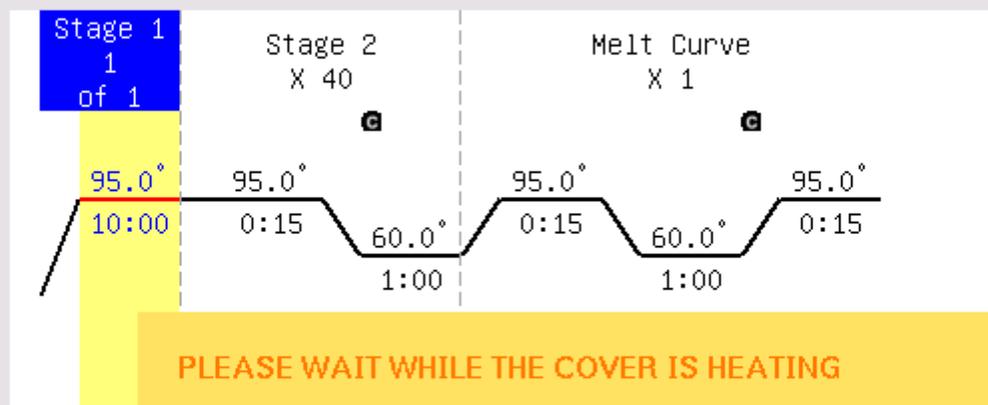


Sample:
31.3 °C

Stage 1

Time Remaining:
02:03:46

Experiment: SYBR_GREEN



PLEASE WAIT WHILE THE COVER IS HEATING

Current Run Temperature:

Required Run Temperature:



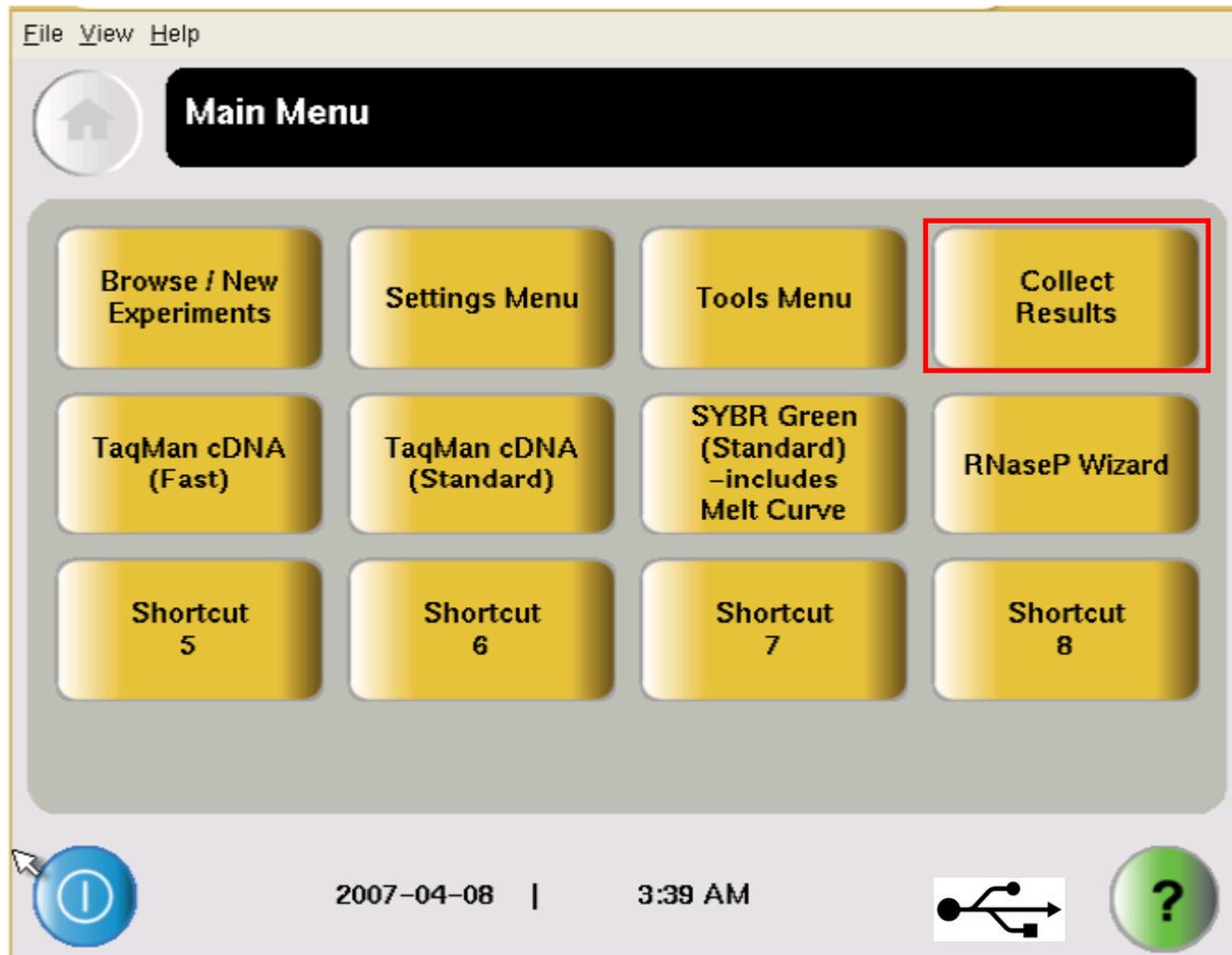
Add Melt Curve

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



StepOne Plus 機器只能暫存一個檔案

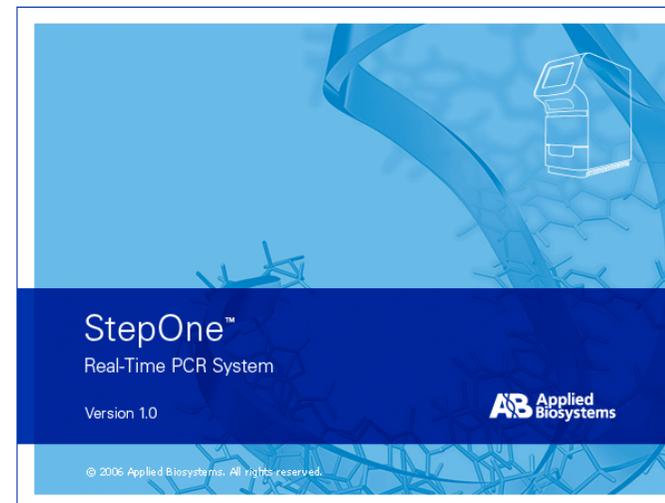
- 插入USB, 待 icon 出現在右下角, 即可點選 **Collect Results** 檔案會自動存到 USB 中



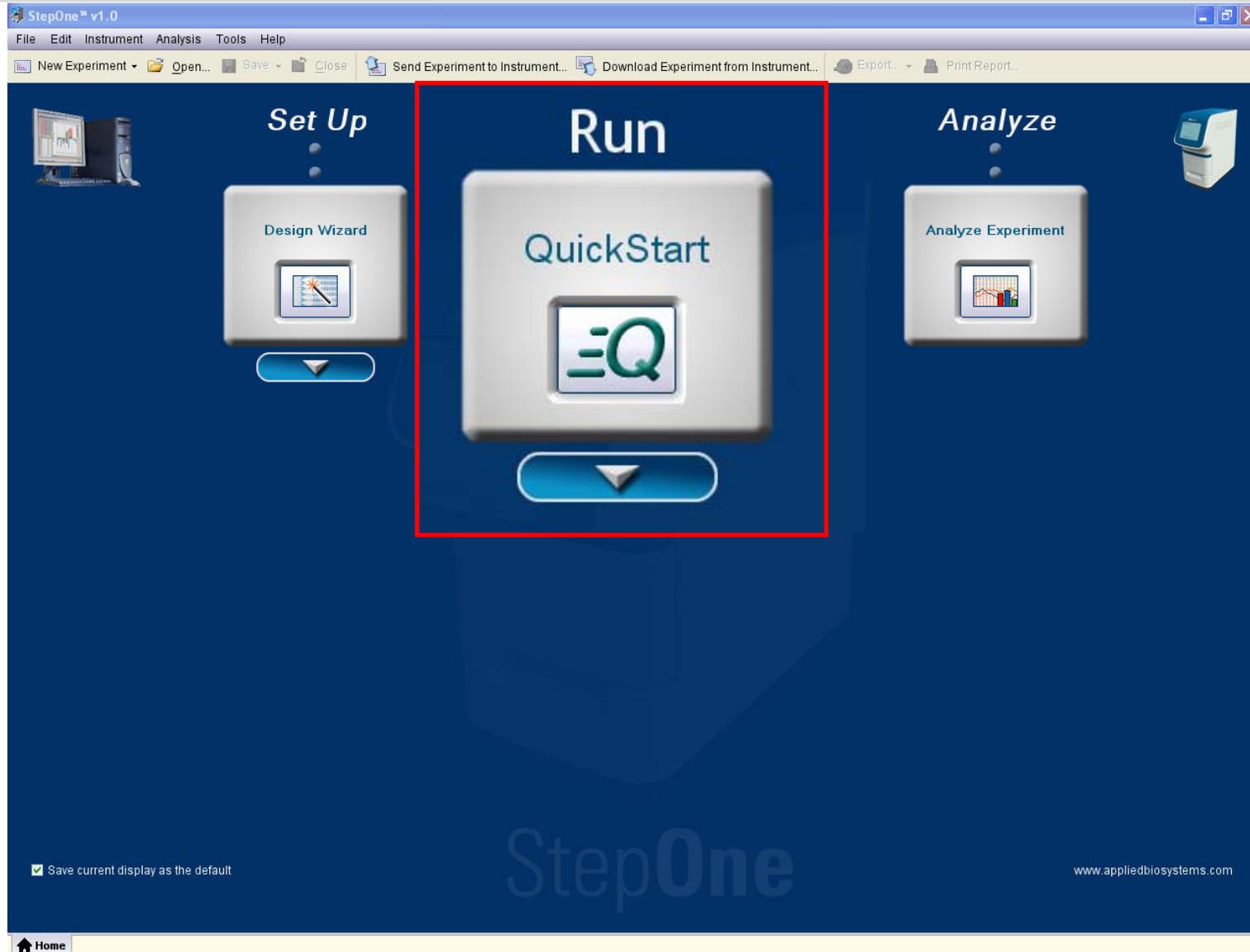
StepOne™ v2.1 software 1280x1024 pixel resolution

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



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: Location:

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 ($\Delta\Delta C_T$)

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

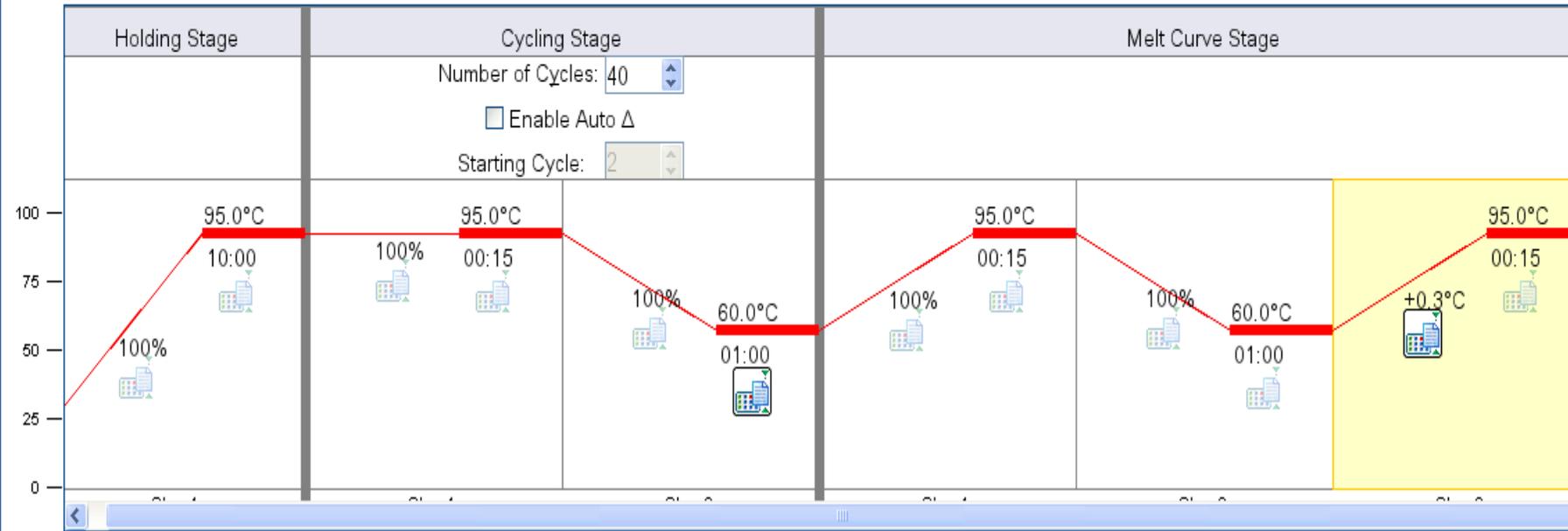
- Setup**
- Experiment Properties
- Plate Setup
- Run Method**
- Reaction Setup
- Materials List

4. **START RUN** 

... 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.

Well μL

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



5. Setup: Plate Setup 定義基因和樣品名稱

The screenshot shows the 'Define Targets and Sample' window in the software. A red box highlights the title 'Define Targets and Sample'. The interface includes a sidebar with 'Setup', 'Experiment Prop...', 'Plate Setup', 'Run Method', 'Reaction Setup', and 'Materials List'. The main area has two tabs: 'Define Targets and Samples' (selected) and 'Assign Targets and Samples'. Below the tabs are two panels: 'Define Targets' and 'Define Samples'. The 'Define Targets' panel has a table with columns: Target Name, Reporter, Quencher, and Co... The 'Define Samples' panel has a table with columns: Sample Name and Co... An 'Assign Targets and Samples' button is at the bottom right. Chinese text with arrows points to the 'Target Name' and 'Sample Name' input fields.

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

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

Define Samples

Sample Name	Co...
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

6-1. Setup: Plate Setup

決定基因和樣品位置 (for ddCt)

Assign Targets and Samples

Define Targets and Samples **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"/>	1	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>

* Mixed U Unknown N Negative Control

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 -

Show in Wells View Legend

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

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: 0 Unknown 0 Negative Control

48 Empty

7. Analyze

Analysis : Amplification Plot

3. Analyze or Re-analyze

1. Graph Type: Log

2. Auto or Manual Threshold: Auto

3. Analyze or Re-analyze Reanalyse

4. Check Threshold Show: Threshold

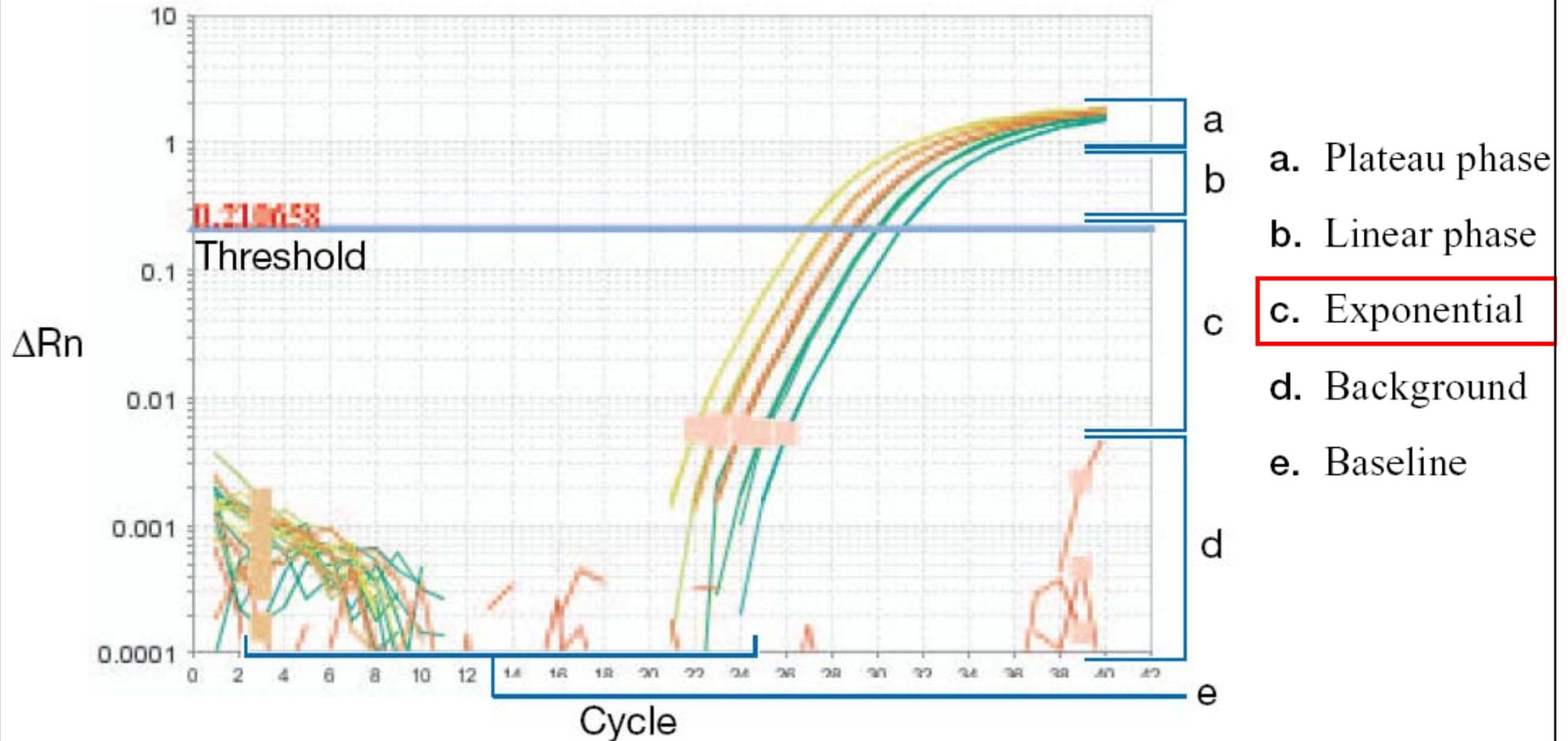
1	2	3	4	5	6	7	8
N TP53 Ct: Unde	N TP53 Ct: Unde	N TP53 Ct: Unde	N GAPDH Ct: Unde	N GAPDH Ct: Unde	N GAPDH Ct: Unde	U TP53 Ct: 30...	U TP53 Ct: 30...
U TP53 Ct: 30...	U GAPDH Ct: 24...	U GAPDH Ct: 24...	U GAPDH Ct: 24...	U TP53 Ct: 30...	U TP53 Ct: 30...	U TP53 Ct: 30...	U GAPDH Ct: 24...
U GAPDH Ct: 24...	U GAPDH Ct: 24...	U TP53 Ct: 30...	U TP53 Ct: 30...	U TP53 Ct: 30...	U GAPDH Ct: 23...	U GAPDH Ct: 23...	U GAPDH Ct: 23...

Wells: **U** 18 Unknown **N** 6 Negative Control **24** Empty

Wells Flagged: 0 Wells Omitted by Analysis: 0 Samples Used: 3 Targets Used: 2

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How to Set the Threshold? Auto or Manual Threshold?



Manual threshold could be used to set **fixed threshold** when doing run to run comparison

Analysis Report

View Plate Layout **View Well Table**

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

Show in Table ▾ Group By ▾

Exp

- Group By ▾
- Target Name
- Sample Name
- Task
- Replicate
- Dye
- Flag
- Ct
- Comments
- Well Position (Row)
- Well Position (Column)
- None

#	Well	Omit	Flag	Sampl...	Target ...	Task	Dyes	Ct	Ct Mean	Ct SD	Quantity
RNase P - STANDARD - 10000.0											
4	B2	<input type="checkbox"/>			RNase P	STANDA...	FAM-NF...	26.874498	26.85865	0.022	10,000
5	B3	<input type="checkbox"/>			RNase P	STANDA...	FAM-NF...	26.834158	26.85865	0.022	10,000
6	B4	<input type="checkbox"/>			RNase P	STANDA...	FAM-NF...	26.867296	26.85865	0.022	10,000
RNase P - STANDARD - 1250.0											
7	C3	<input type="checkbox"/>			RNase P	STANDA...	FAM-NF...	29.93595	29.985449	0.059	1,250
8	C4	<input type="checkbox"/>			RNase P	STANDA...	FAM-NF...	29.9701	29.985449	0.059	1,250
9	C5	<input type="checkbox"/>			RNase P	STANDA...	FAM-NF...	30.050293	29.985449	0.059	1,250
RNase P - STANDARD - 2500.0											
10	B8	<input type="checkbox"/>			RNase P	STANDA...	FAM-NF...	28.973732	28.981377	0.021	2,500
11	C1	<input type="checkbox"/>			RNase P	STANDA...	FAM-NF...	29.005375	28.981377	0.021	2,500
12	C2	<input type="checkbox"/>			RNase P	STANDA...	FAM-NF...	28.965023	28.981377	0.021	2,500
RNase P - STANDARD - 5000.0											
13	B5	<input type="checkbox"/>			RNase P	STANDA...	FAM-NF...	27.843782	27.894386	0.045	5,000
14	B6	<input type="checkbox"/>			RNase P	STANDA...	FAM-NF...	27.907658	27.894386	0.045	5,000
15	B7	<input type="checkbox"/>			RNase P	STANDA...	FAM-NF...	27.931719	27.894386	0.045	5,000
RNase P - STANDARD - 625.0											
16	C6	<input type="checkbox"/>			RNase P	STANDA...	FAM-NF...	31.05255	31.04659	0.01	625
17	C7	<input type="checkbox"/>			RNase P	STANDA...	FAM-NF...	31.052055	31.04659	0.01	625
18	C8	<input type="checkbox"/>			RNase P	STANDA...	FAM-NF...	31.035166	31.04659	0.01	625
non1 - RNase P - UNKNOWN											

Analysis : Gene Expression

Analysis

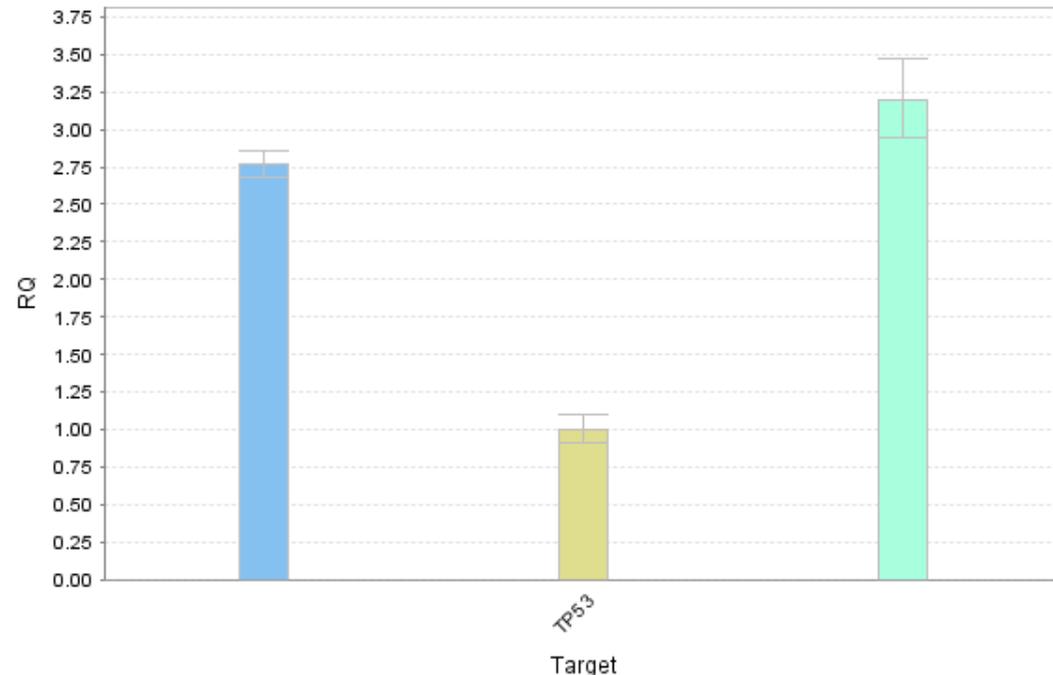
- Amplification Plot
- Gene Expression**
- Multicomponent P...
- Raw Data Plot
- QC Summary
- Multiple Plots View

Gene Expression

Plot Type: RQ vs Target | Graph Type: Linear | Orientation: Vertical Bars



RQ vs Target



Replicate Results Table

Show In Table ▼

#	Omit	Sample	Target	Ct Mean	Δ Ct Mean	Δ Ct SD	Δ Ct SE	$\Delta\Delta$ Ct	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

6-2. Setup: Plate Setup

決定基因和樣品位置 (for Standard curve)

Assign Targets and Samples

Define Targets and Samples Assign Targets and Samples

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

- I 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	<input type="checkbox"/> U <input type="checkbox"/> S <input type="checkbox"/> N	

* 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.

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

● Automatic Standard Curve Setup

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

Define the standard curve * = Required

of Points: 5 Recommended

of Replicates: 3 Recommended

Starting Quantity: Enter the highest or lowest standard quantity for the standard curve.

Serial Factor: Select a value from 1:10 to 10×

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

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

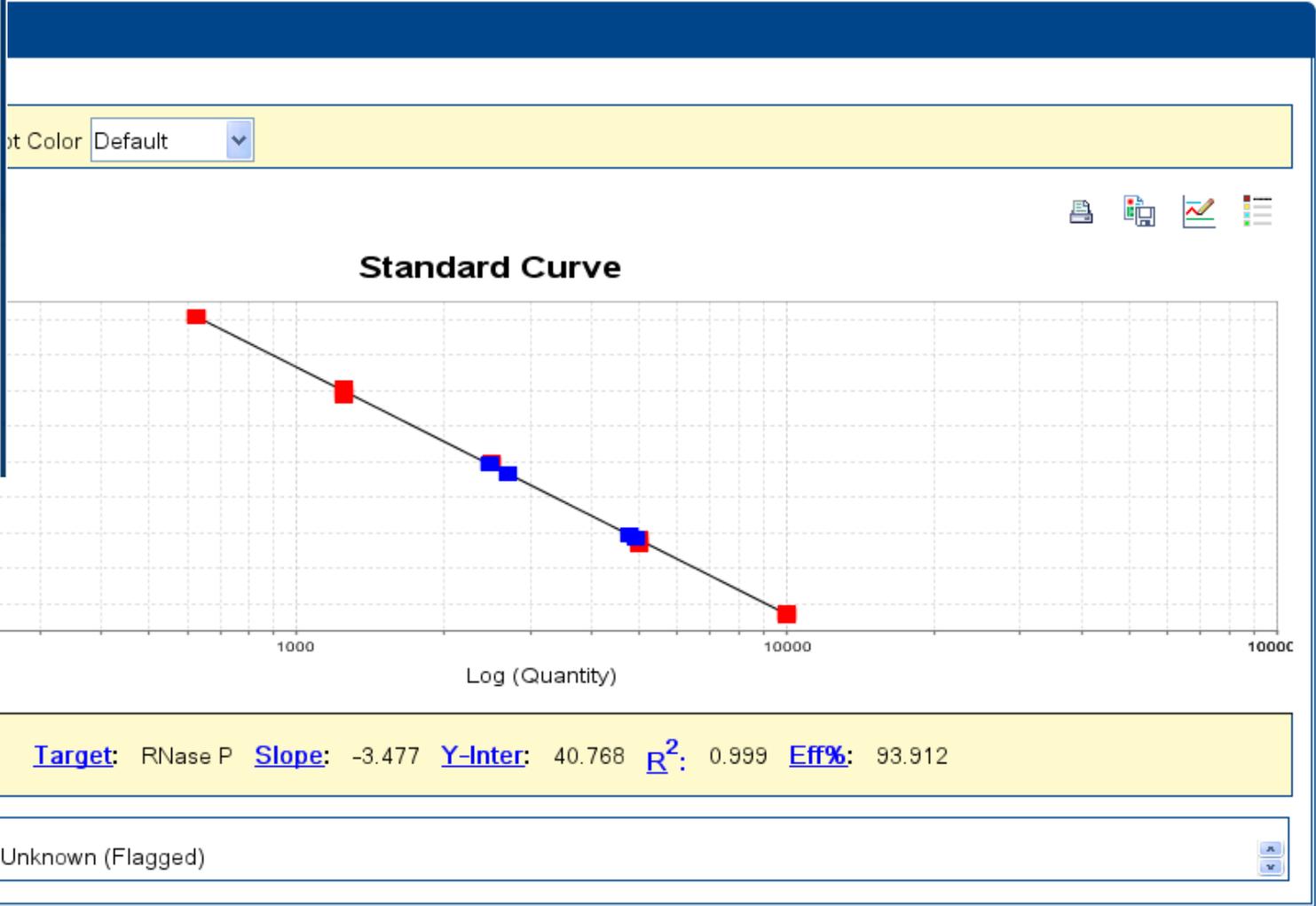
15 Required Wells / 15 Selected Wells

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

Arrange standards in: Columns Rows

Analysis : Standard Curve

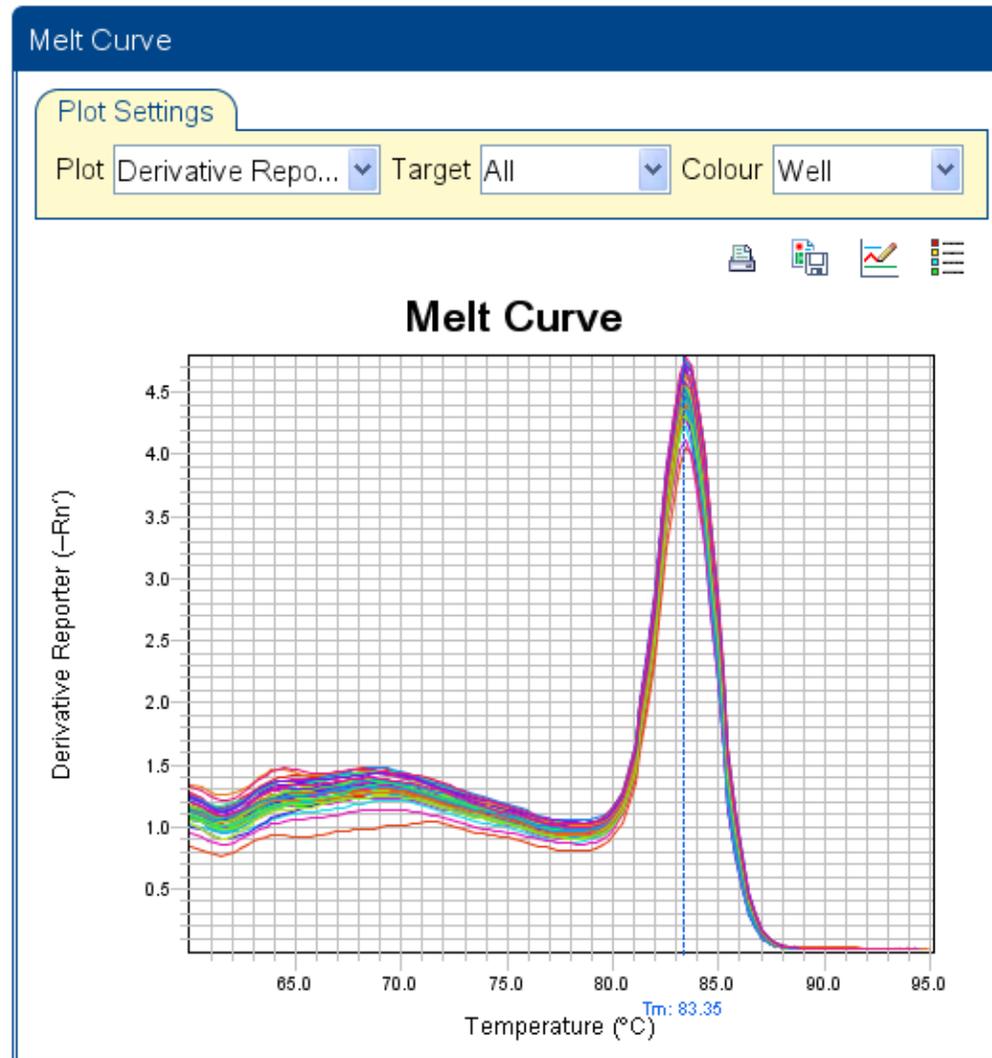
- Analysis**
- Amplification Plot
- Standard Curve**
- Multicomponent P...
- Raw Data Plot
- QC Summary
- Multiple Plots View



Analysis Summary: Total Wells in Pla... 48 Wells Set ... 24 Wells Omitted Manual... 0 Wells Flagg... 0 Wells Omitted by Analys... 0 Samples Us... 2 Targets Us... 1

Analysis : Melt Curve (SYBR Green)

- Setup
- Run
- Analysis**
 - Amplification Plot
 - Standard Curve
 - Melt Curve**
 - Multicomponent ...
 - Raw Data Plot
 - QC Summary
 - Multiple Plots View



QC Summary Help Your Troubleshooting

-  **Analysis**
-  Amplification Plot
-  Standard Curve
-  Multicomponent ...
-  Raw Data Plot
-  **QC Summary**
-  Multiple Plots View

QC Summary

QC 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	
RF FAIL	Baseline algorithm failed	0	

Flag: AMPNC—Amplification in negative control

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

Flag Criteria: Ct < 35.0

Flagged Wells: F1, F3

[View AMPNC Troubleshooting Information](#)



View Plate Layout View W

Select Wells Wit

Show in Wells ▾ 

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

數據和圖形簡易輸出! 超easy~

Export to Excel, PowerPoint or save as jpeg

Export... Print Report...

Export... Send To PowerPoint...

1. Select data to export: Raw Data Multicomponent Data Amplification Data

2. Select one file or separate files: One File *Select to export all data in one file or in separate files for each data type.*

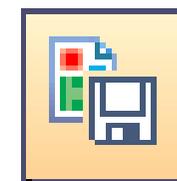
3. Enter export file properties:

Export File Name: Standard Curve Example_data File Type: (*.xls)

Export File Location: C:\Applied Biosystems\7500\experiments

Open file(s) when export is complete

Save current settings as the default



Multiple Plots View

Amplification Plot - ΔRn vs Cycle

Multicomponent Plot

Standard Curve

Gene Expression Plot

Comparative Ct Study: 1. Create Study

不限樣品盤數，但上機條件要相同，且每盤需含 **endogenous control**

The screenshot shows the StepOne Software v2.1 interface. The window title is "StepOne™ Software v2.1". The menu bar includes "File", "Edit", "Instrument", "Analysis", "Tools", and "Help". The toolbar contains "New Experiment", "Open...", "Save", "Close", "Send Experiment to Instrument...", "Download Experiment from Instrument...", "Export...", and "Print Report...".

The main interface is divided into three columns: "Set Up", "Run", and "Analyze".

- Set Up:** Contains three buttons: "Design Wizard" (with a target icon), "Advanced Setup" (with a grid icon), and "Template" (with a folder icon). A blue arrow points up from the "Template" button.
- Run:** Contains one button: "QuickStart" (with a "EQ" icon). A blue arrow points down from the "QuickStart" button.
- Analyze:** Contains two buttons: "Analyze Experiment" (with a bar chart icon) and "Create Study" (with a folder icon). The "Create Study" button is highlighted with a red border. A blue arrow points up from the "Create Study" button.

At the bottom left, there is a checkbox labeled "Save current display as the default" which is checked. At the bottom center, the text "StepOne™ & StepOnePlus™" is displayed. At the bottom right, the text "Applied Biosystems Home" and "Real-Time PCR Decision Tree" is displayed.

Comparative Ct Study: 2. Add Experiment

Study Menu << Study: RQ study Type: unknown # of Experiments: 1 Last Modified: ? Analysis Settings Analyze

Setup
Study Properties
Define Replicates
Analysis

Study Properties

* Study Name: RQ study Analysis: unknown
Comments (Optional):
User Name (Optional): GUEST
Last Modified: Number of Experiments: 1

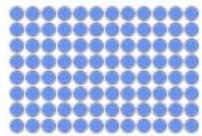
Set Up Experiments (1)
Add Experiment(s) Selected Experiment(s) Hide Filter

Enter a filter query, then click "Apply Filter."
IF Experiment Name = Apply Filter Remove Filter

Experiment Name	Number of Targets	Number of Samp...	Operator	Run Date
Comparative Ct Example.ed	6	4	Applied Biosystems sa...	10/25/2004 12:23:56 GM...

Properties: Comparative Ct Example.ed

Overview Samples Targets



- Negative Control Well
- Standard Well
- Unknown Well
- Empty Well

Comparative Ct Study:

3. Add or Edit Biological Group

若有biological replicates, 請點選 [Add Biological Group]
沒有的話請跳到 4. Analyze & check threshold

The screenshot shows the 'Study Menu' on the left with 'Define Replicates' highlighted in red. The main area displays 'Study: RQ study', 'Type: unknown', and '# of Experiments: 1'. An instruction box says 'Add biological replicate groups to the study as needed.' Below this, a 'Set Up Biological Replicate Groups' section contains three buttons: 'Add Biological Group' (highlighted in red), 'Edit Biological Group', and 'Delete Biological Group'. A table with columns 'Biological Group Name', 'Color', '# of Replicates', and 'Comments' is present but empty. To the right, a 'Properties' panel includes fields for 'Biological Group Name:', 'Color: [input]', 'Comments:', and '# of Replicates:'. Below these is a table with 'Sample' and 'Target' columns. At the bottom, there is a 'Used on Plate(s):' field.

Study Menu <<

Study: RQ study Type: unknown # of Experiments: 1 Last Modified: ? Analysis Settings Analyze

Instructions: Add biological replicate groups to the study as needed.

Set Up Biological Replicate Groups

Add Biological Group Edit Biological Group Delete Biological Group

Biological Group Name	Color	# of Replicates	Comments
-----------------------	-------	-----------------	----------

Properties

Biological Group Name:

Color:

Comments:

of Replicates:

Sample	Target
--------	--------

Used on Plate(s):

Comparative Ct Study: 3. Add or Edit Biological Group

1. 輸入 Biological Group Name

Biological Group Name: 8, 255, 222

2. 圈選這個 Biological Group 的 wells

3. Click >>

4. 從下拉式選單選擇另外一盤，並重複 steps 2 and 3, 直到把這個 Biological Group 的所有樣品都選進來

5. 點此 [Save & Add Another Group], 重覆 step 1~4, 設定另一個 Biological Group

Wells: 48 Unknown 0 Standard 0 Negative Control 48 Empty

Plate	Target
Brain2	OGDH
Brain1	OSGEP
Brain2	18S
Brain1	18S
Brain2	EGR3
Brain1	SERPING1
Brain2	MAOB
Brain1	OSGEP
Brain2	EGR3
Brain1	OGDH
Brain2	MAOB
Brain1	SERPING1

Comparative Ct Study:

4. Analyze & check threshold

確認Threshold是否設在Exponential phase

Amplification Plot Settings

Plot Type: ΔRn vs Cycle | Graph Type: Log | Plot Color: Target

Save current settings as the default

Options

Target: SERPING1 | Threshold: Auto (0.370235) | Auto Baseline

Show: Threshold | Baseline Start: Well | Target | Baseline End: Well | Target

48 Wells Selected | Hide unselected data from plot

Well Results Data

#	Sample	Target	Experim...	Well	Omit
1	Brain2	18S	Comparative	C1	<input type="checkbox"/>
2	Brain2	18S	Comparative	D1	<input type="checkbox"/>
3	Brain2	EGR3	Comparative	C3	<input type="checkbox"/>
4	Brain2	EGR3	Comparative	D3	<input type="checkbox"/>
5	Brain2	MAOB	Comparative	C5	<input type="checkbox"/>
6	Brain2	MAOB	Comparative	D5	<input type="checkbox"/>
7	Brain2	OGDH	Comparative	C2	<input type="checkbox"/>
8	Brain2	OGDH	Comparative	D2	<input type="checkbox"/>
9	Brain2	OSGEP	Comparative	C4	<input type="checkbox"/>
10	Brain2	OSGEP	Comparative	D4	<input type="checkbox"/>
11	Brain2	SERPING1	Comparative	C6	<input type="checkbox"/>
12	Brain2	SERPING1	Comparative	D6	<input type="checkbox"/>
13	Kidney2	18S	Comparative	C7	<input type="checkbox"/>
14	Kidney2	18S	Comparative	D7	<input type="checkbox"/>

5. View Gene Expression Plot by Technical Replicates

檢視 technical replicate group 的 2^{-ddCt} 結果

Gene Expression

Plot Settings
 Plot Type: RQ vs Sample | Graph Type: Linear | Orientation: Vertical Bars
 Save current settings as the default

RQ vs Sample

Y-axis: RQ (0.0 to 20.0)
 X-axis: Sample (Brain1, Brain2, Kidney1, Kidney2, Liver1, Liver2, Unknown1, Unknown2)

Legend:
 18S (Red), EGR3 (Orange), MAOB (Green), OGDH (Teal), OSGEP (Blue), SERPING1 (Purple)

0 Replicates Selected | Hide unselected data from plot

Replicate Results Data

Technical Replicates | Biological Replicates

Show In Table | Add BioGroup | Endo Controls | Ref Sample

#	Omit	Sample	Target	ΔCt Mean
1		Brain1	18S	
2		Brain1	EGR3	16.9
3		Brain1	MAOB	14.9
4		Brain1	OGDH	15.8
5		Brain1	OSGEP	18.4
6		Brain1	SERPING1	16.0
7		Brain2	18S	
8		Brain2	EGR3	27.9538
9		Brain2	MAOB	17.4
10		Brain2	OGDH	15.8
11		Brain2	OSGEP	18.4
12		Brain2	SERPING1	16.0
13		Kidney1	18S	
14		Kidney1	EGR3	16.9
15		Kidney1	MAOB	14.9

Well Results Data

Show In Table | Group Results

#	Sample	Target	Experiment	Well	Omit	CT

Easily
choose
Endo
Controls

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