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

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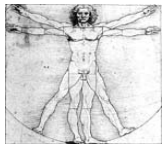
Field Application Scientist

同步定量PCR之應用



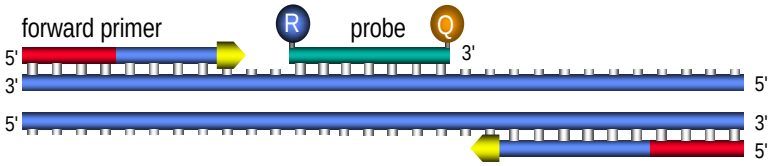
基因定量

- 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

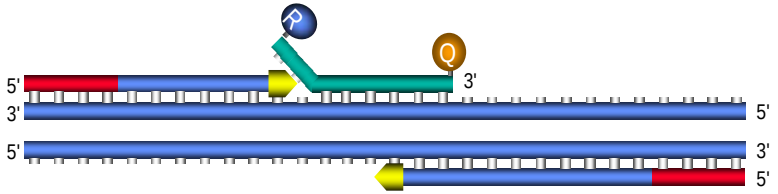
- 基因型研究
  - **-- SNP**與疾病關聯性
  - **-- CNV**與疾病關聯性

TaqMan® probe chemistry: Fluorogenic 5′ Nuclease assay

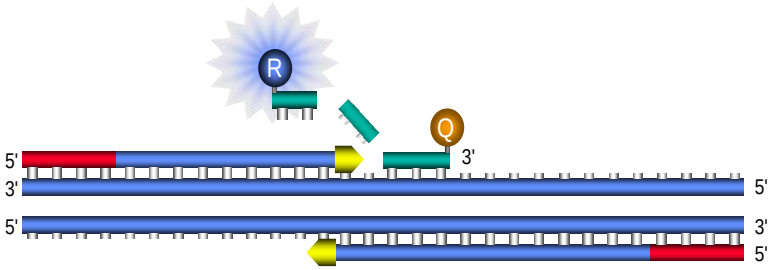


1. Polymerisation

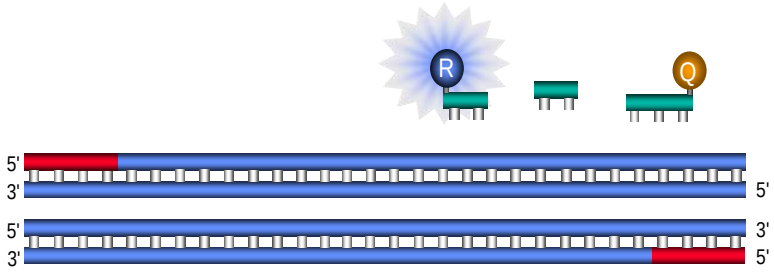
reverse primer



2. Strand displacement



3. Cleavage

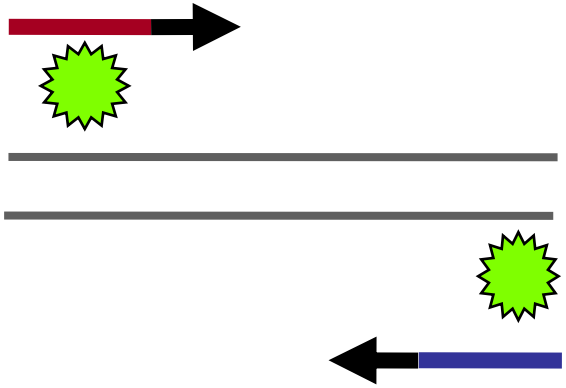


4. Polymerisation completed

R = Reporter
Q = Quencher

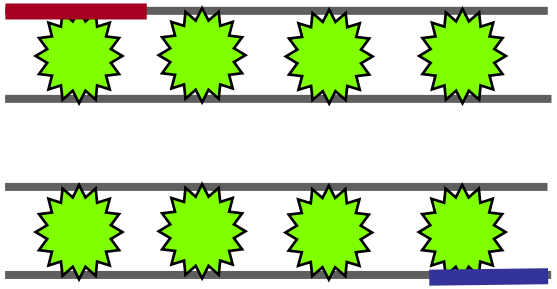
SYBR Green 1®

- dsDNA minor-groove binding dye



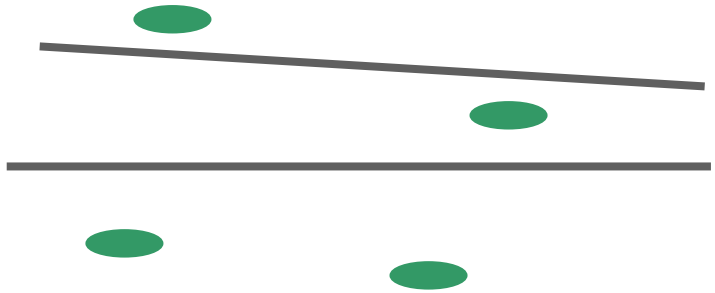
Polymerization

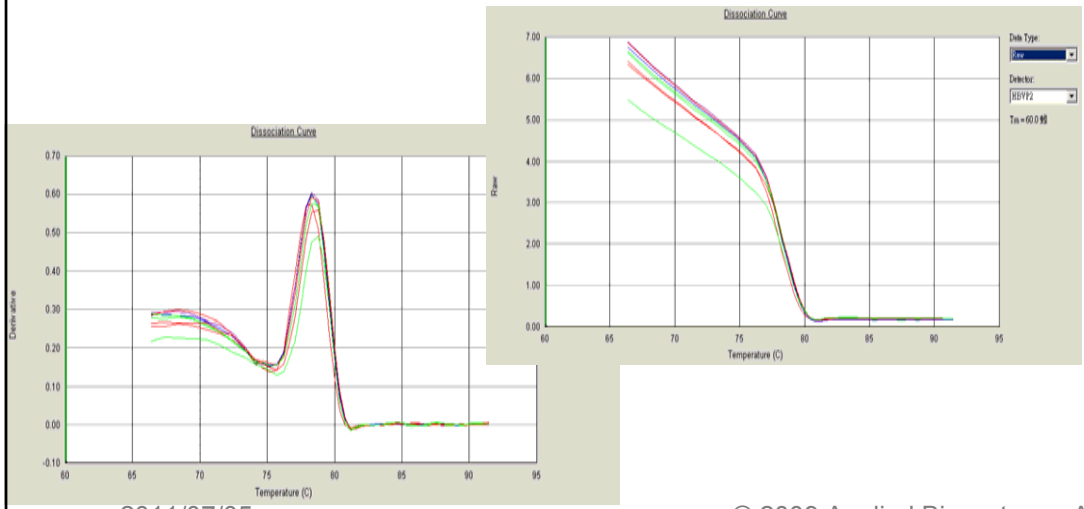
PCR

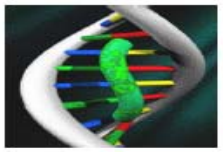


Polymerization Complete

Denaturation



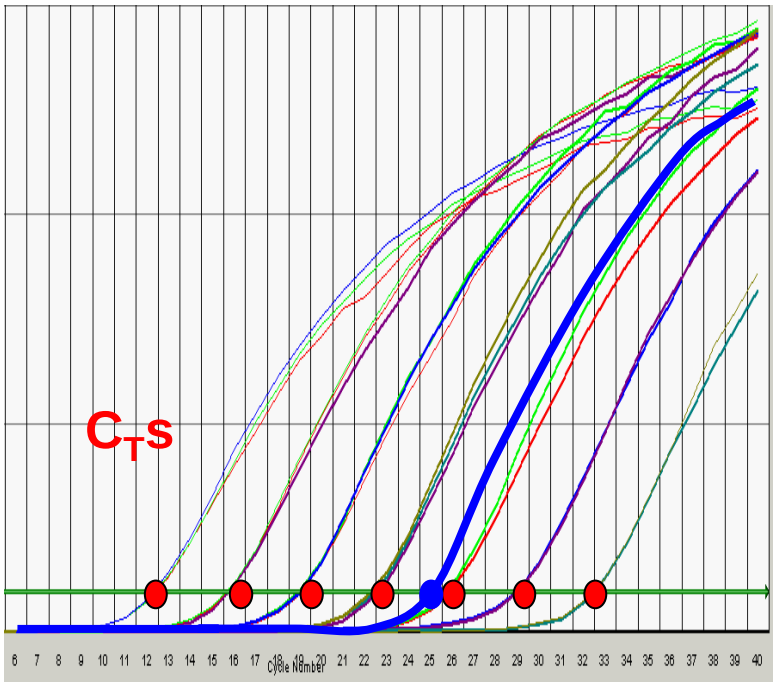


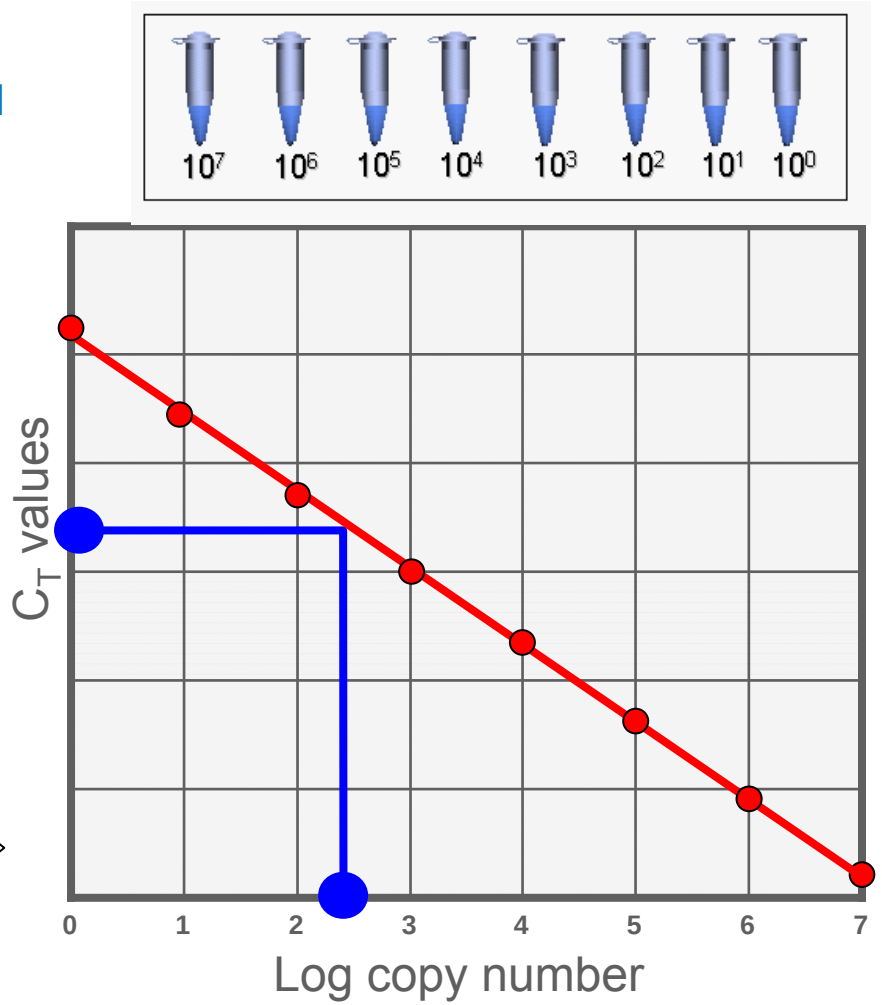
	TaqMan® Probe	SYBR® Green 1 Dye
		
Specificity	• Highly specific • Probe Hybridization	• Non-specific
Sensitivity	• Very High	• Very High
Flexibility	• Mutiplex PCR • SNP detection • +/- application	• No Probe is required • Screening tool
Optimization	• Ready to use 20x primer/probe mix - no need to optimize • Gold standard for MAQC • PCR efficiency 100% ±10%	• 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

相對定量(Relative Quantitation)

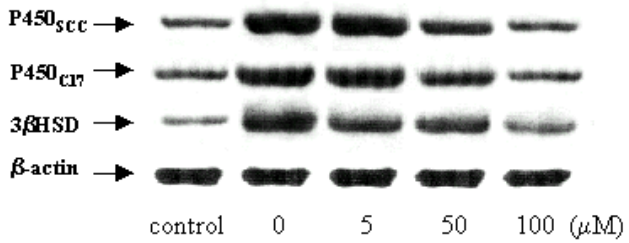
To determine fold differences of a target nucleic acid in a starting material with statistical confidence.

-- 1. △△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 !!



Comparative Ct Method

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

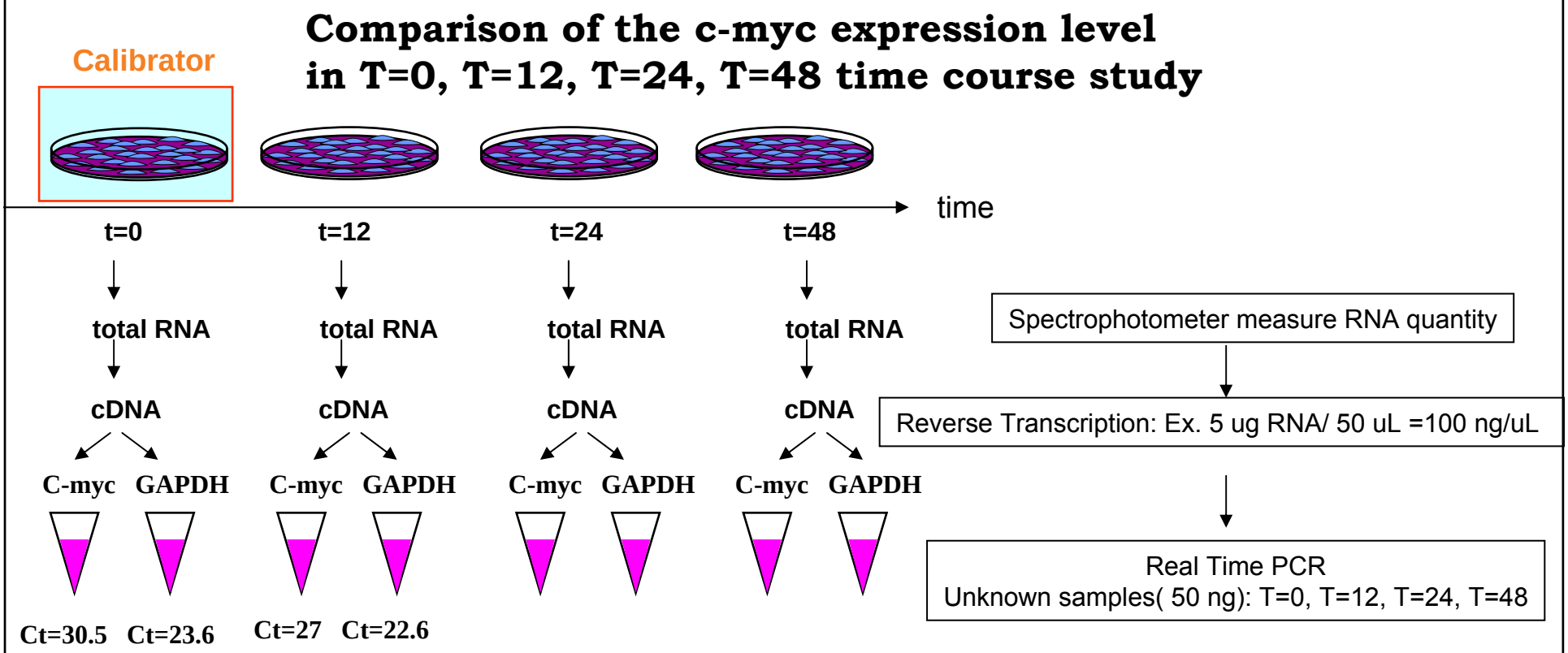
ΔCt Sample – ΔCt Reference = **ΔΔCt**

step 3: use the formula

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

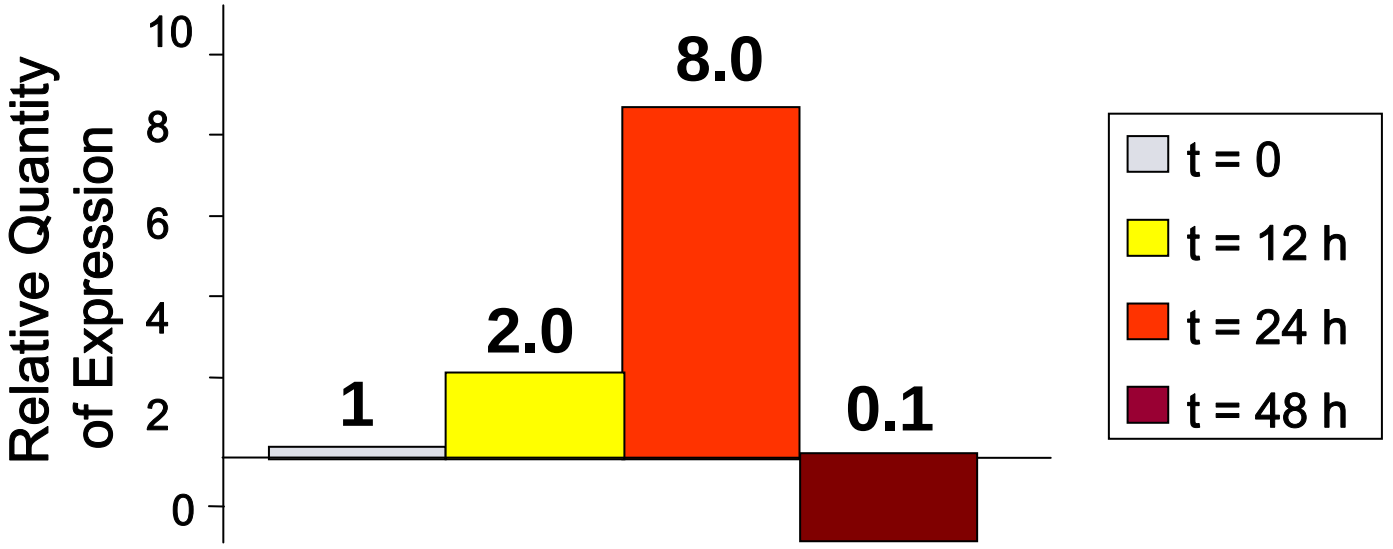
A reference sample is a sample to which unknown samples are compared (ex. untreated sample or control).

Comparative Ct Method

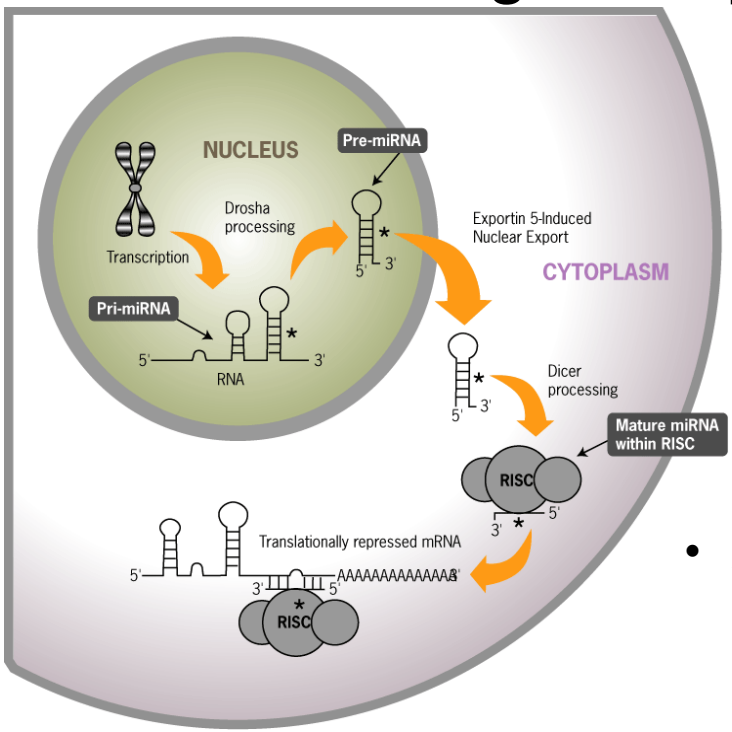


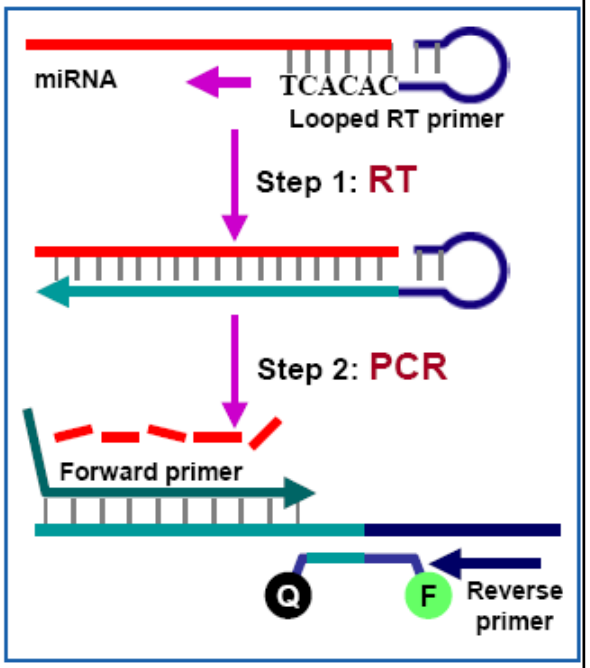
$\Delta\Delta C_t$ Calculations (Comparative C_t)

	c-Myc	GAPDH	ΔC_t	$\Delta\Delta C_t$	$2^{-\Delta\Delta Ct}$
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



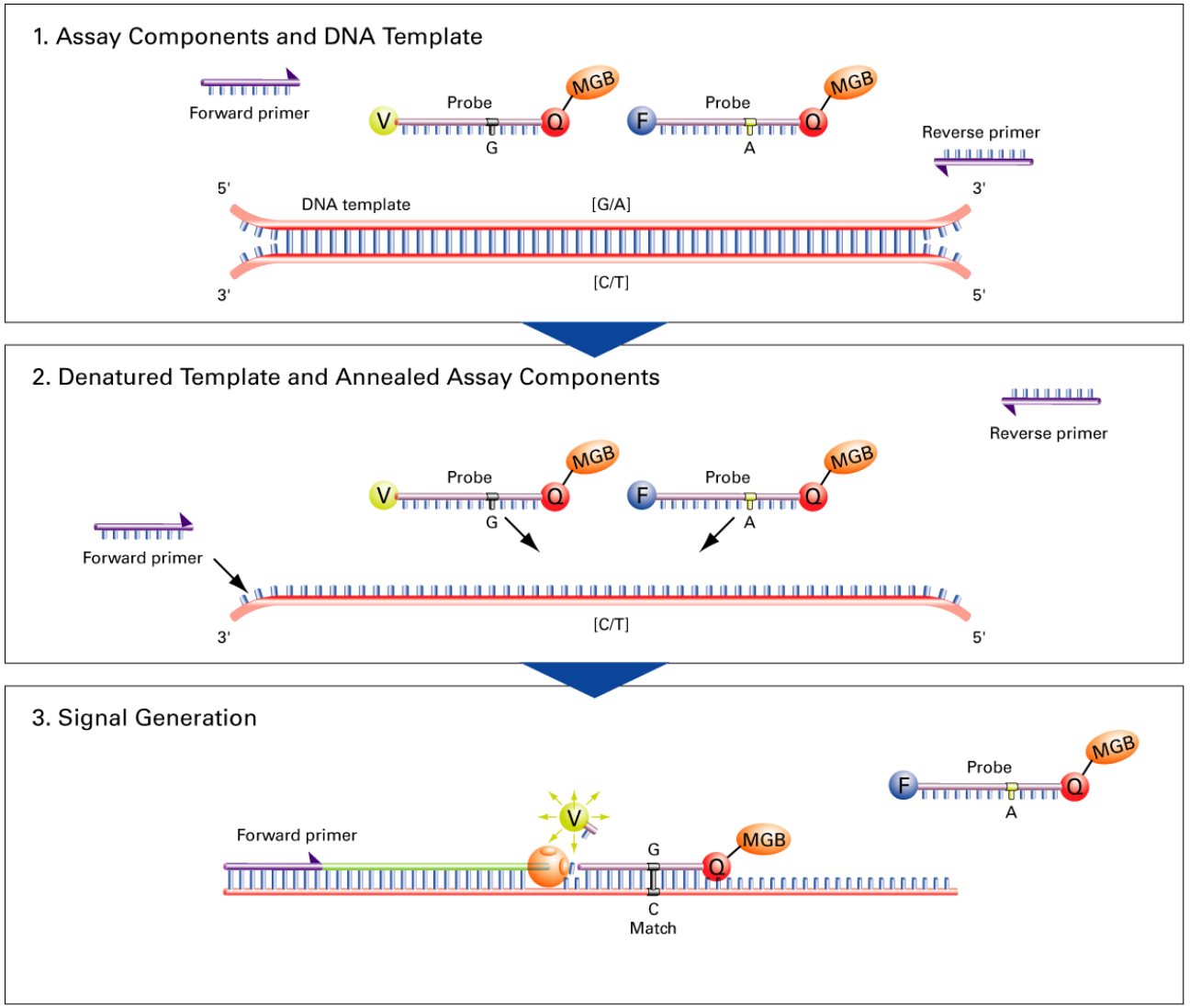


Mature miRNA

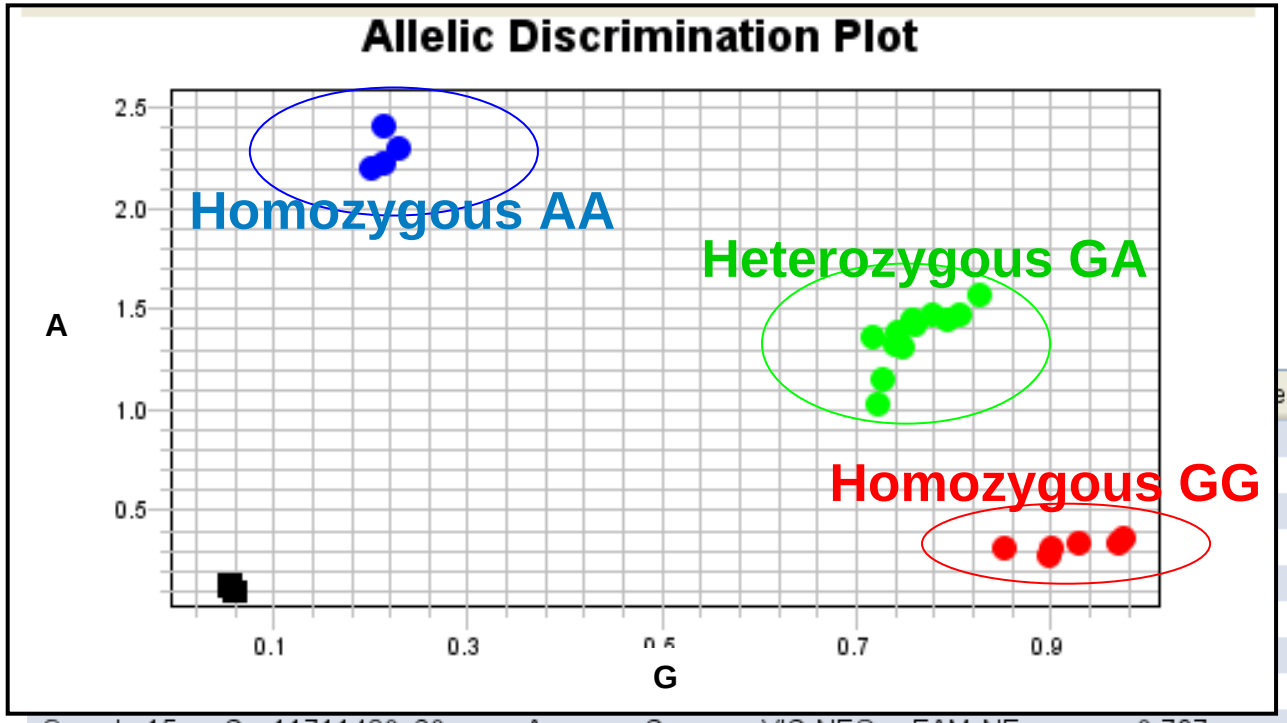
UGAUUGAGCCGUGUCAAUAU

- 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

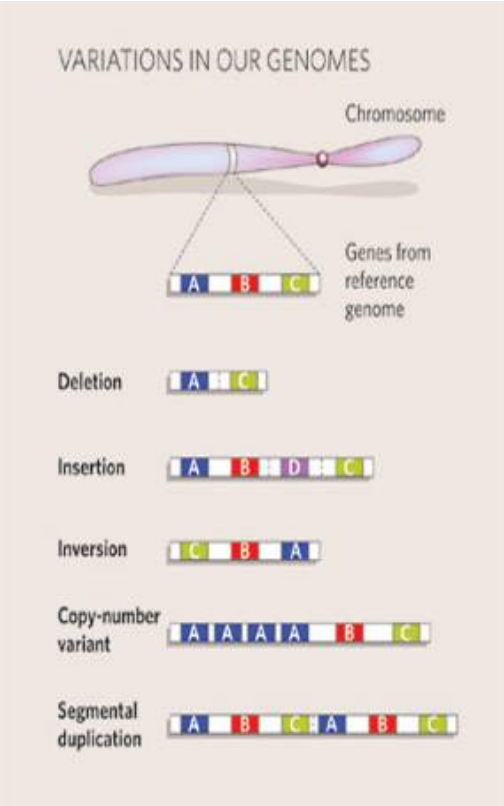


Allelic Discrimination (SNP) data



							e 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	Heterozygous 1/2
							1.206	3,942.024	Heterozygous 1/2
							1.624	3,956.087	Heterozygous 1/2
Sample 15	C__11711420_30	...	A	C	VIC-NFQ...	FAM-NF...	0.767	1.526	3,849.214	Heterozygous 1/2
Sample 16	C__11711420_30	...	A	C	VIC-NFQ...	FAM-NF...	0.748	1.478	3,793.905	Heterozygous 1/2
Sample 17	C__11711420_30	...	A	C	VIC-NFQ...	FAM-NF...	0.709	1.086	3,820.435	Heterozygous 1/2
Sample 18	C__11711420_30	...	A	C	VIC-NFQ...	FAM-NF...	0.736	1.371	3,945.303	Heterozygous 1/2
Sample 19	C__11711420_30	...	A	C	VIC-NFQ...	FAM-NF...	0.795	1.528	4,026.388	Heterozygous 1/2
Sample 2	C__11711420_30	...	A	C	VIC-NFQ...	FAM-NF...	0.84	0.364	3,737.053	Homozygous 1/1
Sample 20	C__11711420_30	...	A	C	VIC-NFQ...	FAM-NF...	0.888	0.37	4,099.657	Homozygous 1/1
Sample 3	C__11711420_30	...	A	C	VIC-NFQ...	FAM-NF...	0.189	2.251	3,643.652	Homozygous 2/2
Sample 4	C__11711420_30	...	A	C	VIC-NFQ...	FAM-NF...	0.963	0.421	3,826.976	Homozygous 1/1
Sample 5	C__11711420_30	...	A	C	VIC-NFQ...	FAM-NF...	0.705	1.418	3,982.397	Heterozygous 1/2
Sample 6	C__11711420_30	...	A	C	VIC-NFQ...	FAM-NF...	0.729	1.386	4,048.287	Heterozygous 1/2
Sample 7	C__11711420_30	...	A	C	VIC-NFQ...	FAM-NF...	0.916	0.393	4,015.545	Homozygous 1/1
Sample 8	C__11711420_30	...	A	C	VIC-NFQ...	FAM-NF...	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

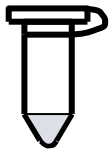
Workflow of TaqMan® Copy Number Assays

FAM™-labeled
TEST ASSAY

1

VIC®-labeled
CONTROL ASSAY
(i.e.: RNase P)

2



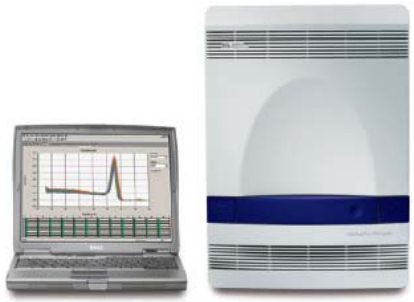
gDNA
1 ng / µL PCR rxn

3

TaqMan
Master Mix

4

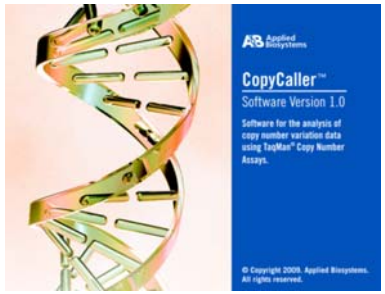
Standard TaqMan protocol



qPCR

4 replicates
per gDNA sample

CopyCaller



CopyCaller™ Software-輕鬆獲得CNV結果

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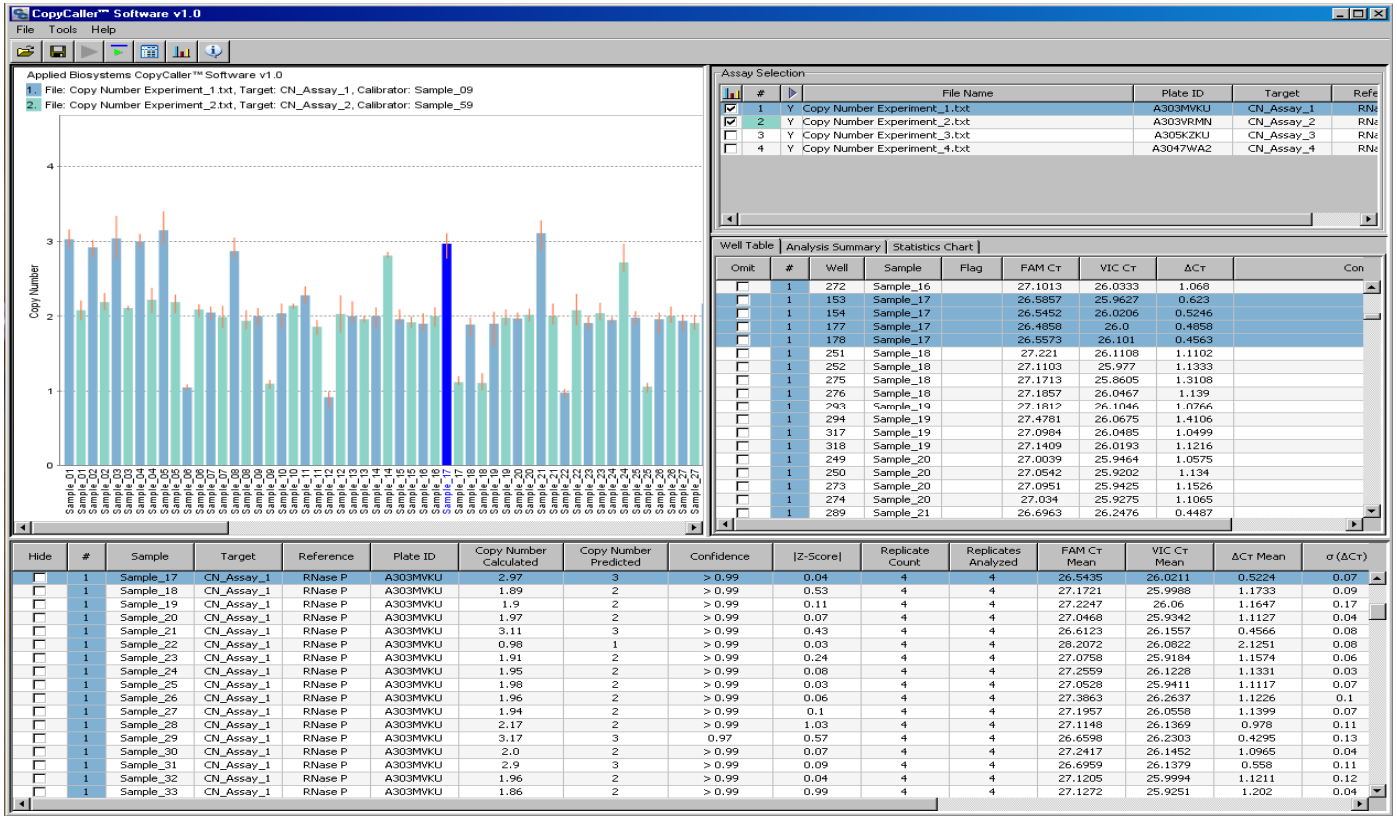
★ > 1.6M Pre-Designed TaqMan Copy Number Assays available



- **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

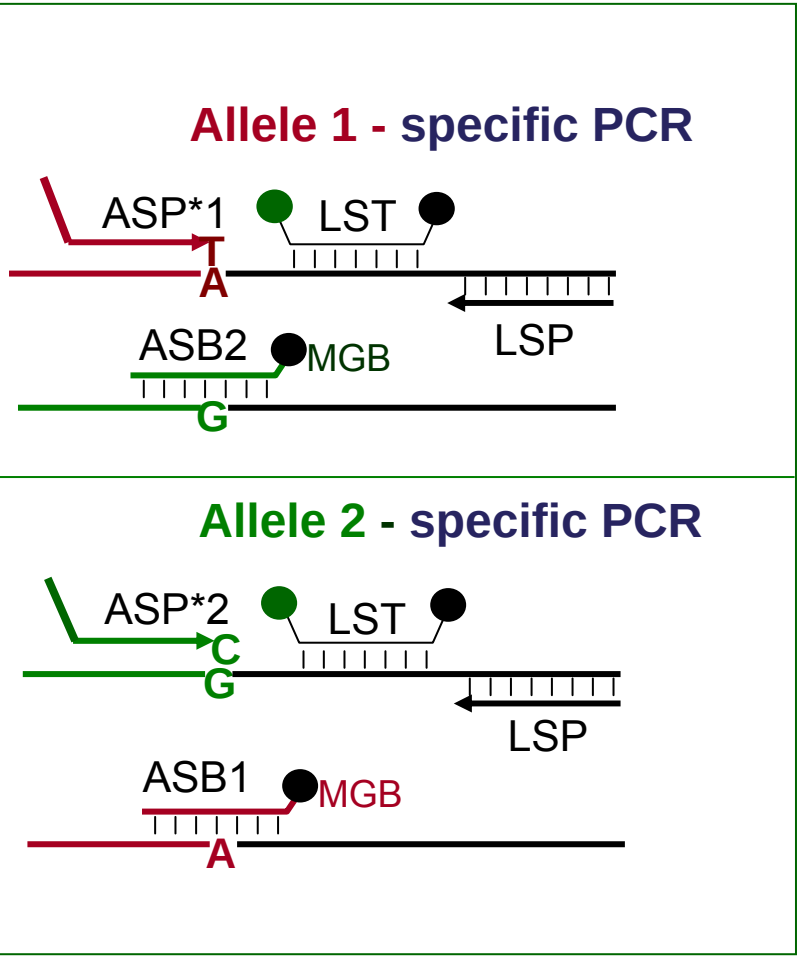
A pair of assays:

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

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Conventional AS-PCR

gDNA template	Primer base at 3′ C	T	A
G	27.2	28.1	37.3
A	28.5	28.1	37.1
T	28.1	30.5	27.3

Ave. ΔCt ($Ct_{mm} - Ct_{pm}$) = 4.1

castPCR

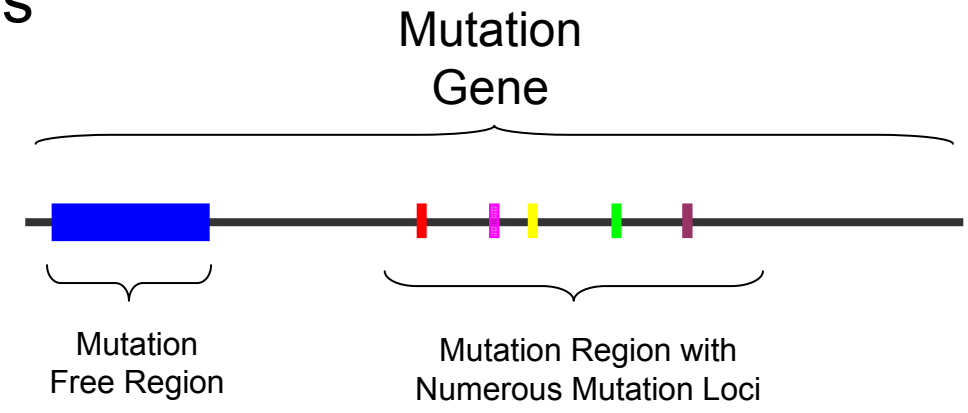
gDNA template	Primer base at 3′ C	T	A
G	28.5	48.2	50.0
A	48.5	28.5	50
T	50	50	28.1

Ave. ΔCt ($Ct_{mm} - Ct_{pm}$) = 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

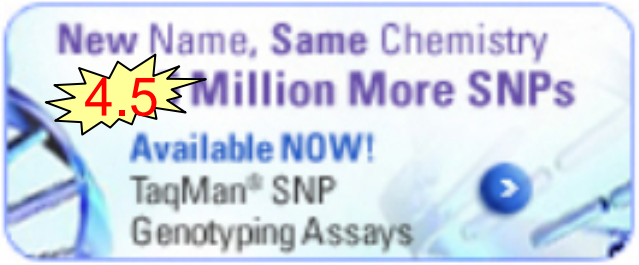
如何設計Real Time PCR的Primers/ Probe

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

Custom TaqMan Assays Service

- *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 Expresson assay??

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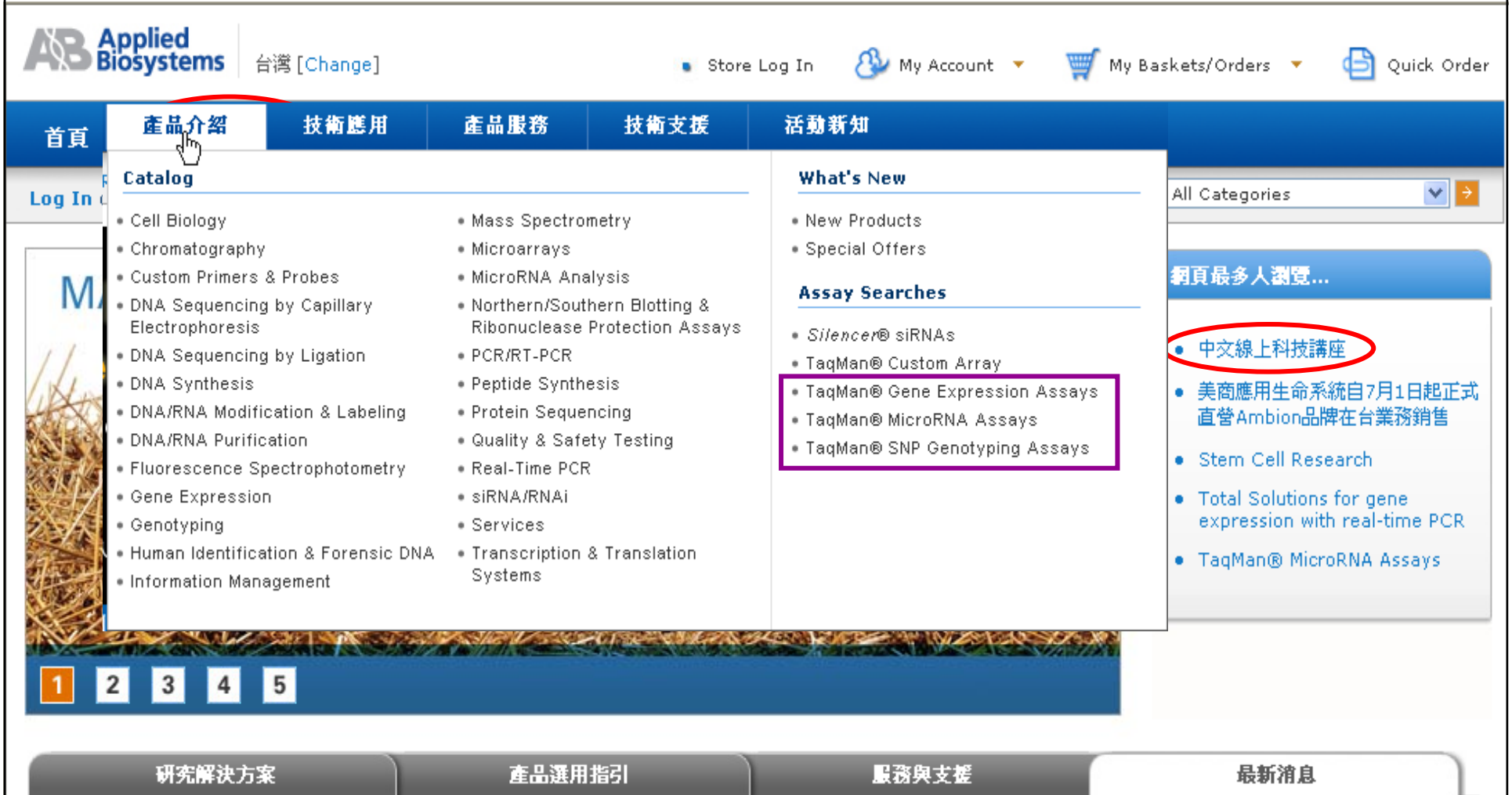
www.appliedbiosystems.com.tw



TaqMan® Array Gene Signature Plates

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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 a Regulation
- Development and Stem Cells
- ECM Matrix and Adhesior
- 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.

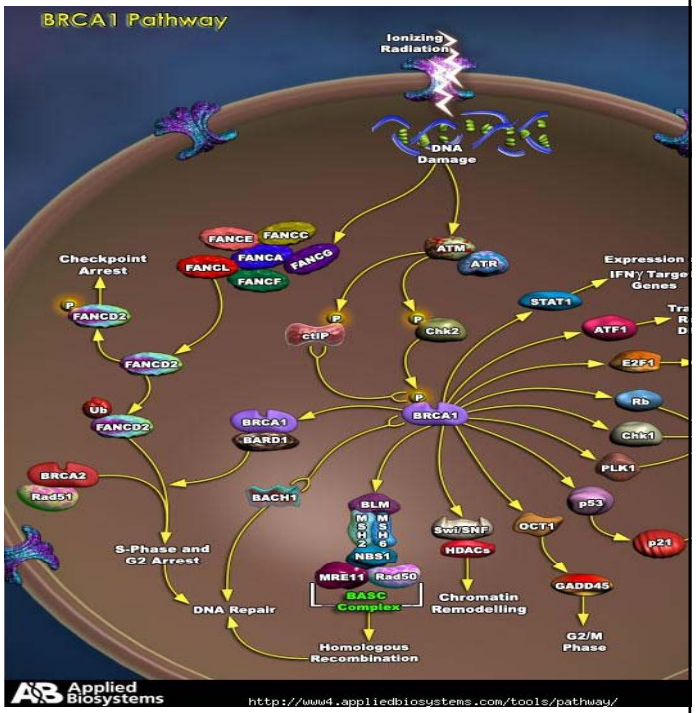
The panel of assays in this plate was selected in cooperation with the International Stem Cell Initiative (2007 Nature Biotechnology. 25:803-816). The assays target genes which are validated as markers for the characterization of human embryonic stem cell identity, and which assess variations between 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	Hs0015637
B	Hs00164004_m1	Hs00156568_m1	Hs00201350_m1	Hs00275636_m1	Hs00170025_m1	Hs00251859_m1	Hs00157258_m1	Hs0017187
C	Hs00170454_m1	Hs00176573_m1	Hs00277509_m1	Hs00232764_m1	Hs00255287_s1	Hs00241459_m1	Hs00544355_m1	Hs0017140
D	Hs00220998_m1	Hs00157674_m1	Hs00917999_g1	Hs00747223_g1	Hs00744391_s1	Hs00232128_m1	Hs00169095_m1	Hs0070513
E	Hs00236830_m1	Hs00158126_m1	Hs00174029_m1	Hs00196158_m1	Hs00300550_m1	Hs00158620_m1	Hs00267056_m1	Hs0076412
F	Hs02387400_g1	Hs00707120_s1	Hs00159598_m1	Hs00415443_m1	Hs00271352_s1	Hs00383230_g1	Hs00187067_m1	Hs0026596
G	Hs00193638_m1	Hs00742896_s1	Hs00829813_s1	Hs00603586_g1	Hs00234119_m1	Hs00194498_m1	Hs00231692_m1	Hs0017381
H	Hs00538143_m1	Hs00300531_m1	Hs00610080_m1	Hs00356930_m1	Hs02339499_g1	Hs00162669_m1	Hs00232708_m1	Hs0016594

Gene Symbol	1	2	3	4	5	6	7	8
A	18S	GAPDH	HPRT1	GUSB	ACTC1	AFP	BXDC2	CD34
B	COL1A1	COL2A1	COMMD3,BMI1	CRABP2	CTNNB1	DDX4	DES	DNMT3B
C	FGF5	FLT1	FN1	FOXA2	FOXD3	GABRB3	GAL	GATA4
D	GDF3	GFAP	GRB7	HBB	HBZ	MNX1	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 form 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

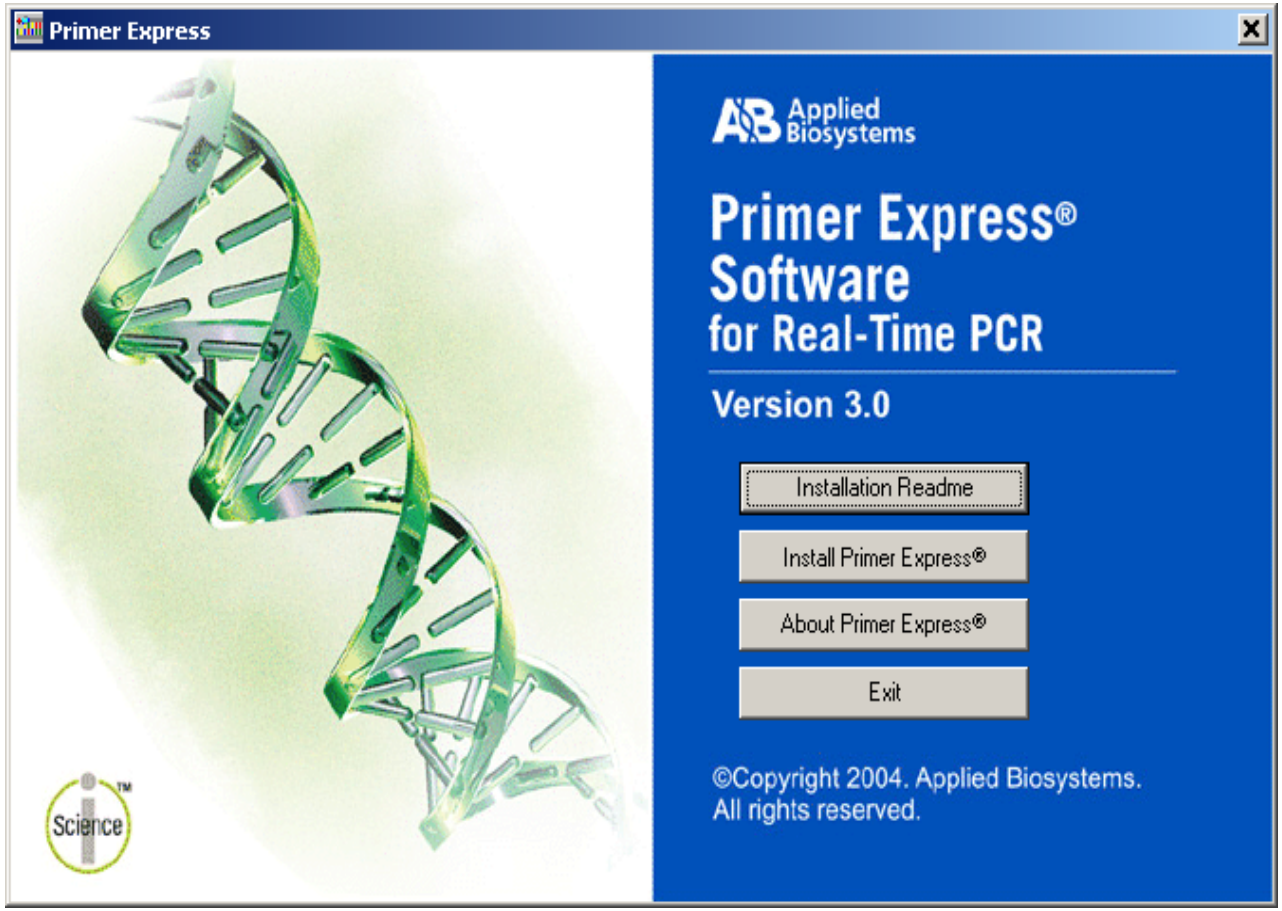
Close Window

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	580	BARD1	BRCA1 associated RING domain 1	s1887, s1886, s1885 /	Hs00194427_m1

定量PCR Primers/ Probe設計軟體

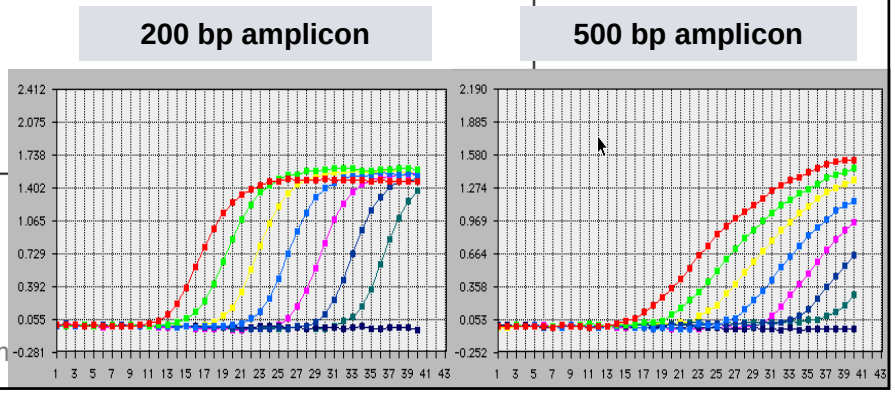


清楚明確的 TaqMan Probe & Primer 設計規範

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℃ (Quantification assay) 65-67℃ (Allelic Discrimination assay)	Tm 值: 58-60℃
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

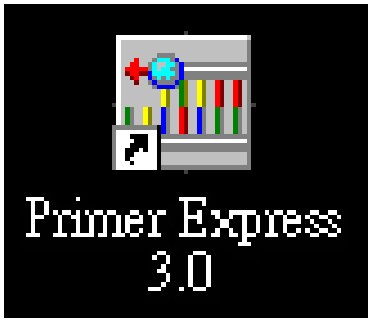
b: 參數可選擇設定

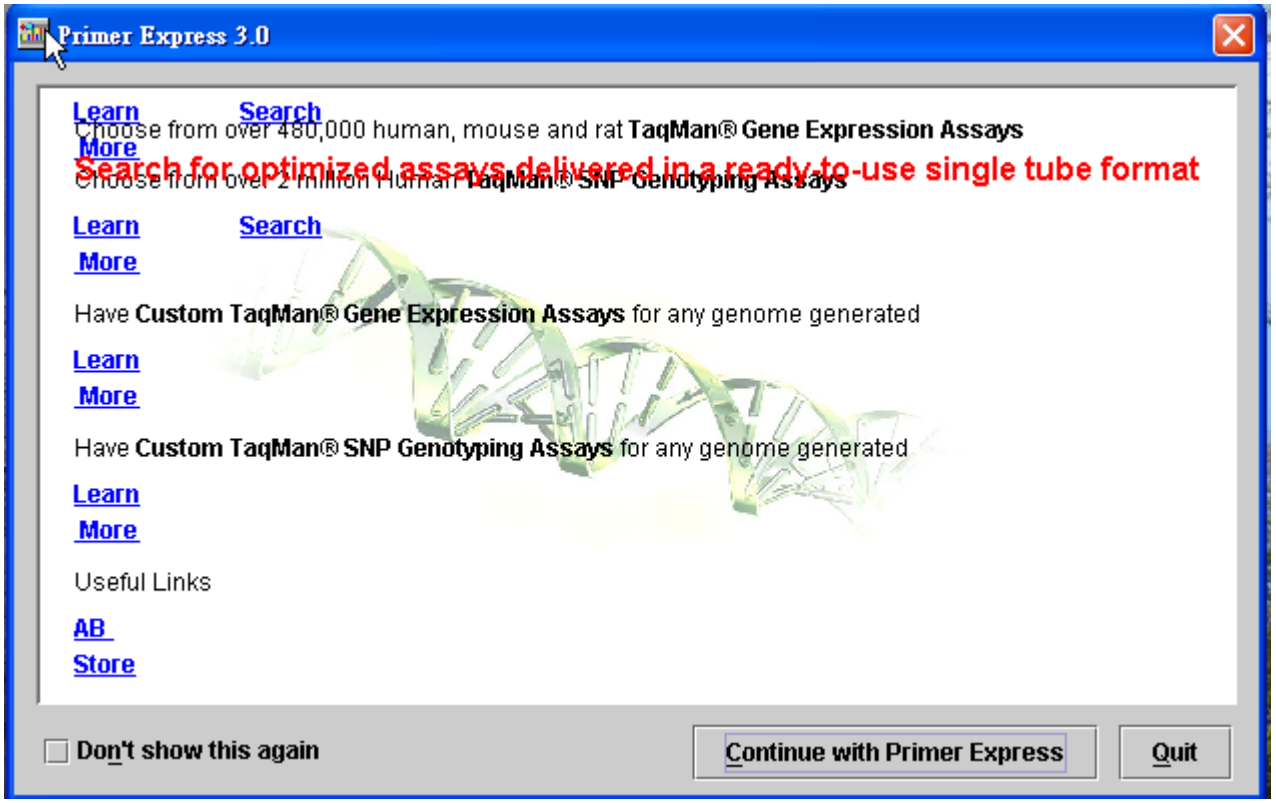


SYBR Green experiment procedure

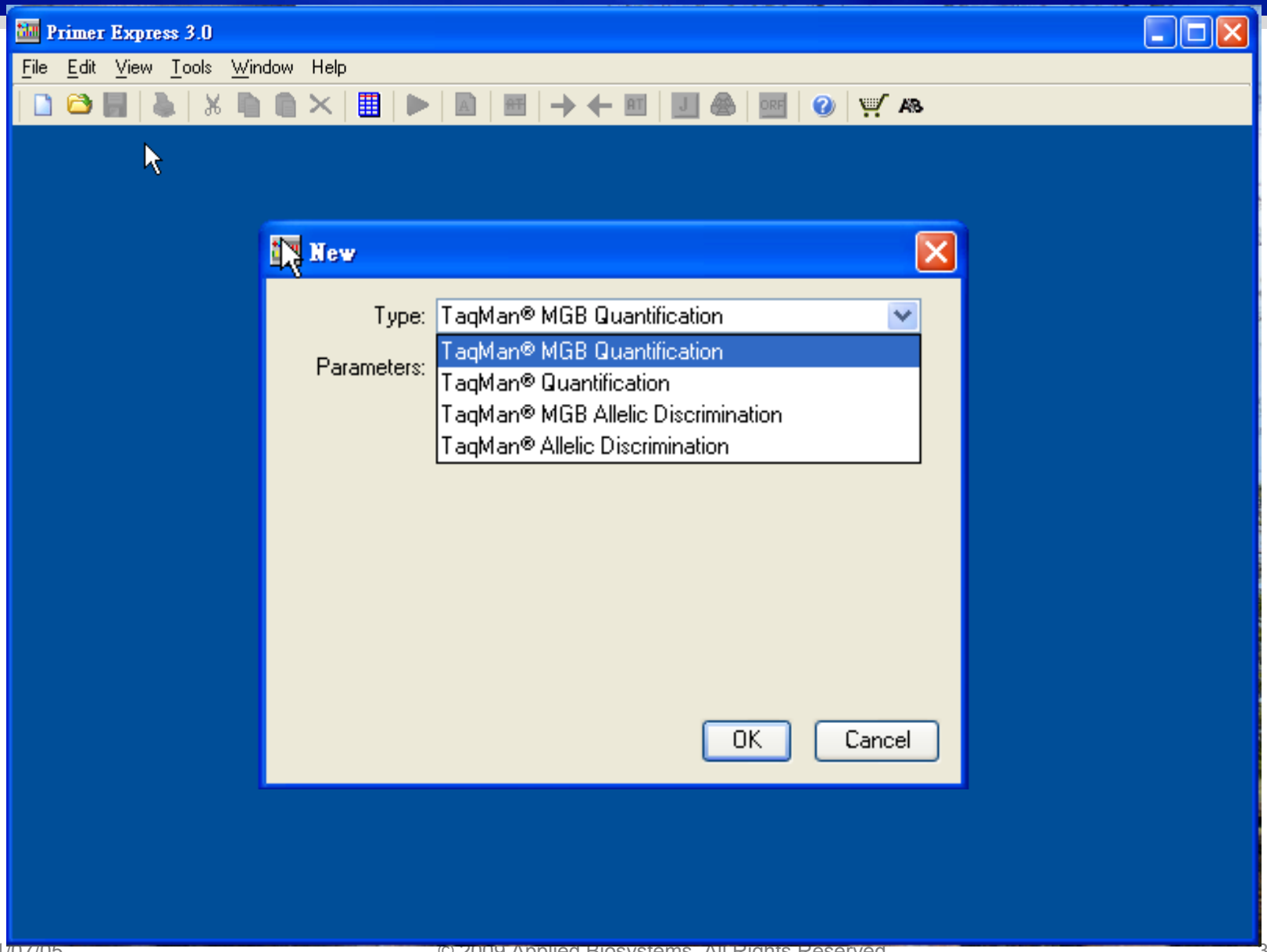
1. **Primer conc. Optimization**
   - Primer Final conc. 100-300 nM
   - No primer dimer or non-specific product involved
2. **PCR Primer Efficiency Validation**
   - Sample serial dilution to run standard curve for target gene and endogenous control gene
3. **Real sample run for each gene**

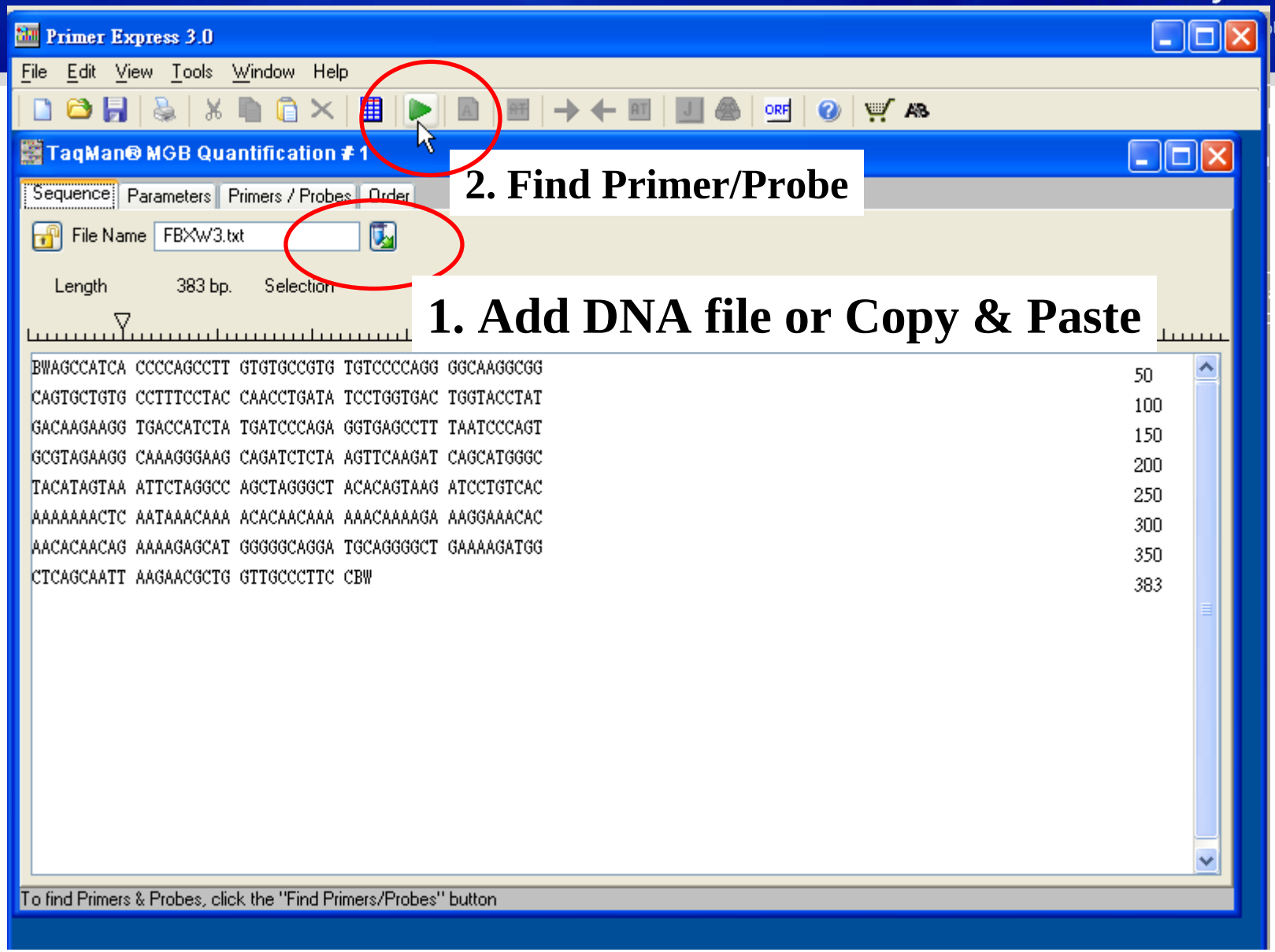
Primer Express 3.0 Operation



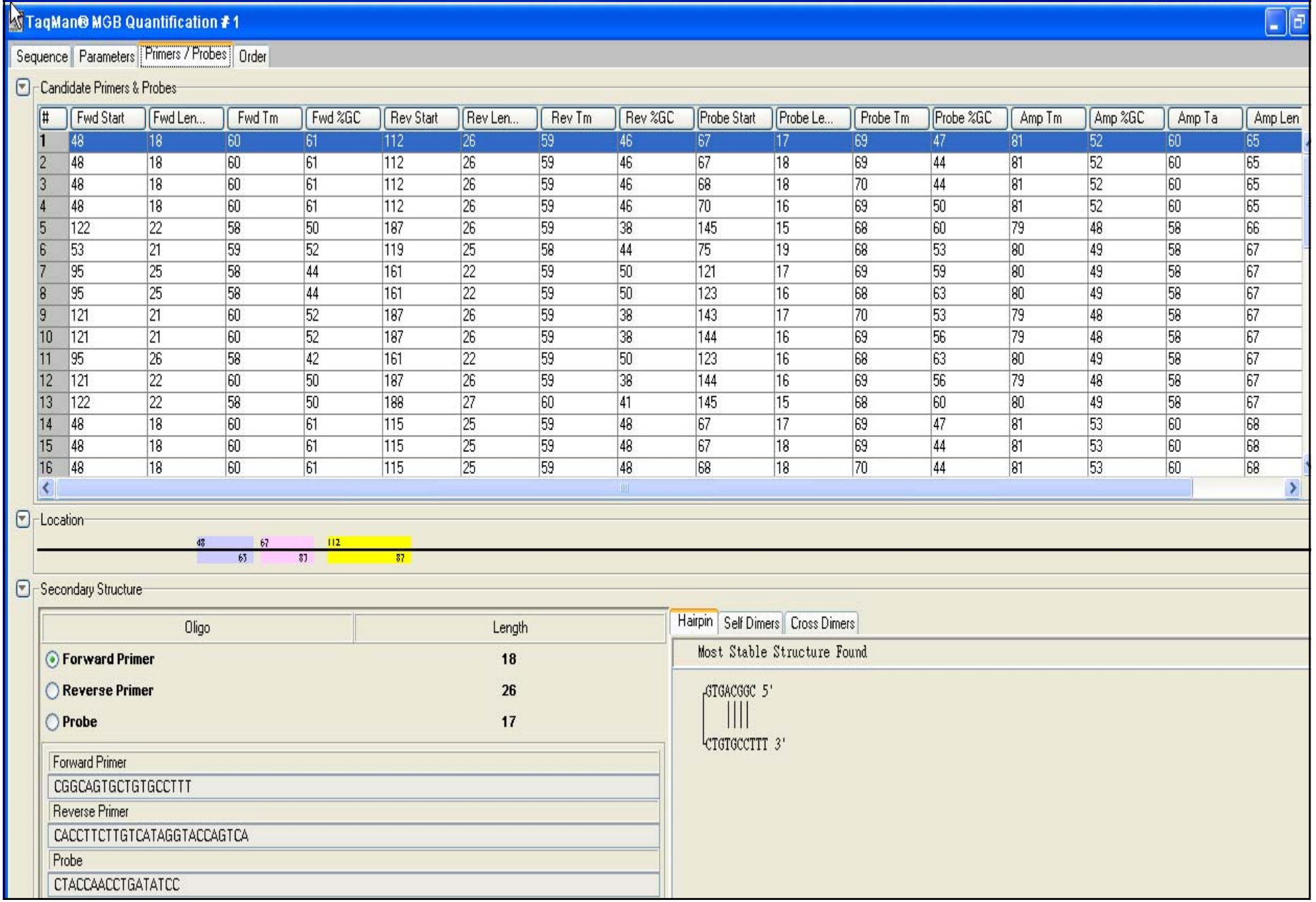


Primer Express 3.0 Operation



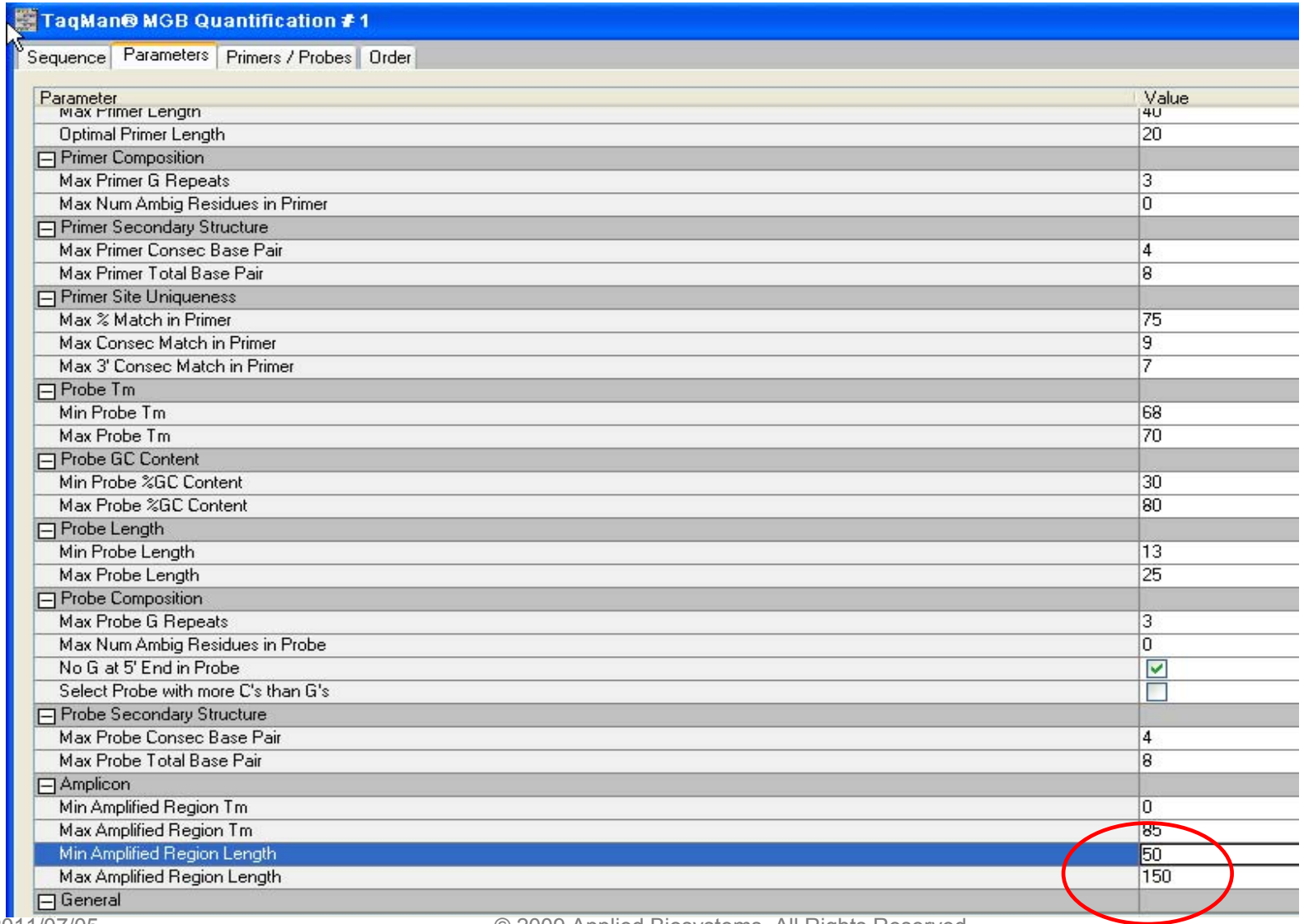


Result

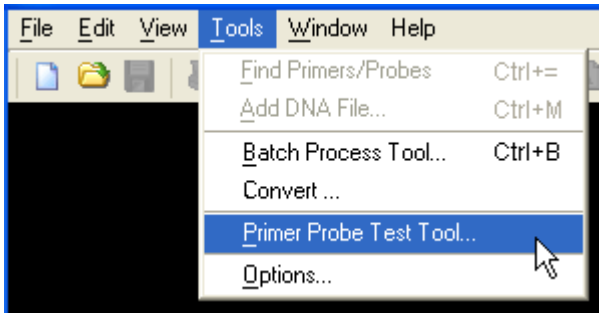


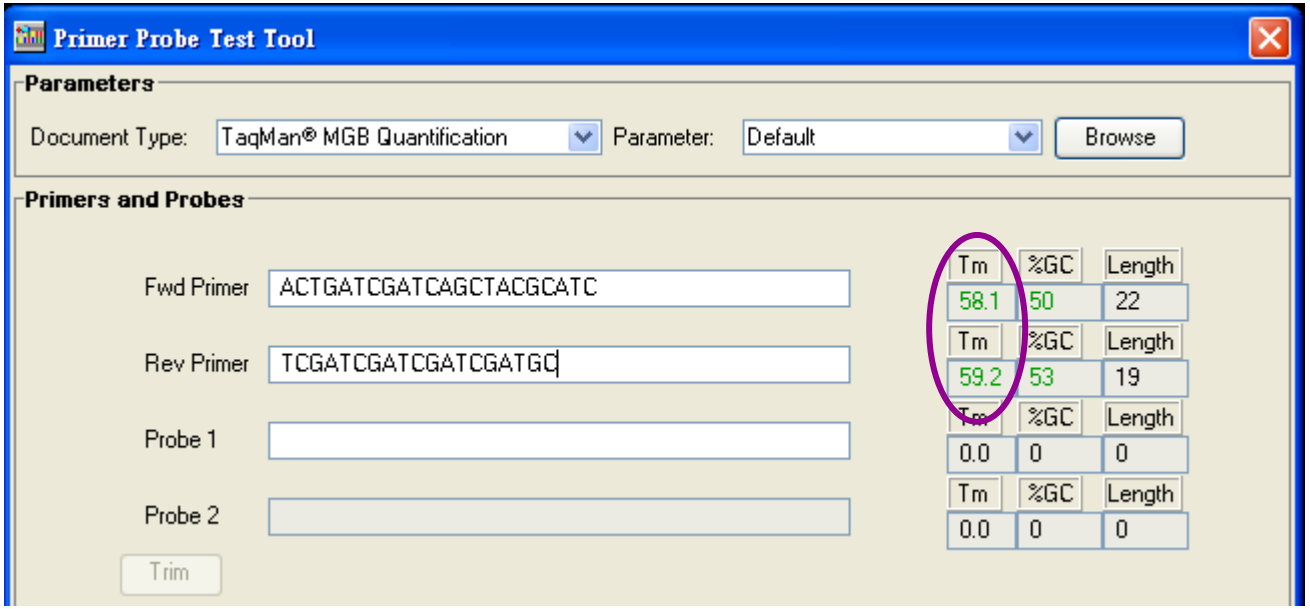
#	Fwd Start	Fwd Len...	Fwd Tm	Fwd %GC	Rev Start	Rev Len...	Rev Tm	Rev %GC	Probe Start	Probe Le...	Probe Tm	Probe %GC	Amp Tm	Amp %GC	Amp Ta	Amp Len
1	48	18	60	61	112	26	59	46	67	17	69	47	81	52	60	65
2	48	18	60	61	112	26	59	46	67	18	69	44	81	52	60	65
3	48	18	60	61	112	26	59	46	68	18	70	44	81	52	60	65
4	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

Design Parameter



Check Tm of primers





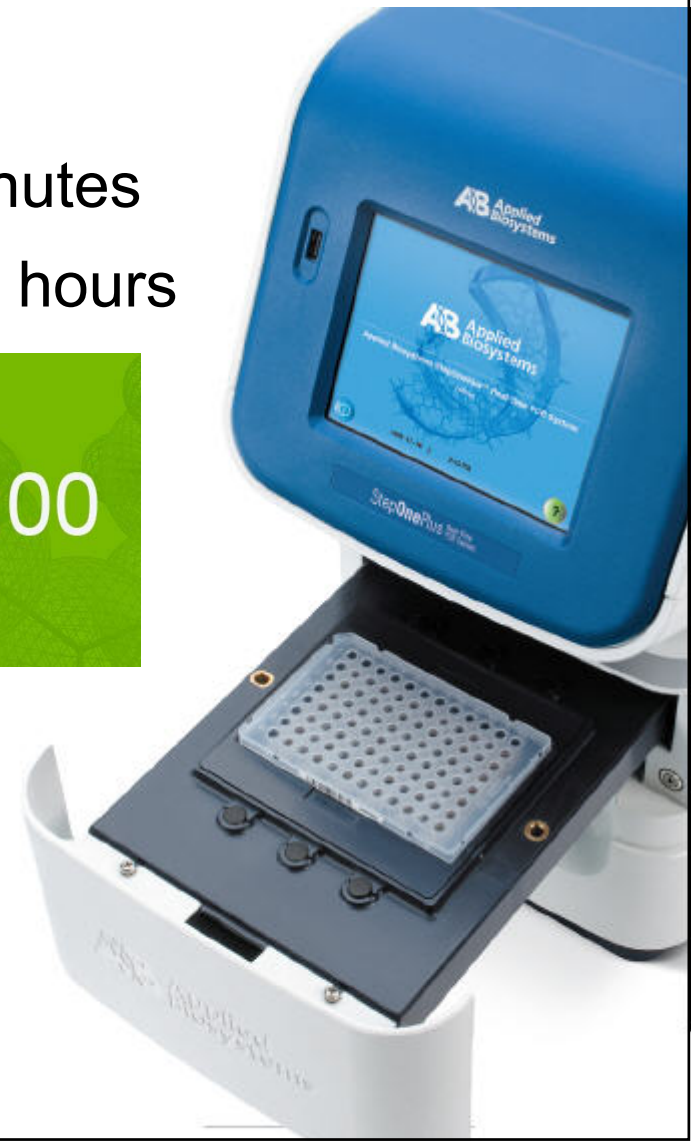
Standalone (PC-Free) Operation 簡易三步驟!

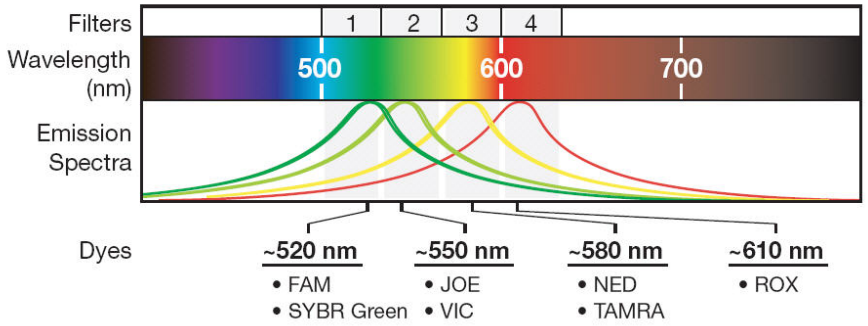
1. Start the run from the touchscreen
2. After run, download the file (.eds) to your PC
3. Analyze your data

StepOnePlus™ Real-Time PCR System
The Basics

Applied Biosystems
part of life technologies

- 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

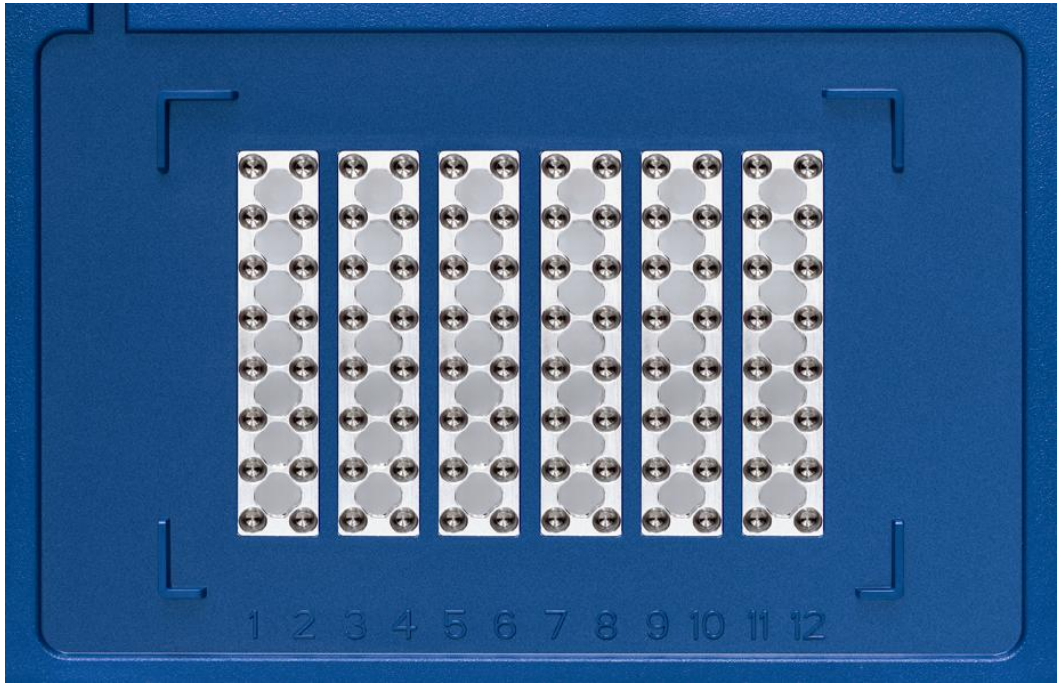


*Image from Veriti Thermal Cycler

StepOnePlus™ Real-Time PCR System
The Basics

Applied Biosystems
part of life technologies

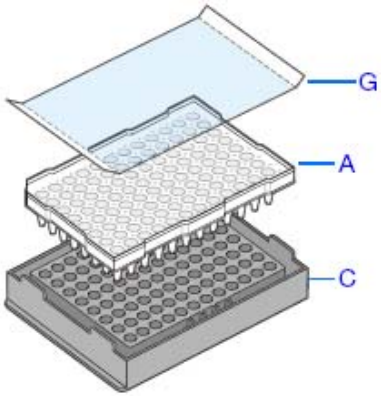
- Supported consumables:
  - **P/N 4346907**

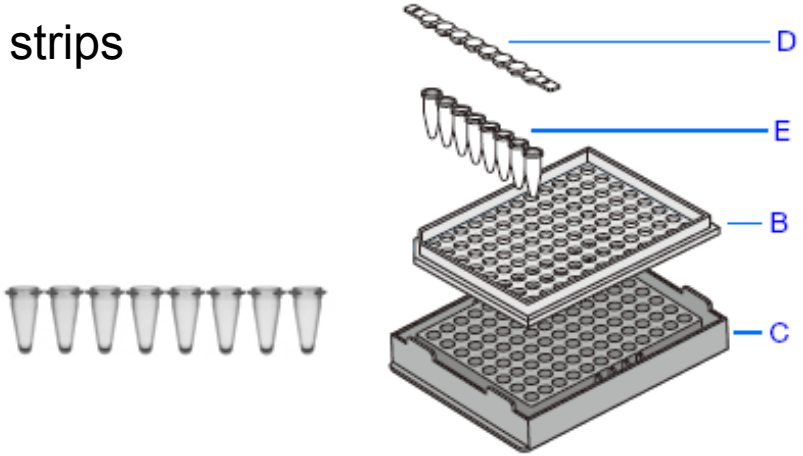
    Fast 96-Well Reaction Plate (0.1 mL) -10 plates
  - **P/N 4360954**

    Optical Adhesive Film - 25 films
  - **P/N 4358293**

    Fast 8-Tube Strip (0.1 mL) - 125 strips
  - **P/N 4323032**

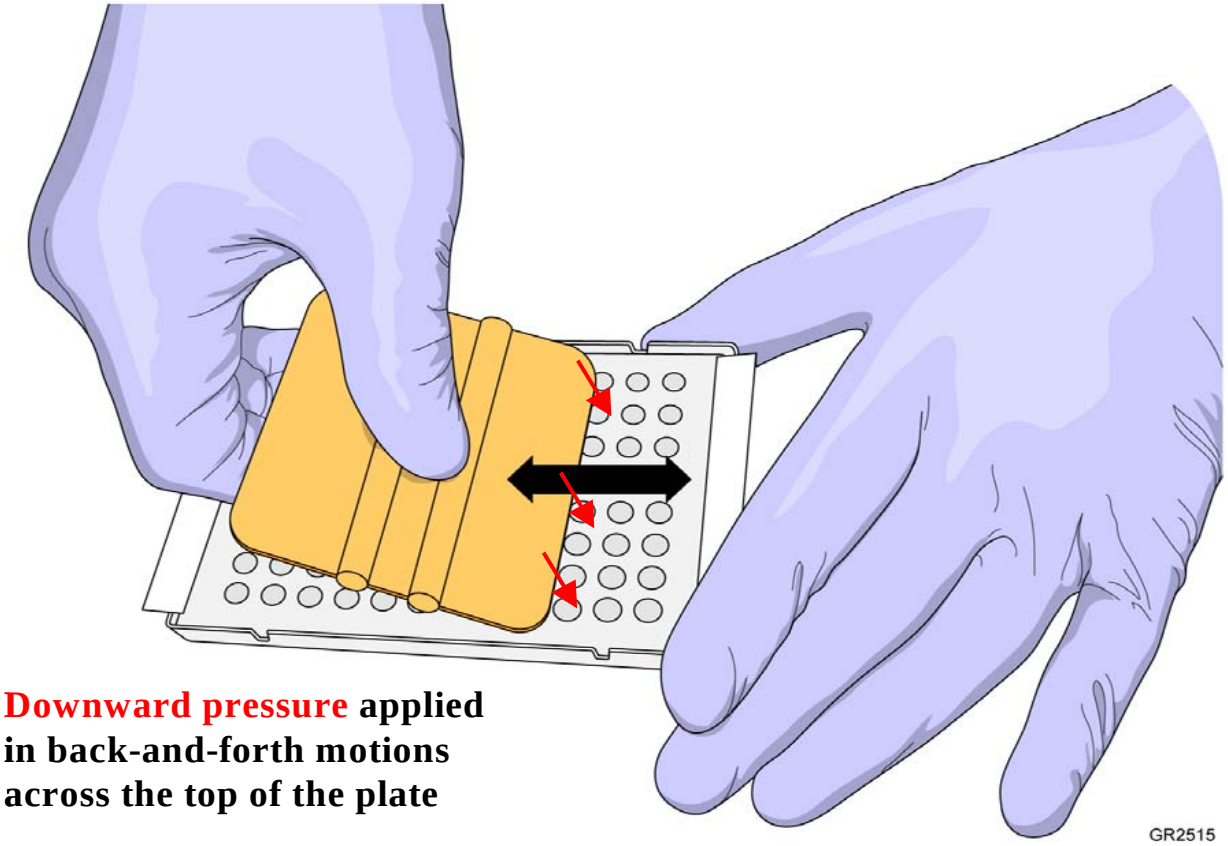
    Optical 8-Cap Strip - 300 strips





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

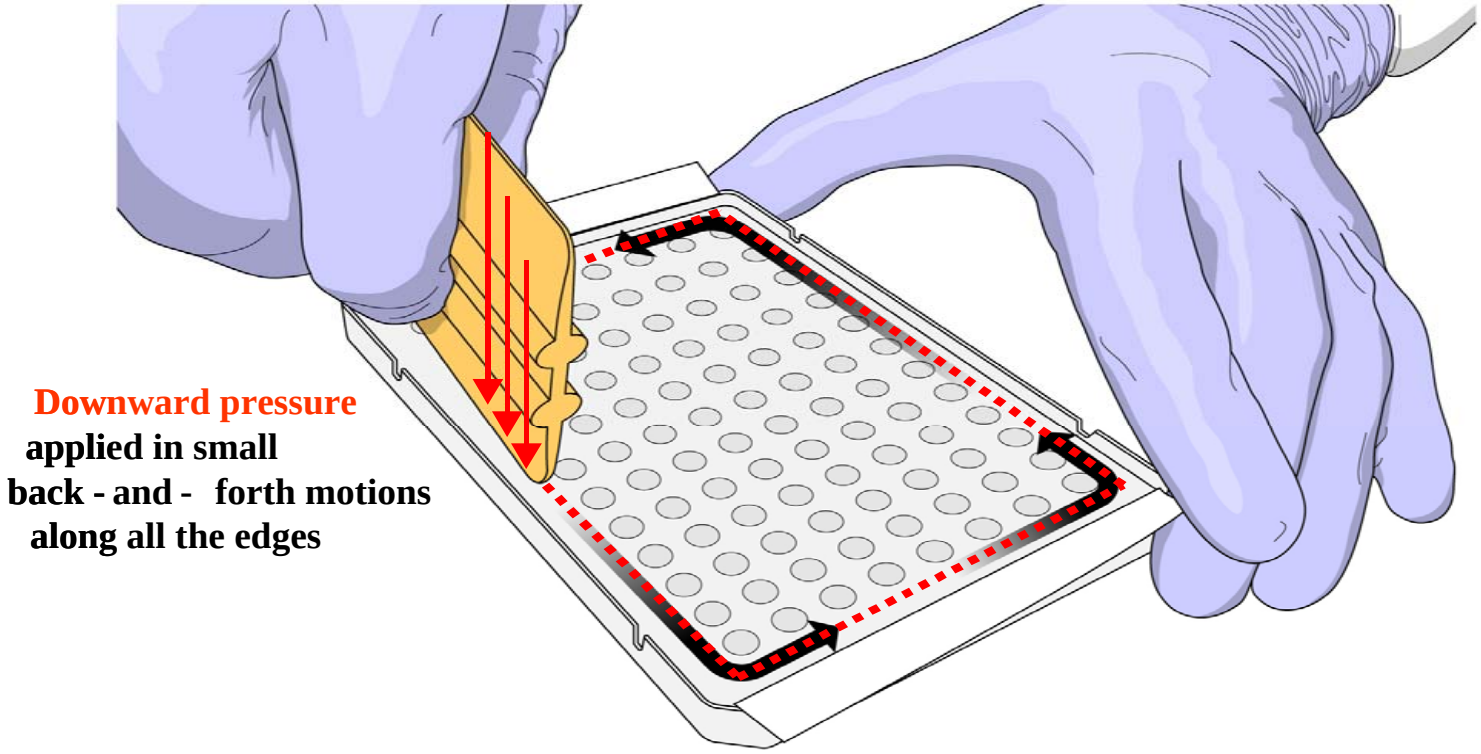
The flat edge of an applicator is rubbed back-and-forth along the length of the plate with a significant downward pressure to form a complete seal on top the wells



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

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

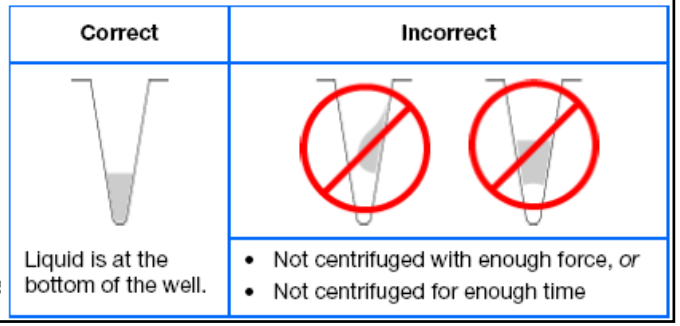
The end of an applicator is rubbed around all the outside edges of the plate with a significant downward pressure to form a complete seal around the outside wells



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

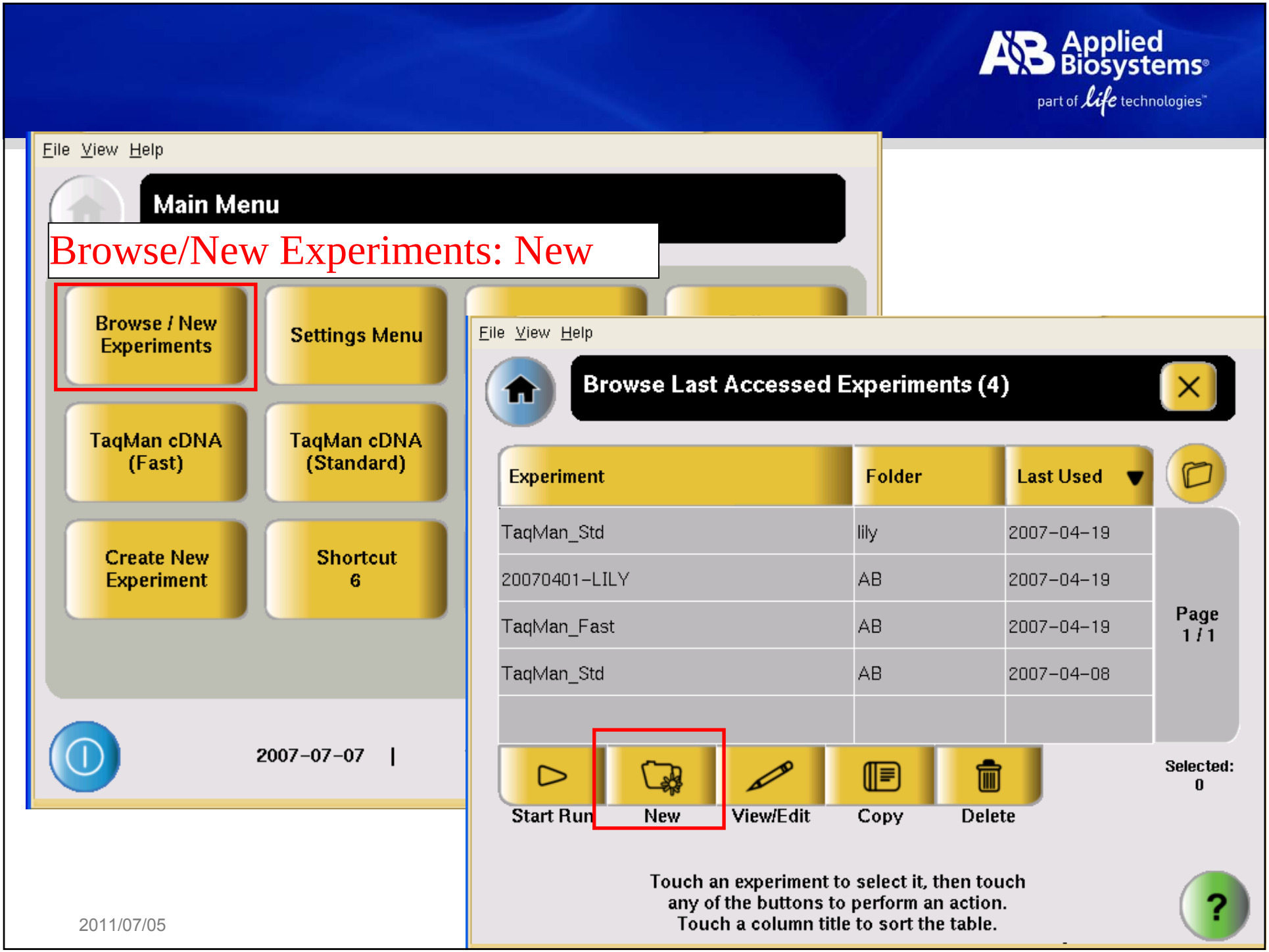
StepOnePlus™ Operation Notes

- 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

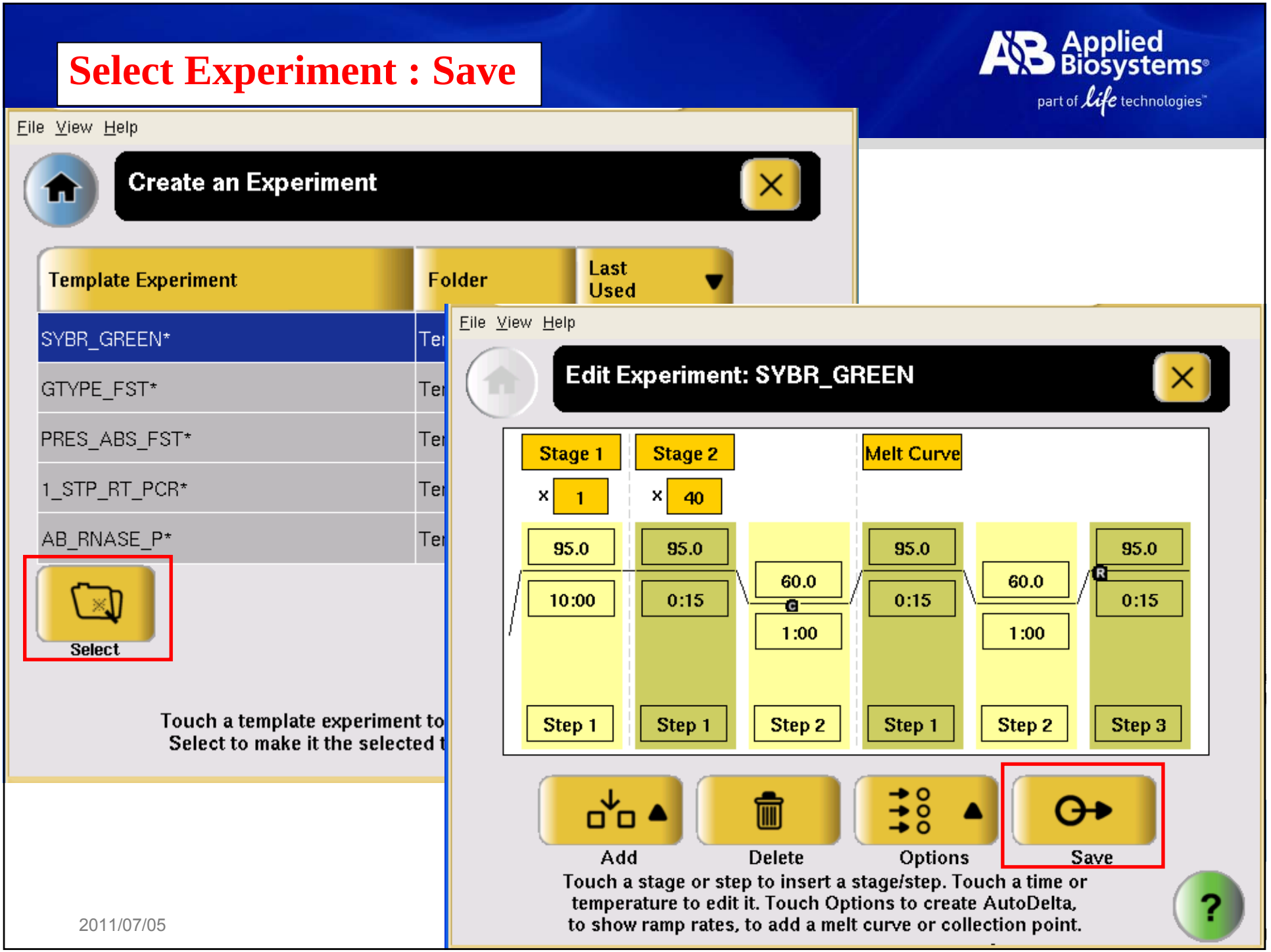


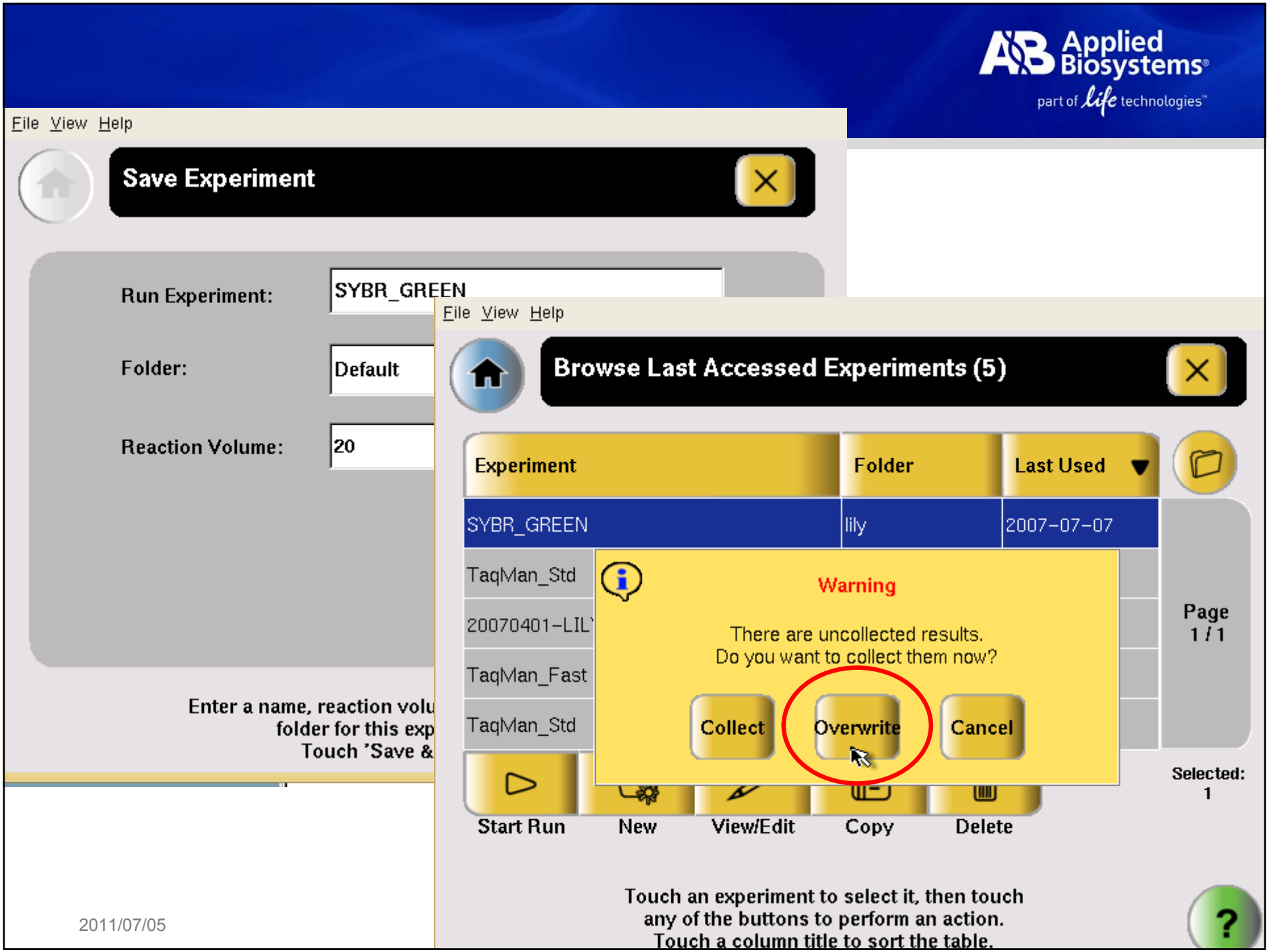
Standalone Operation (單機操作)

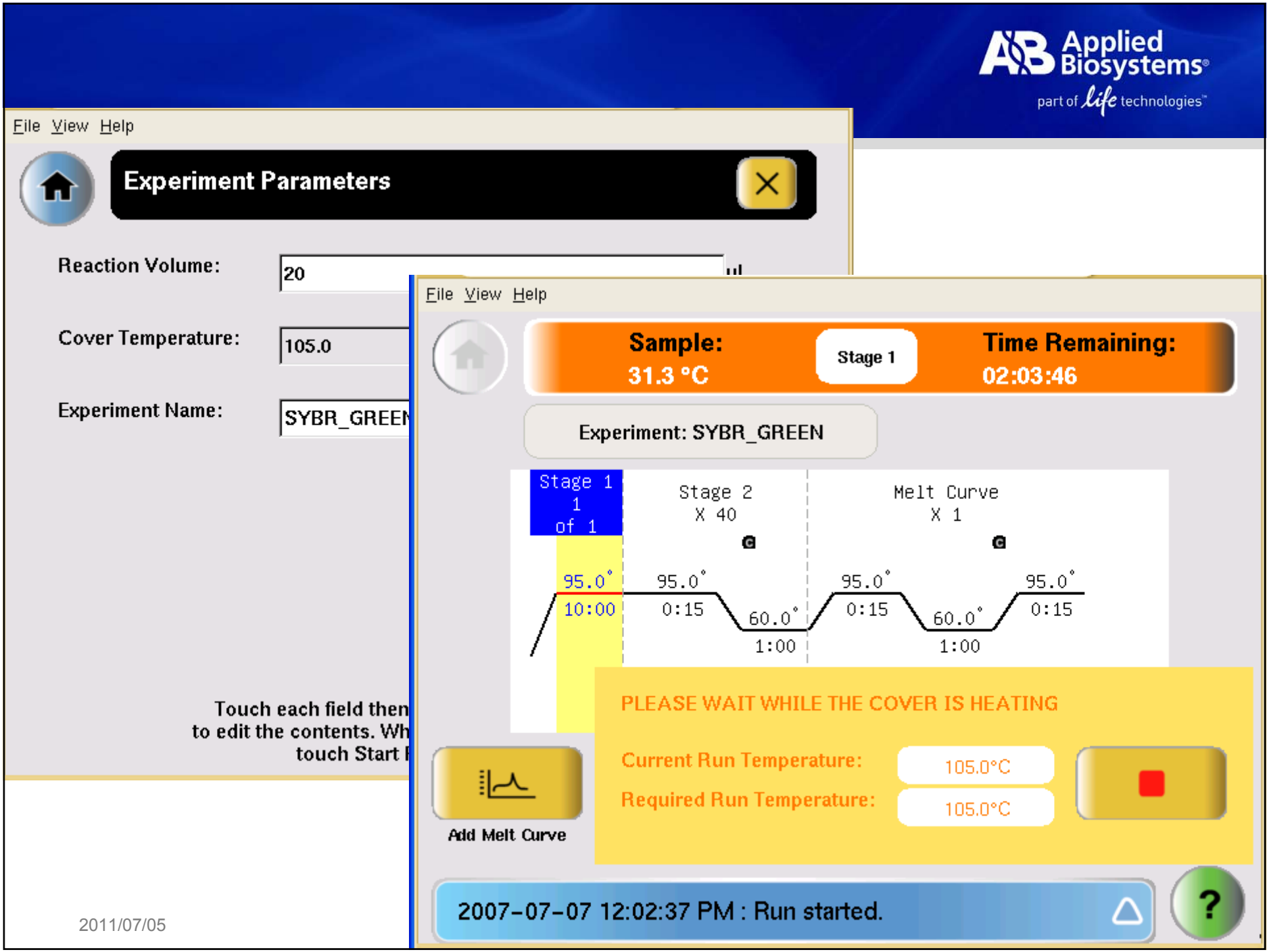
- Soft power button on the LCD touchscreen


Applied Biosystems
part of life technologies
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
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

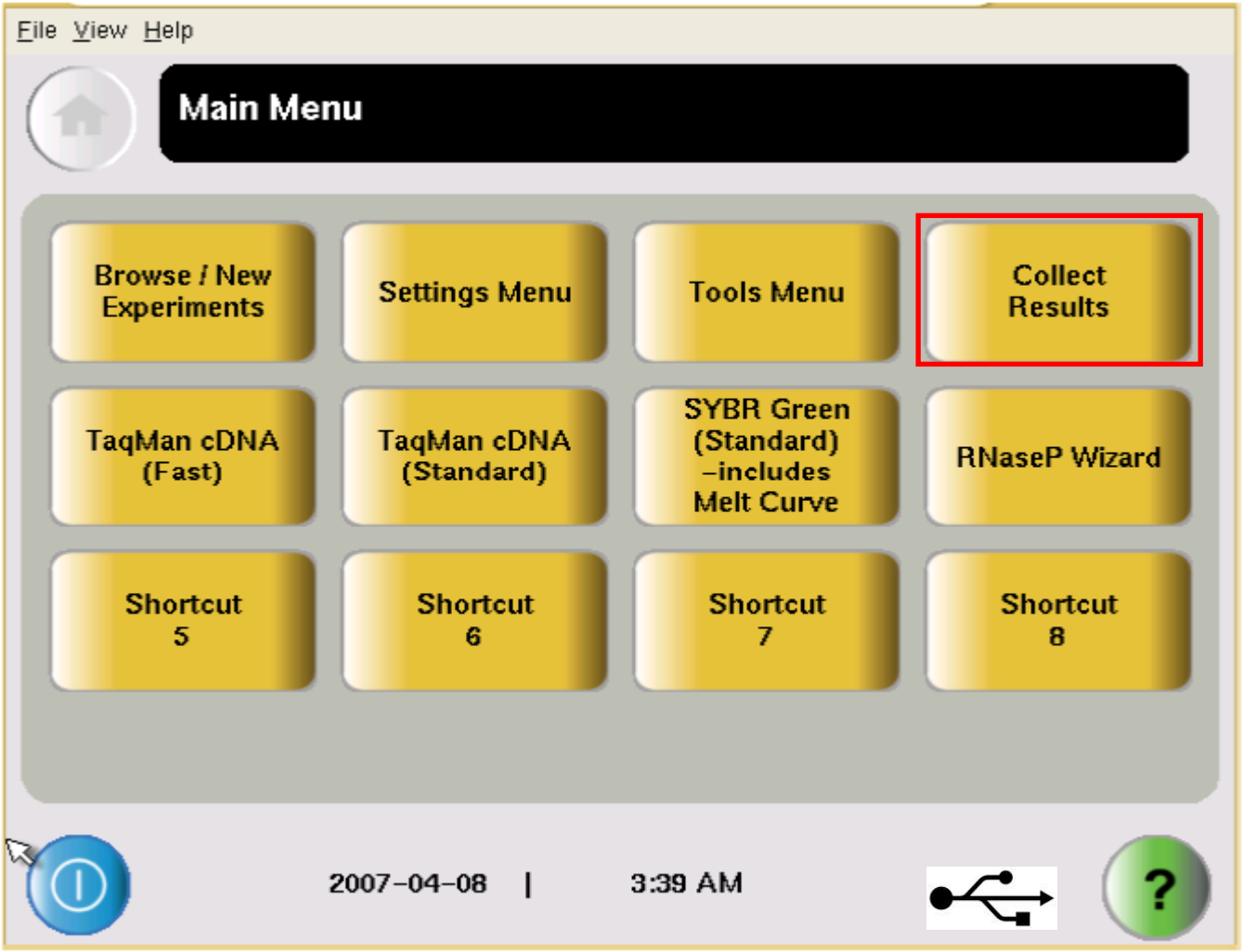



Applied Biosystems
part of life technologies
File View Help
Save Experiment
Run Experiment:
SYBR_GREEN
Folder:
Default
Reaction Volume:
20
Enter a name, reaction volu
folder for this exp
Touch 'Save &
File View Help
Browse Last Accessed Experiments (5)
Experiment
Folder
Last Used
SYBR_GREEN
lily
2007-07-07
TaqMan_Std
20070401-LIL
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.
2011/07/05


File View Help
Experiment Parameters
Reaction Volume:
20
Cover Temperature:
105.0
Experiment Name:
SYBR_GREEN
Touch each field then
to edit the contents. Wh
touch Start
2011/07/05
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
95.0°
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.
?

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

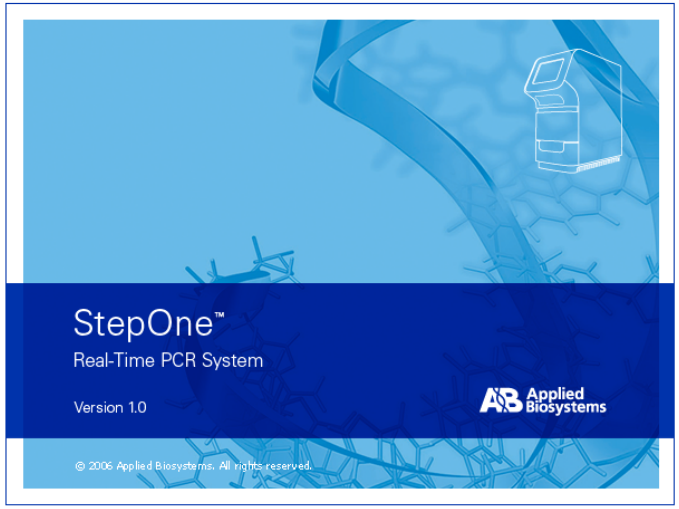


StepOne™ v2.1 software

1280x1024 pixel resolution

一套軟體可以符合全方位的應用

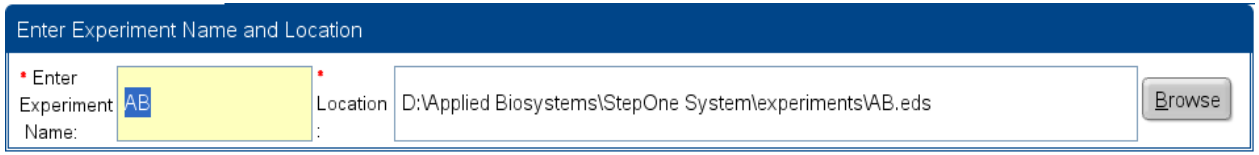
- 絕對定量 Quantification - Standard Curve
- 相對定量 Quantification – Comparative Ct (△△Ct)
- 相對定量 Quantification - Relative Standard Curve
- Melting Curve Analysis
- Genotyping
- Presence/Absence



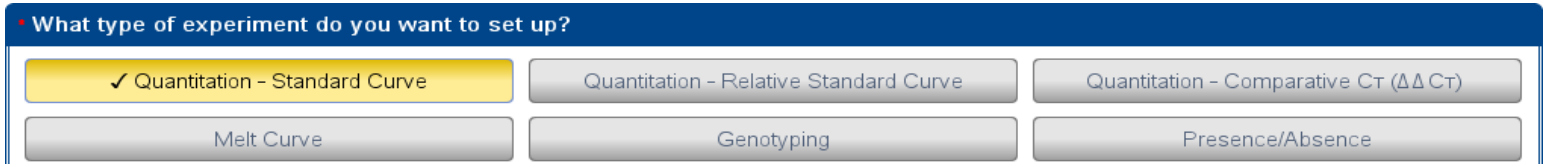
1. Run: QuickStart

2. Setup: Experiment Properties

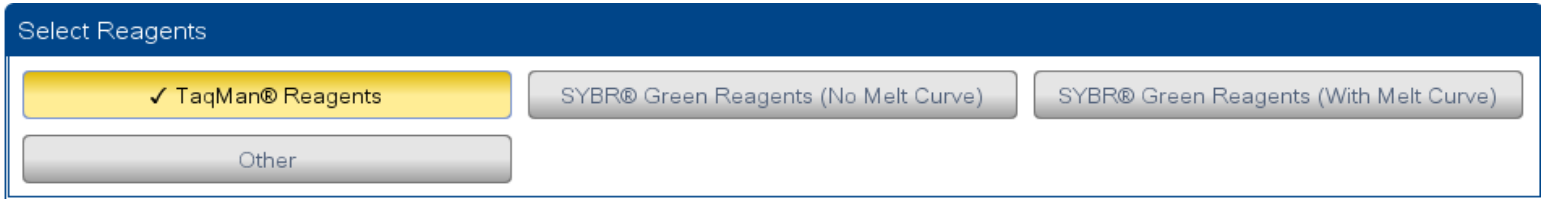
a. Experiment Name 及檔案儲存位置



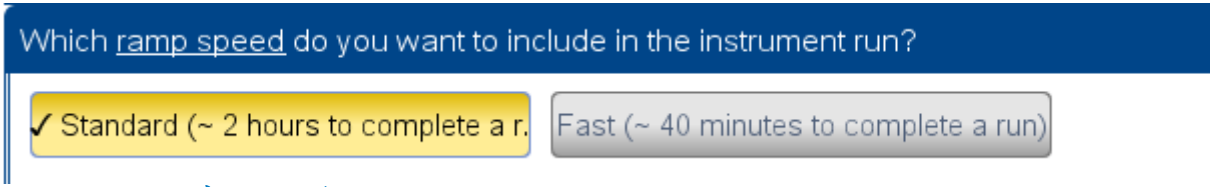
b.選擇 Experiment Type



c.選擇使用螢光系統

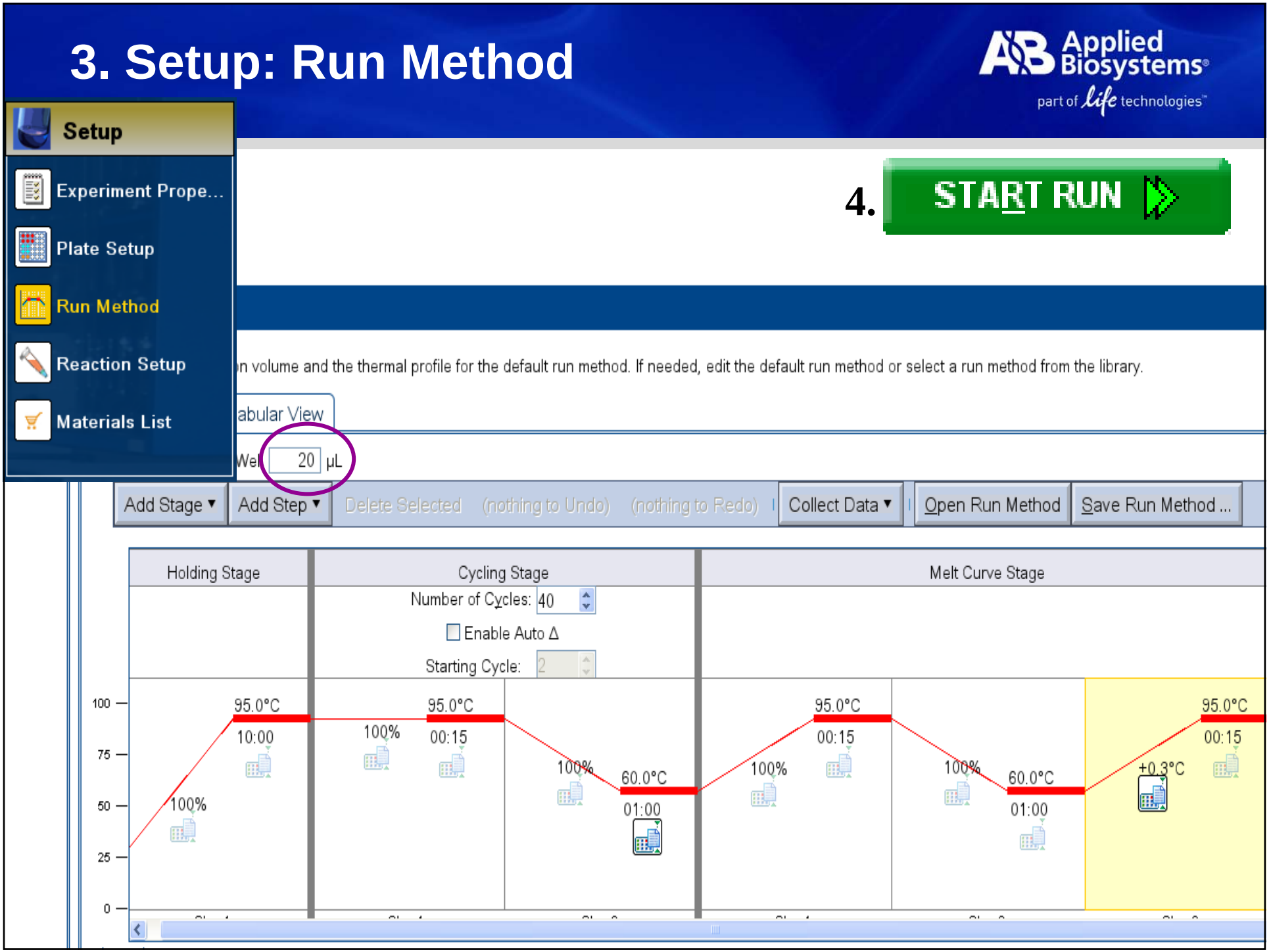


d.選擇Ramp Speed

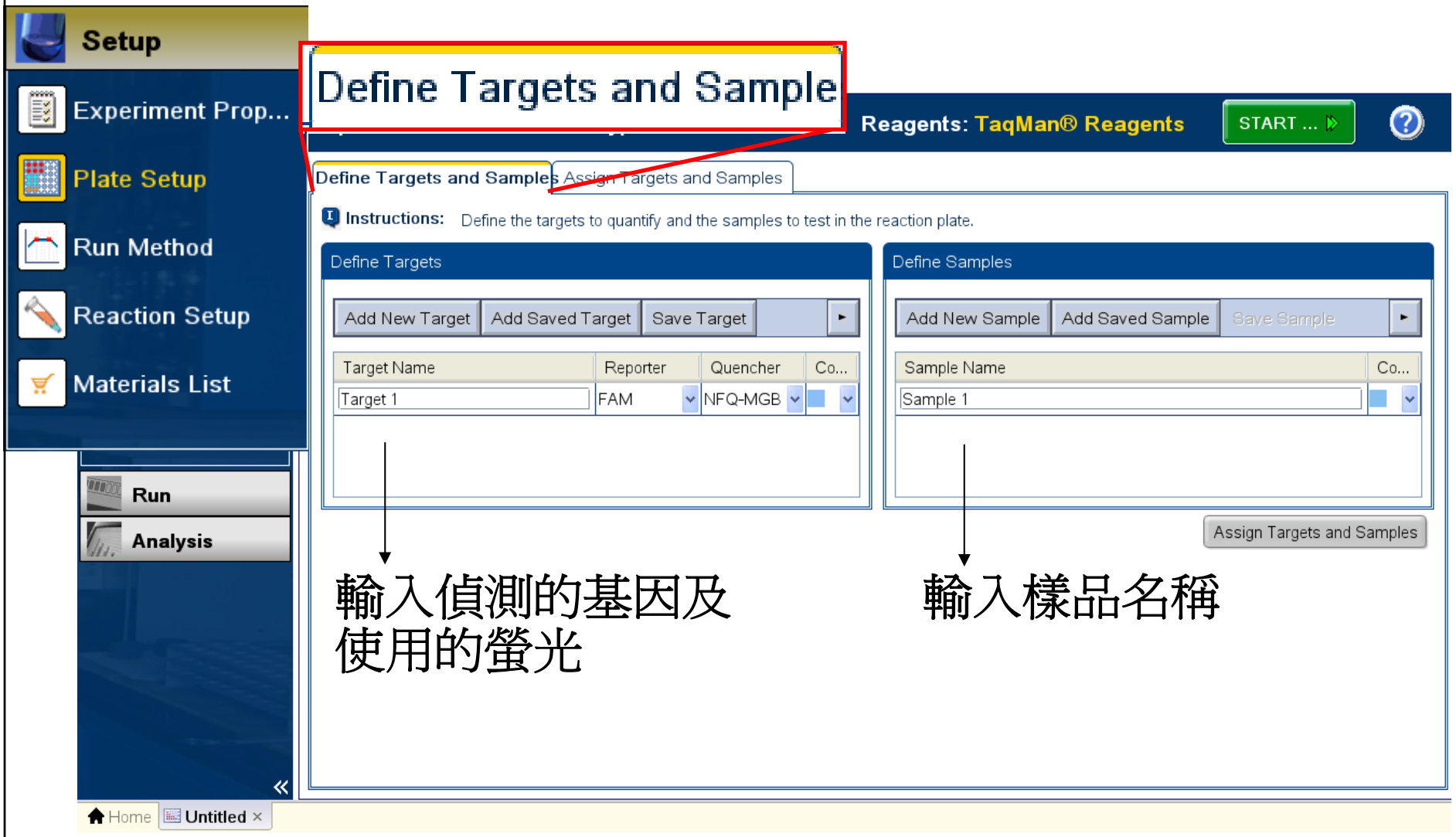


e.選擇實驗樣品種類

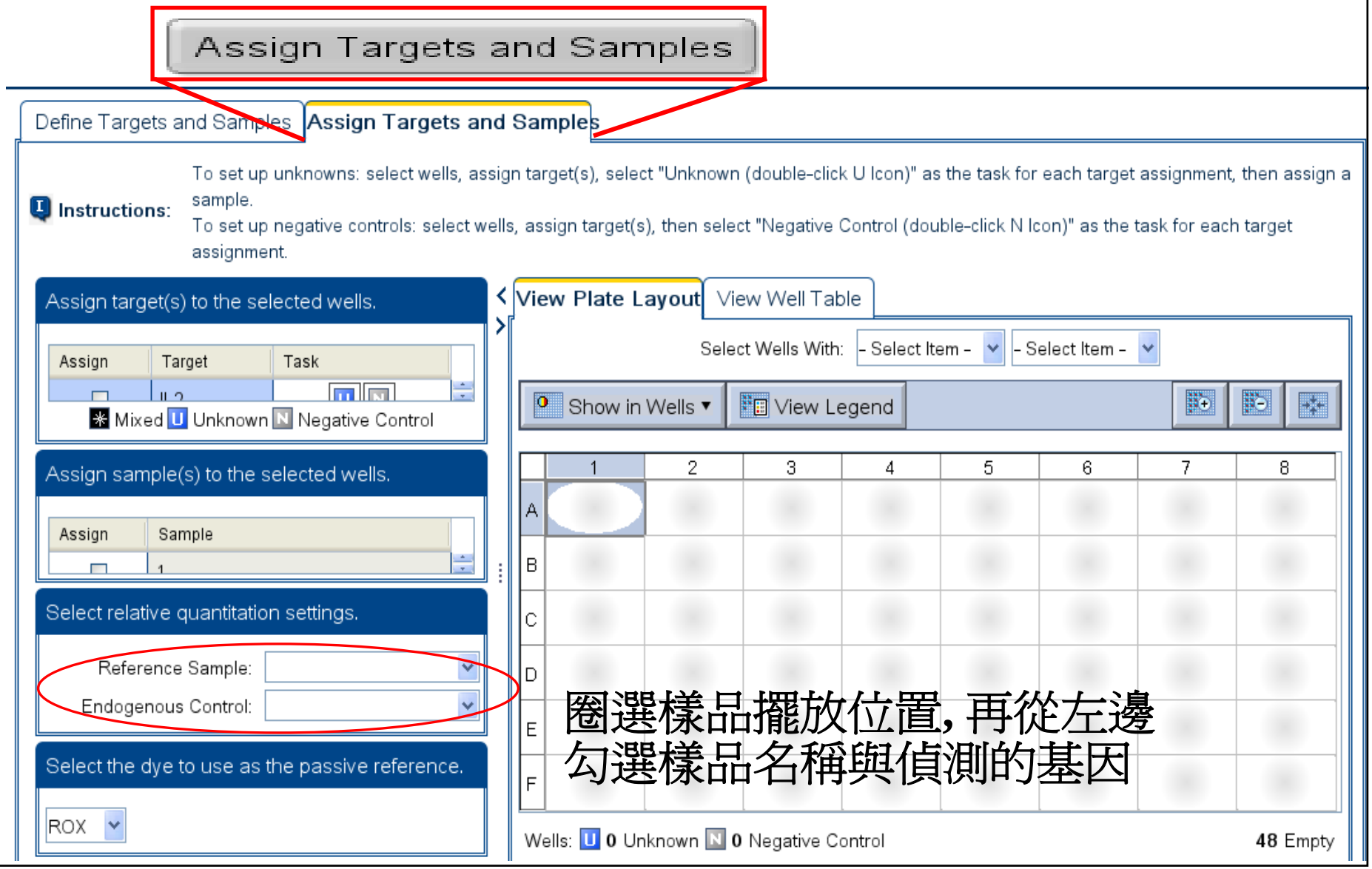
3. Setup: Run Method



5. Setup: Plate Setup 定義基因和樣品名稱

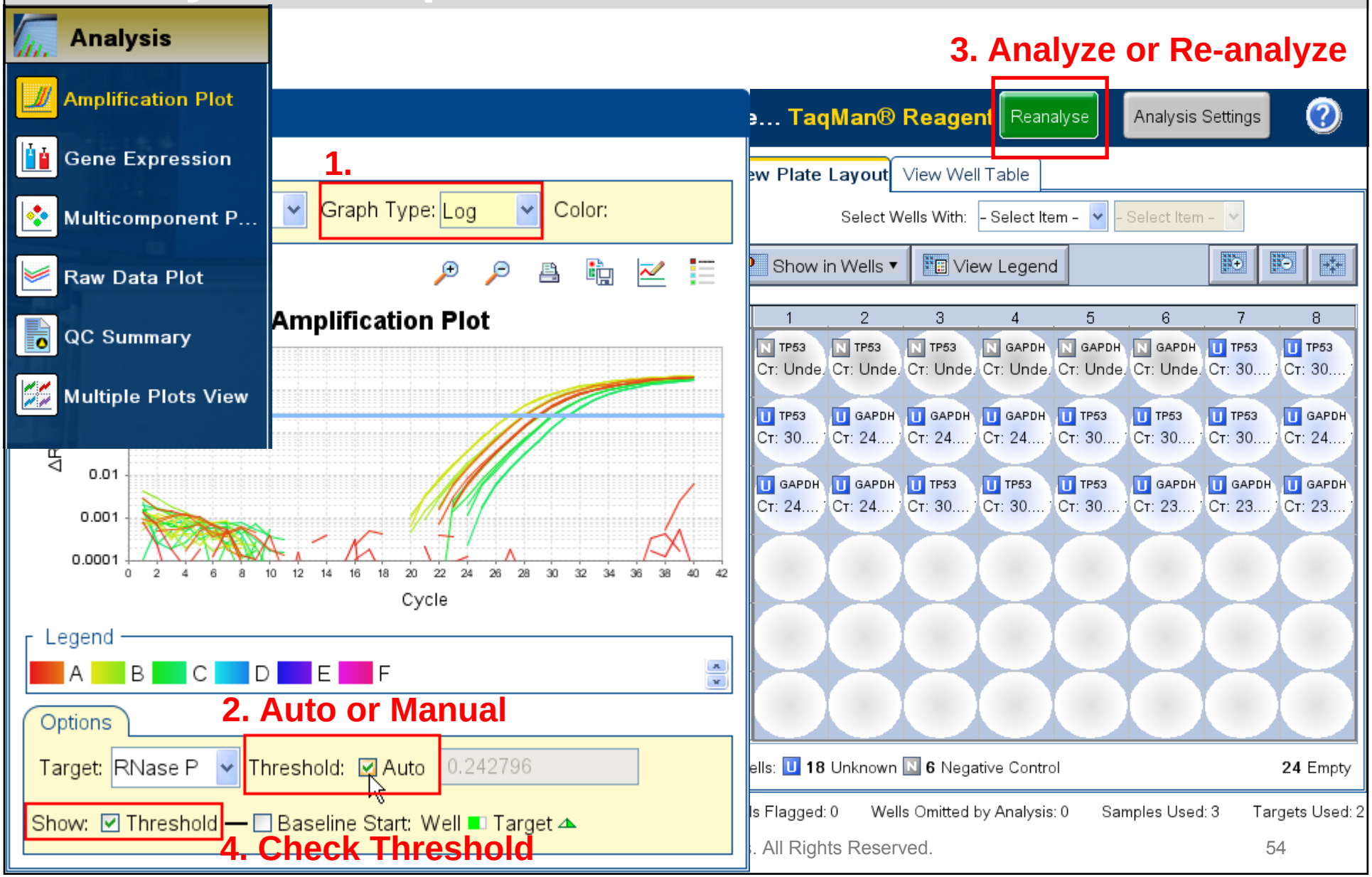


6-1. Setup: Plate Setup
決定基因和樣品位置 (for ddCt)

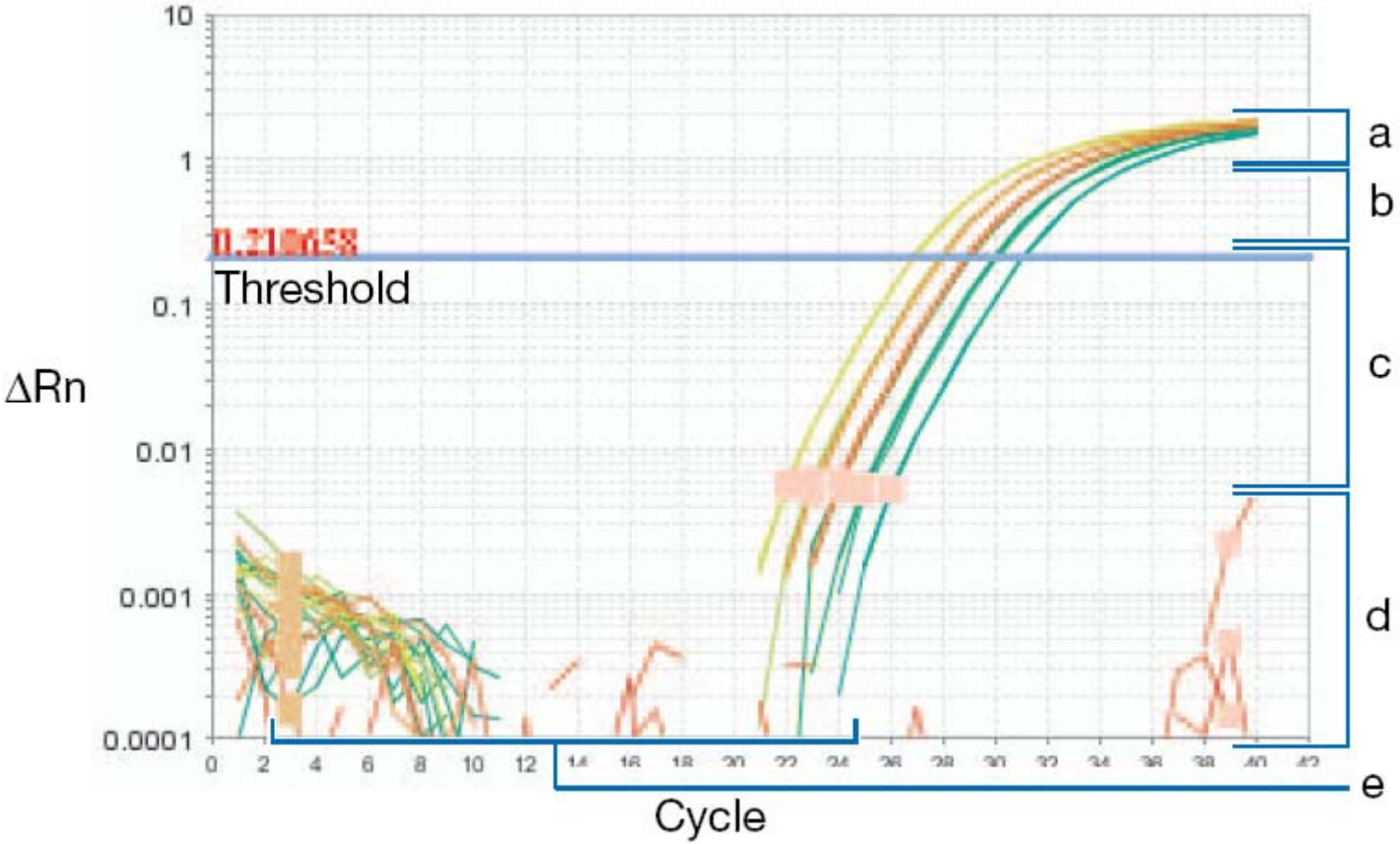


圈選樣品擺放位置, 再從左邊
勾選樣品名稱與偵測的基因

7. Analyze
Analysis : Amplification Plot



How to Set the Threshold? **Auto or Manual Threshold?**



a. Plateau phase

b. Linear phase

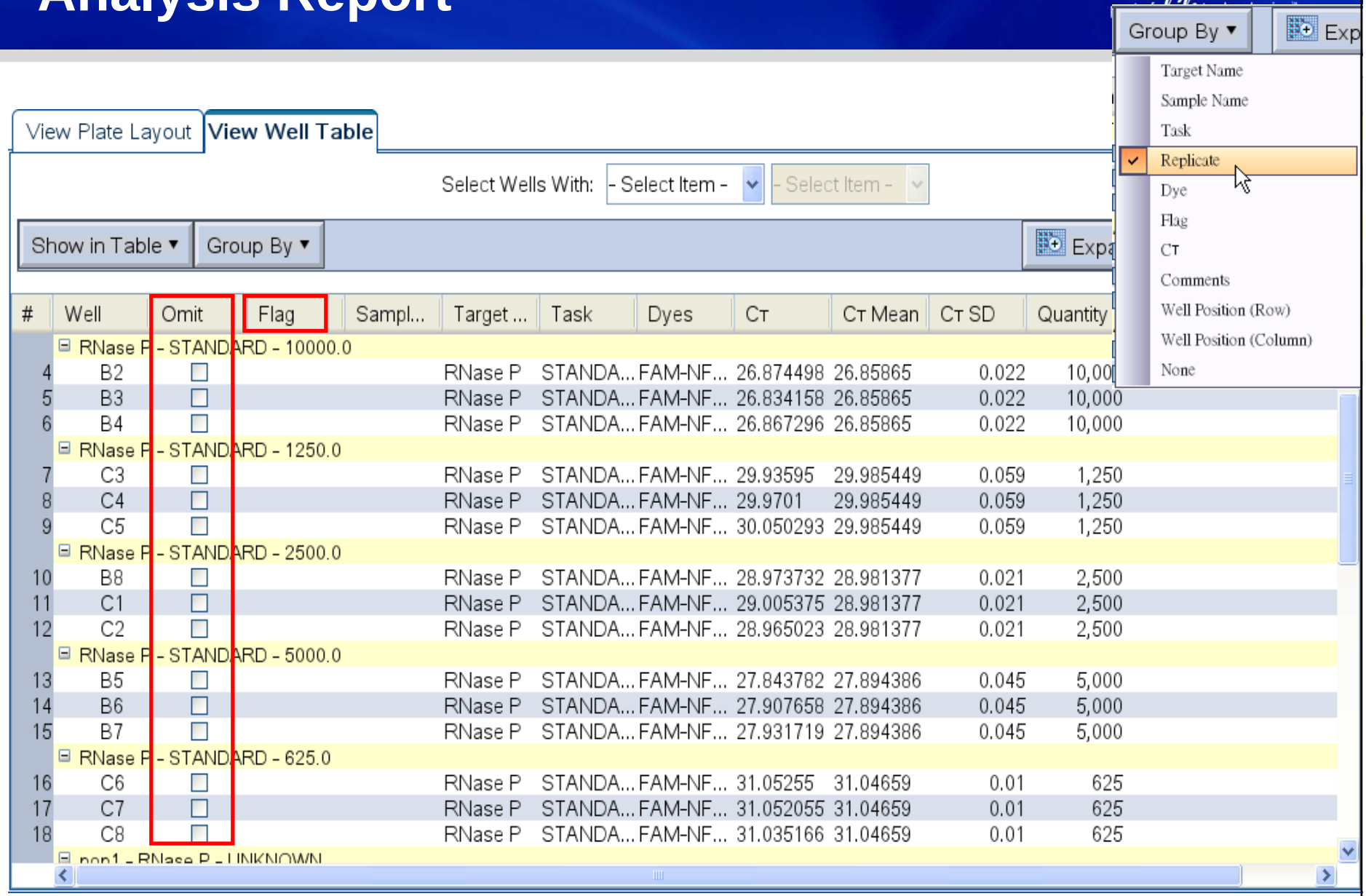
c. Exponential

d. Background

e. Baseline

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

Analysis Report



Analysis : Gene Expression

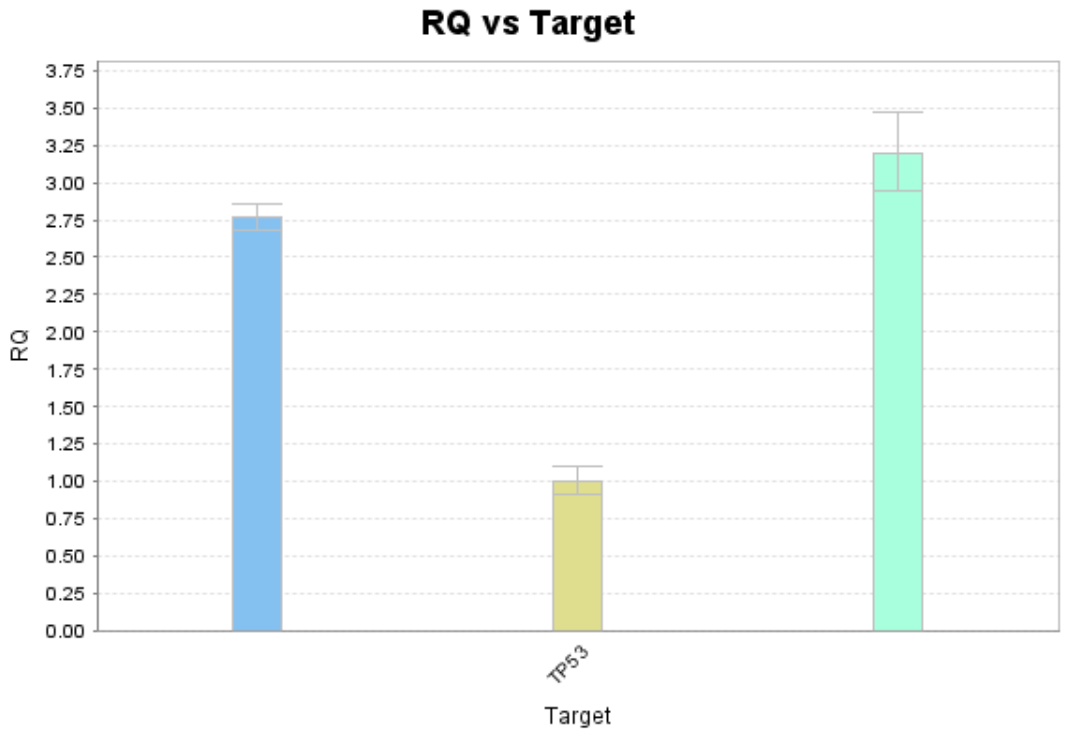
Applied Biosystems
part of life technologies

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

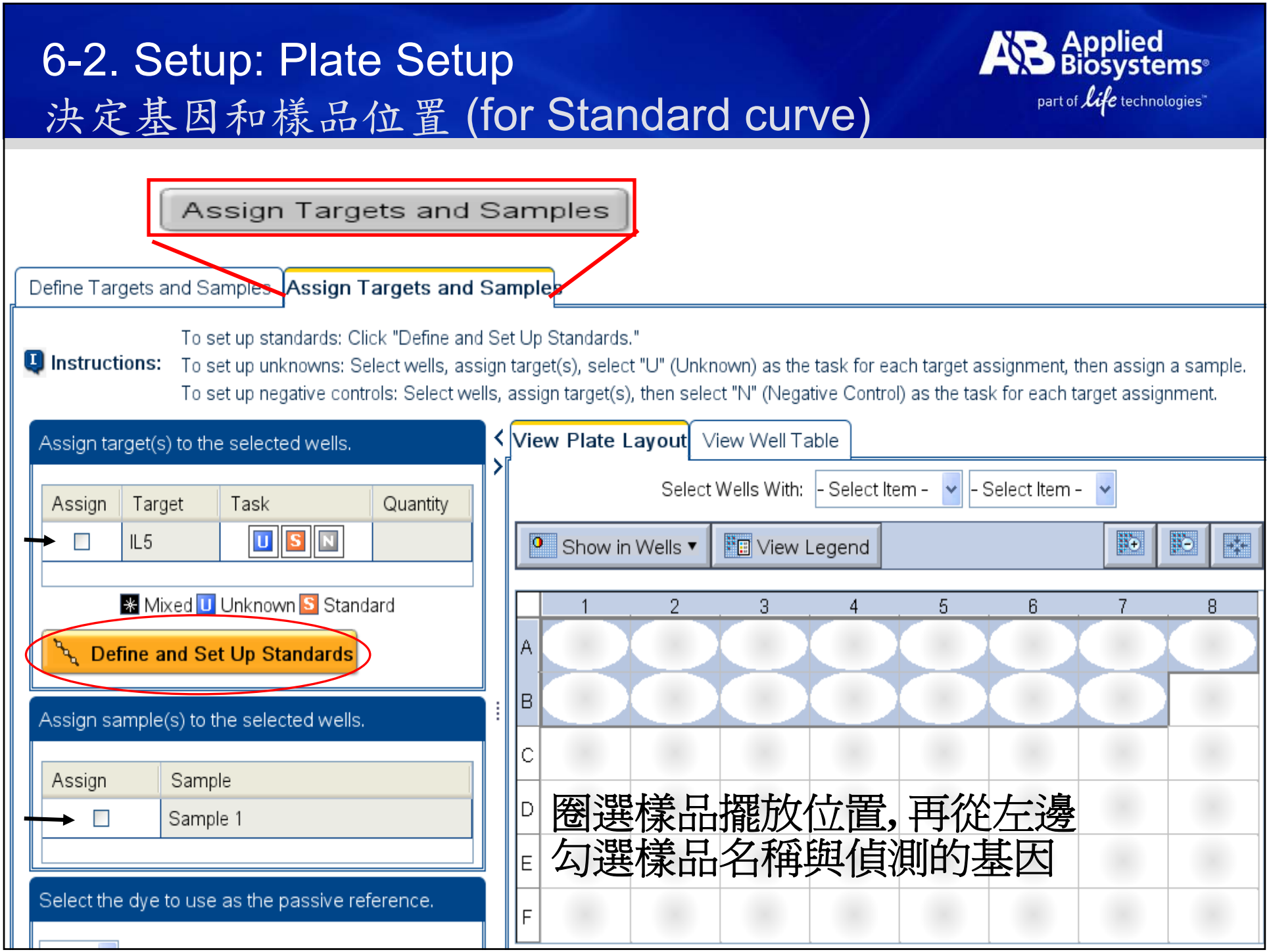


Replicate Results Table

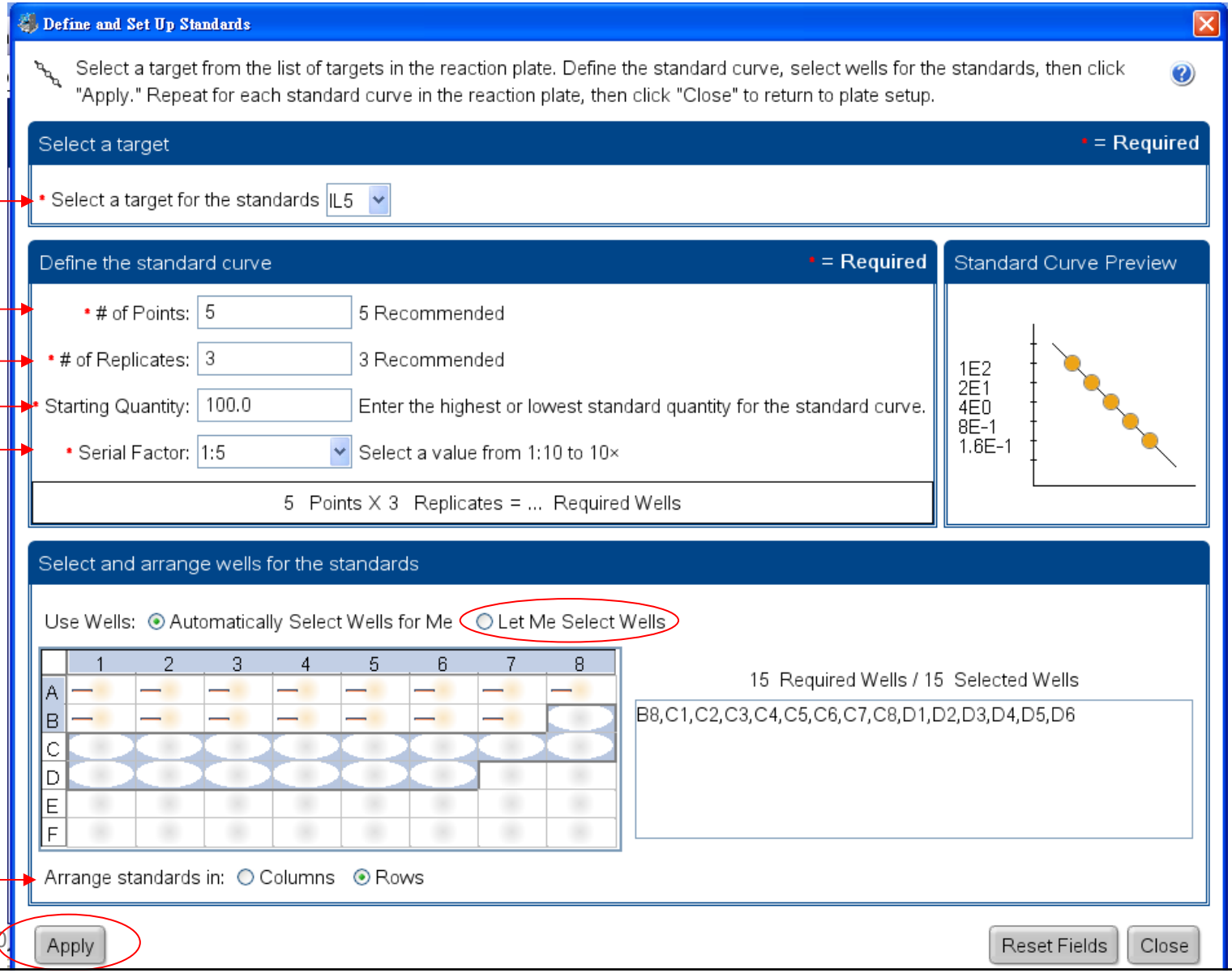
Show In Table

#	Omit	Sample	Target	CT Mean	ΔCT Mean	ΔCT SD	ΔCT SE	ΔΔCT	RQ	RQ Min	RQ Max
1	☐	Liver	TP53	30.69	6.015	0.03	0.017	-1.47	2.77	2.68	2.863
2	☐	Kidney	TP53	30.696	5.807	0.073	0.042	-1.677	3.198	2.948	3.469
3	☐	Brain	TP53	30.912	7.484	0.083	0.048	0	1	0.912	1.097

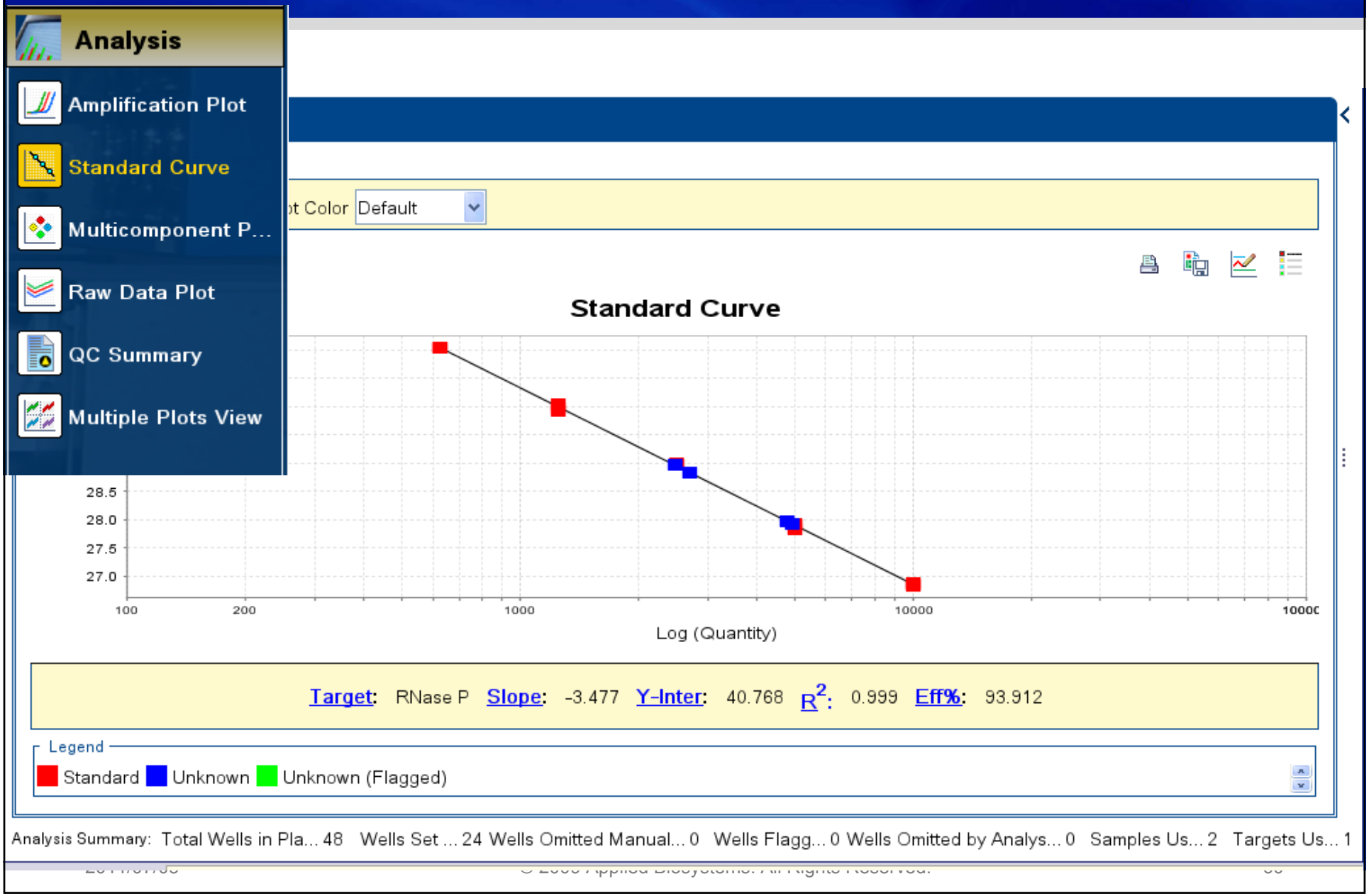
6-2. Setup: Plate Setup
決定基因和樣品位置 (for Standard curve)



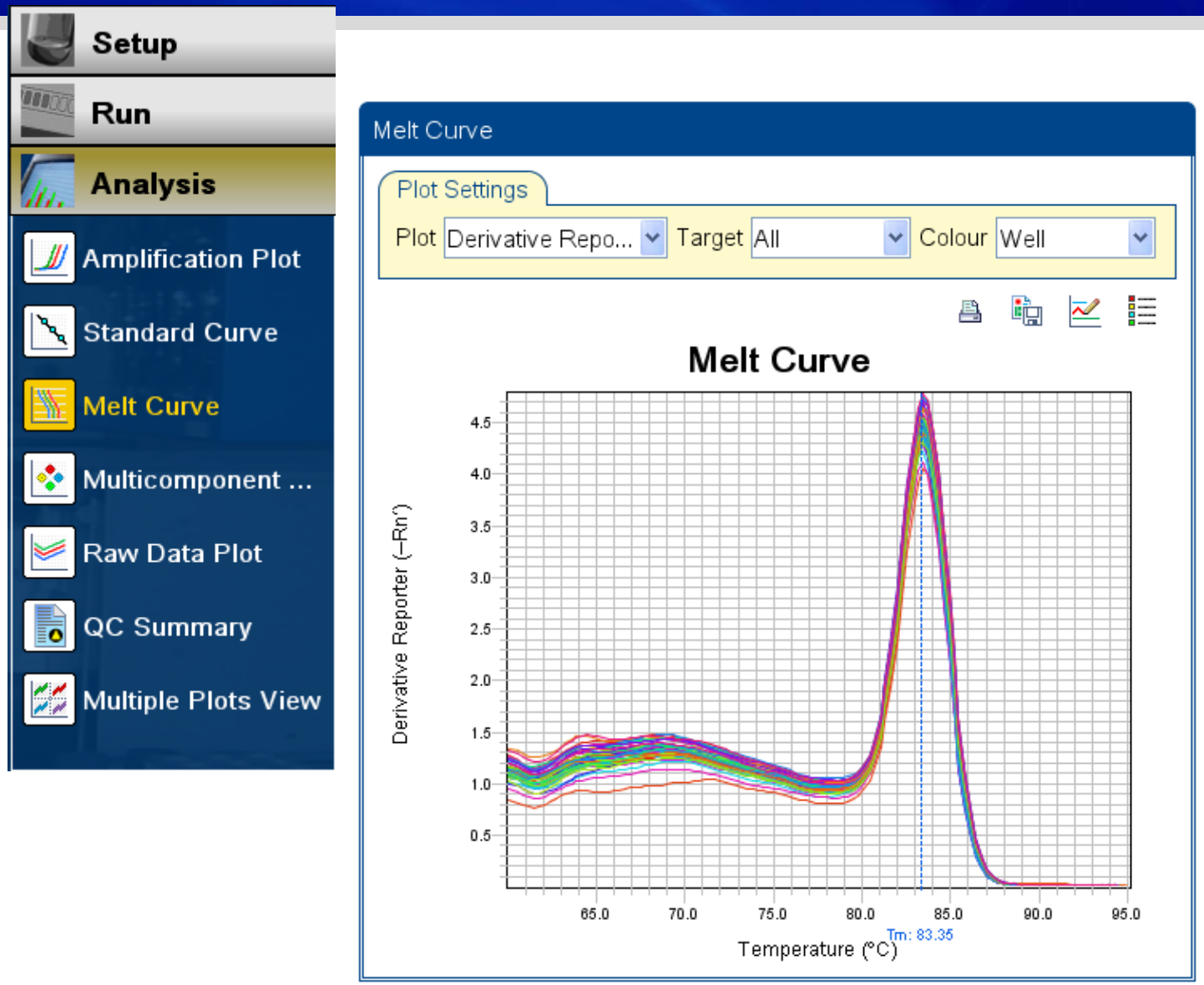
Automatic Standard Curve Setup



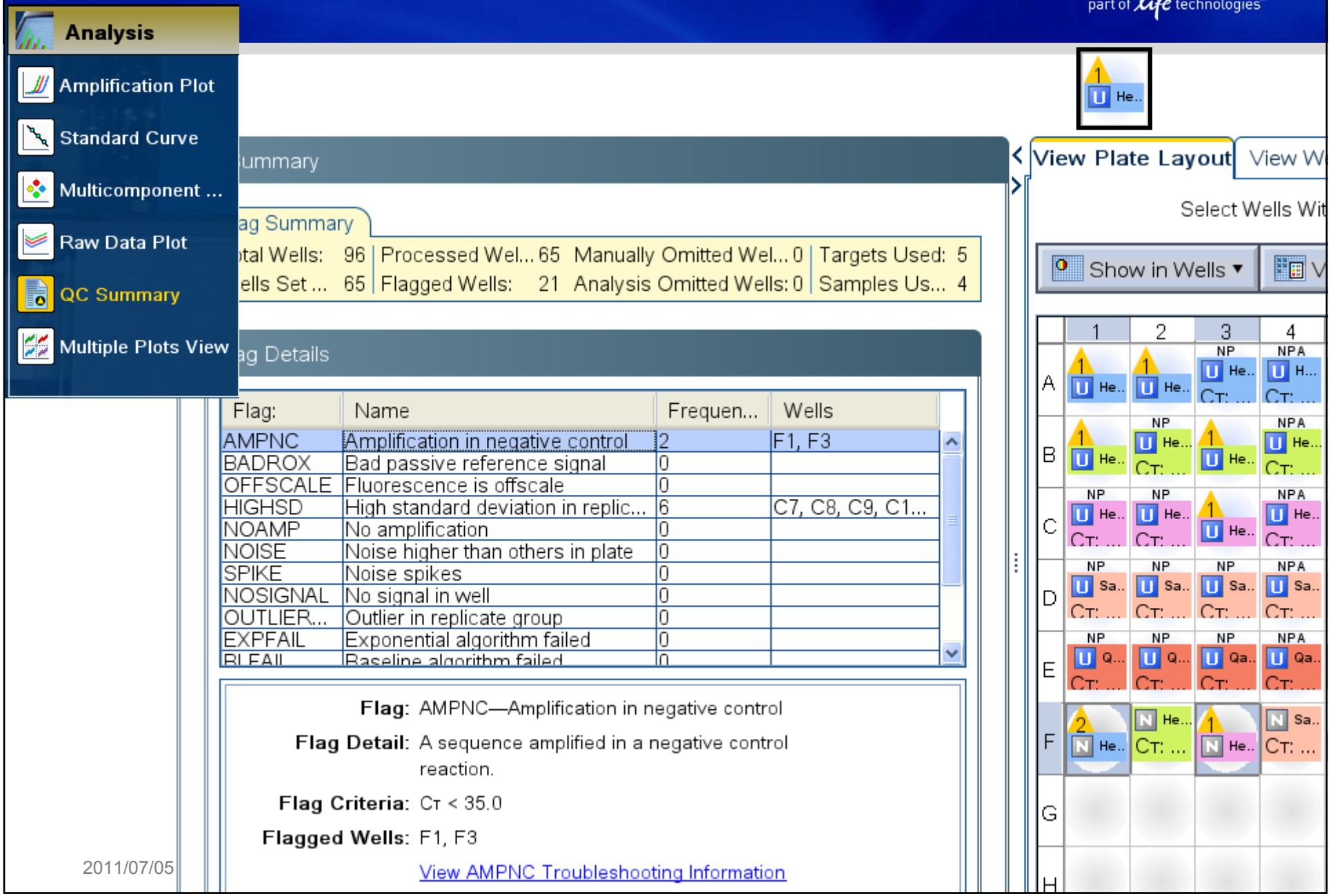
Analysis : Standard Curve



Analysis : Melt Curve (SYBR Green)

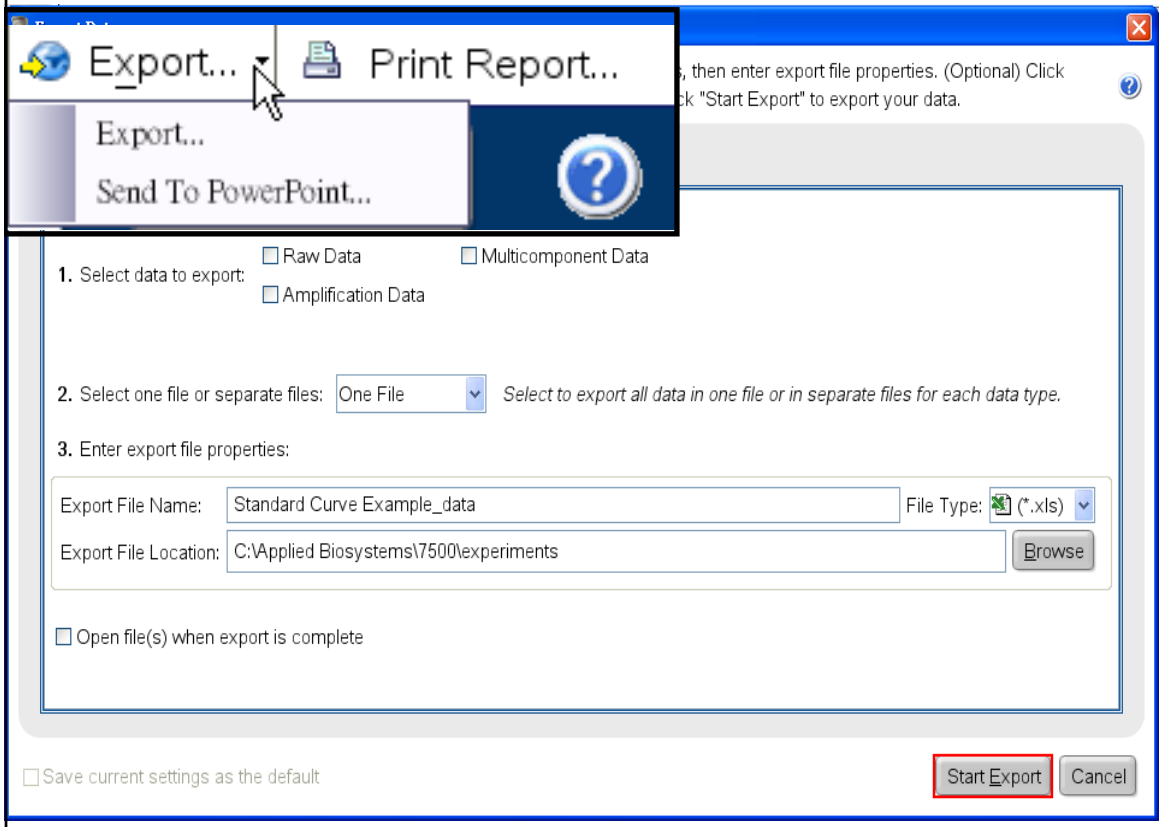


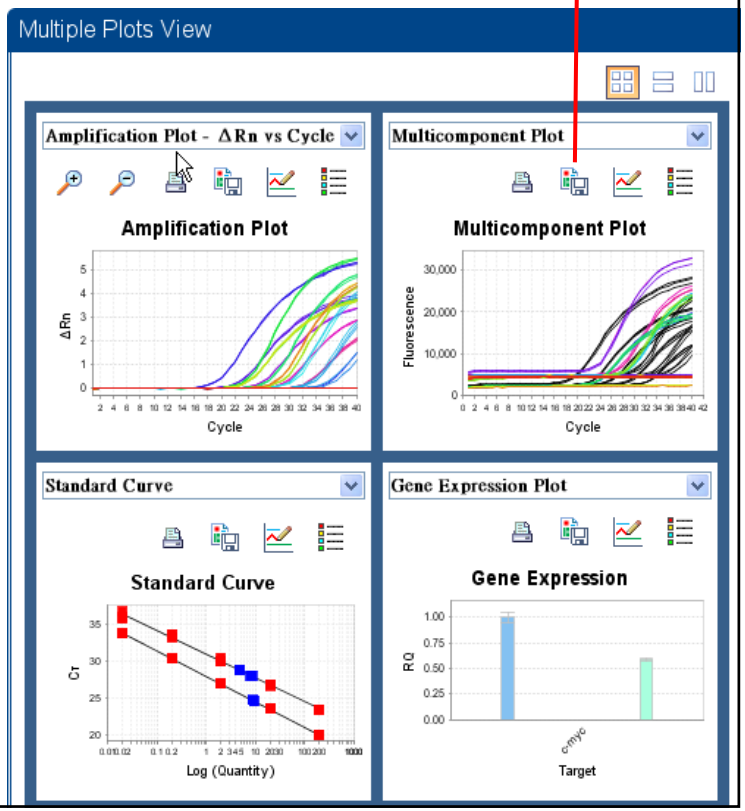
QC Summary Help Your Troubleshooting



數據和圖形簡易輸出! 超easy~

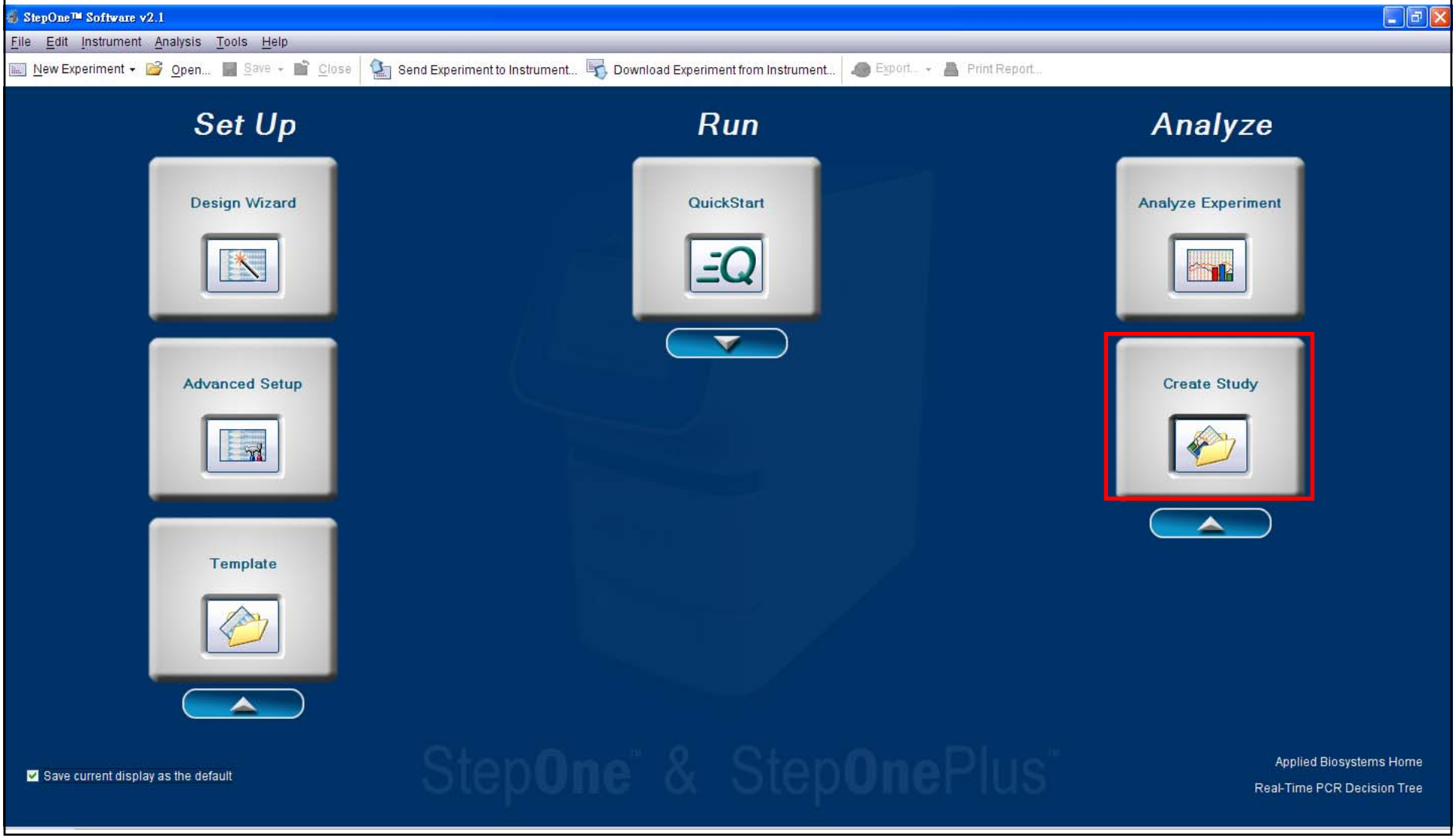
Export to Excel, PowerPoint or save as jpeg



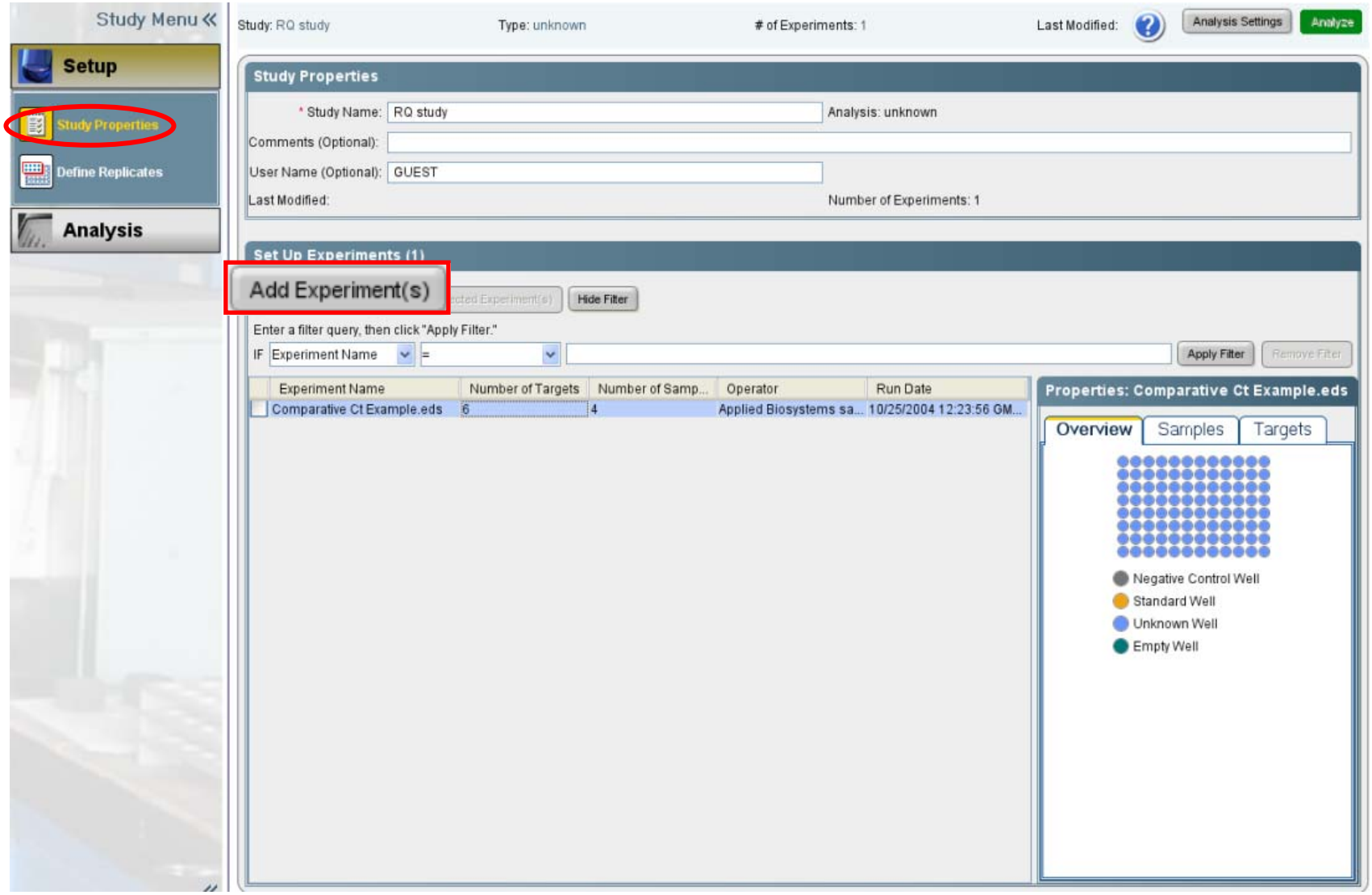


Comparative Ct Study:
1. Create Study

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

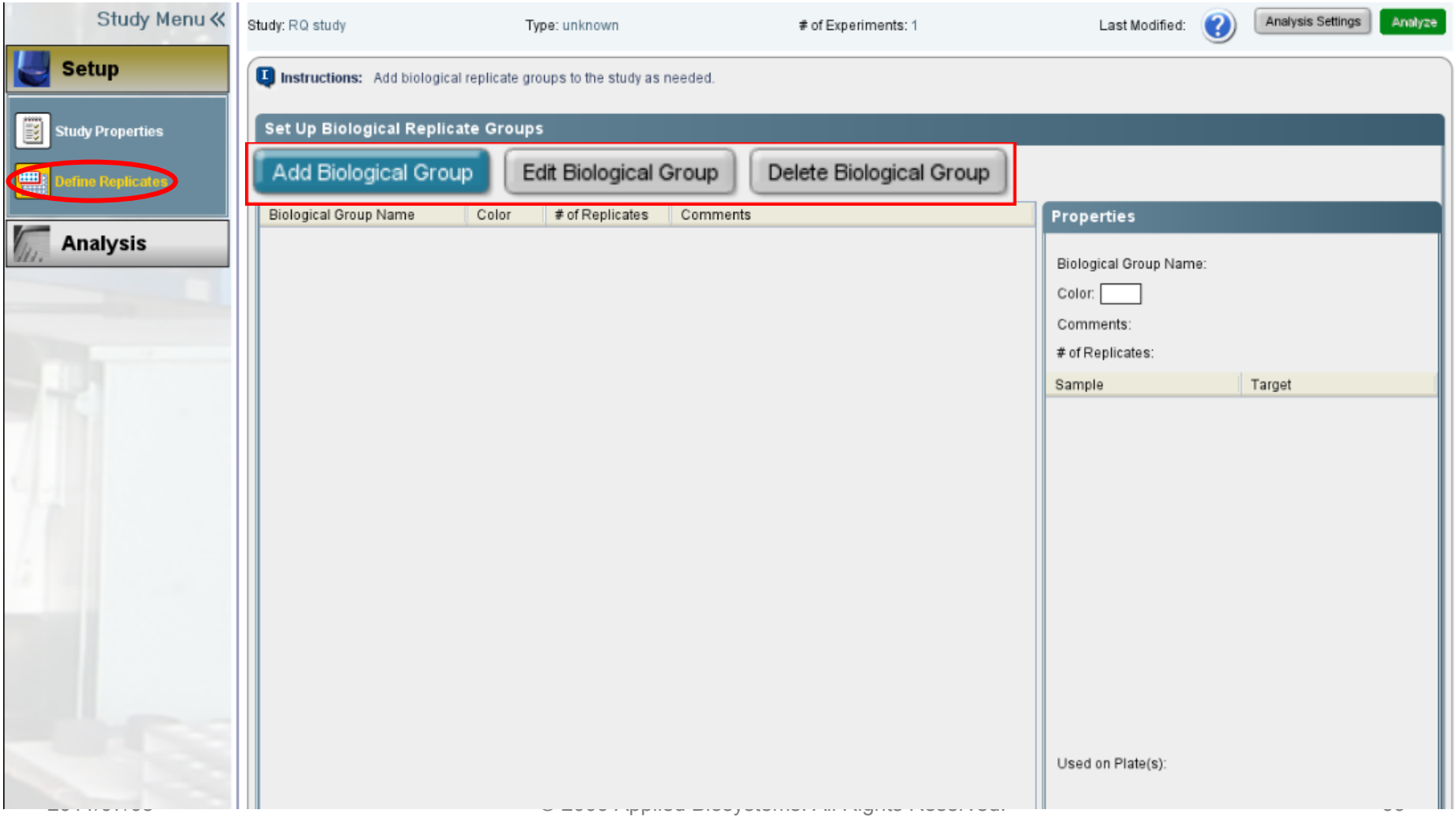


Comparative Ct Study:
2. Add Experiment

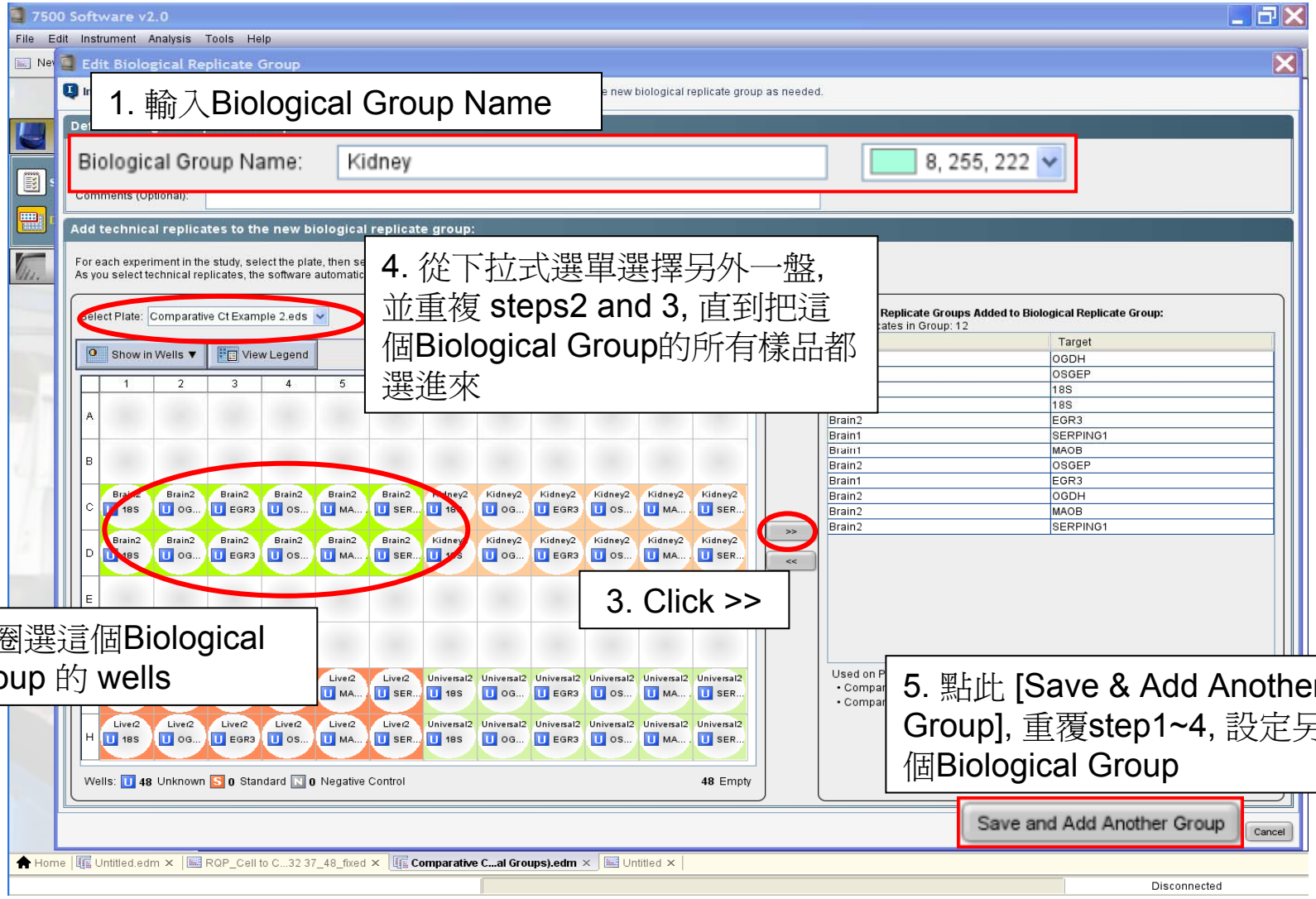


Comparative Ct Study:
3. Add or Edit Biological Group

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

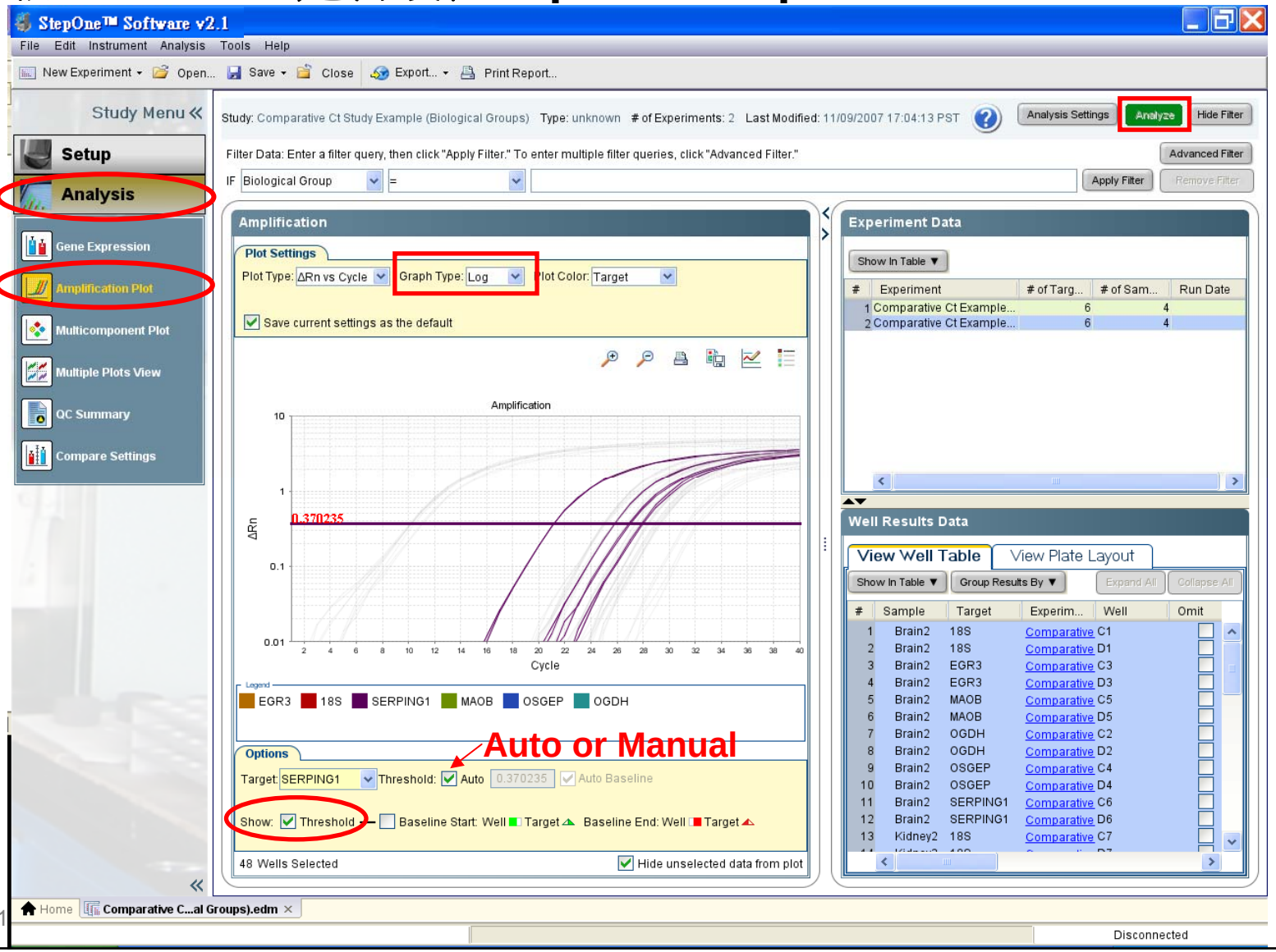


Comparative Ct Study:
3. Add or Edit Biological Group



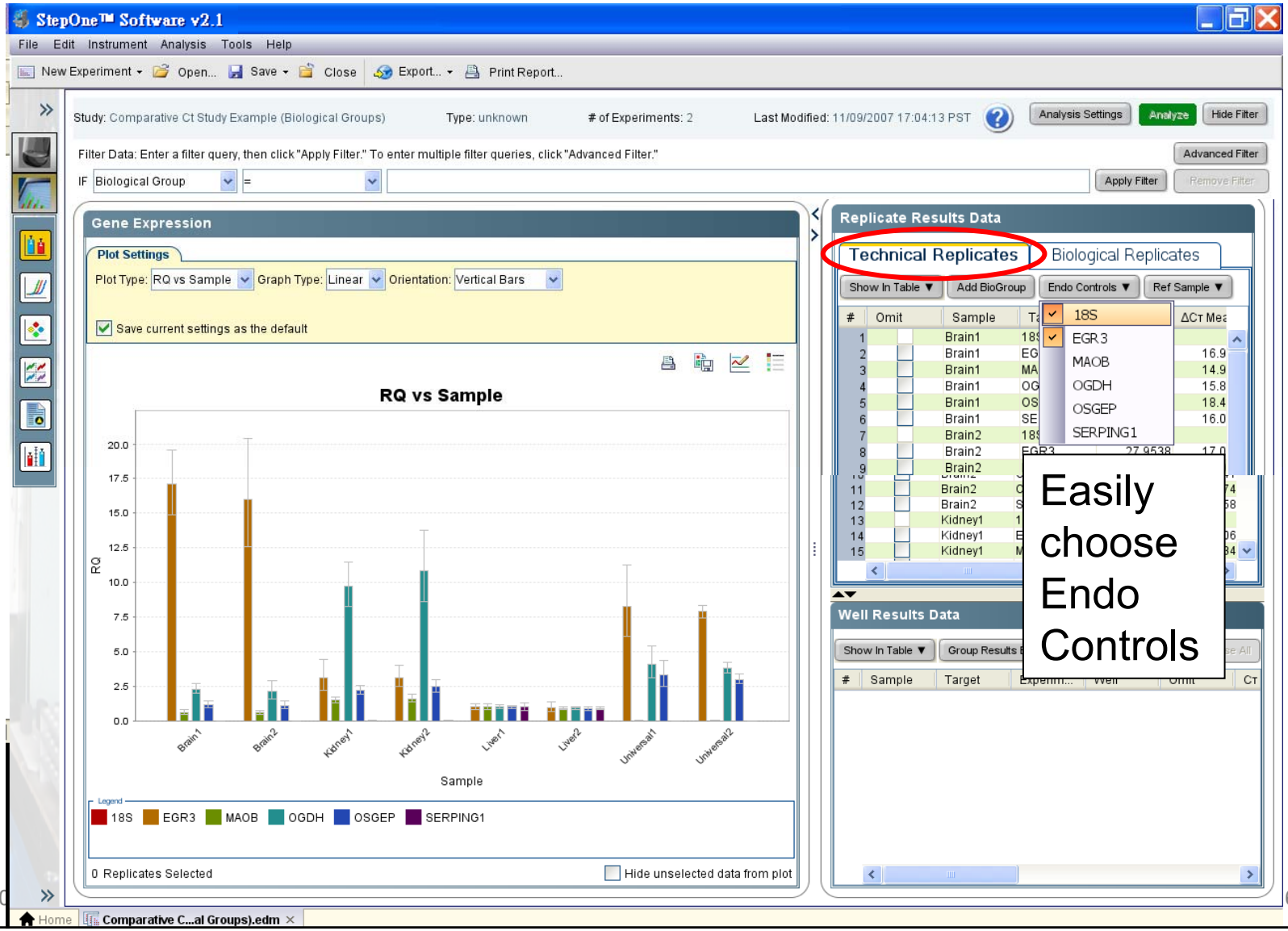
Comparative Ct Study:
4. Analyze & check threshold

確認Threshold是否設在Exponential phase



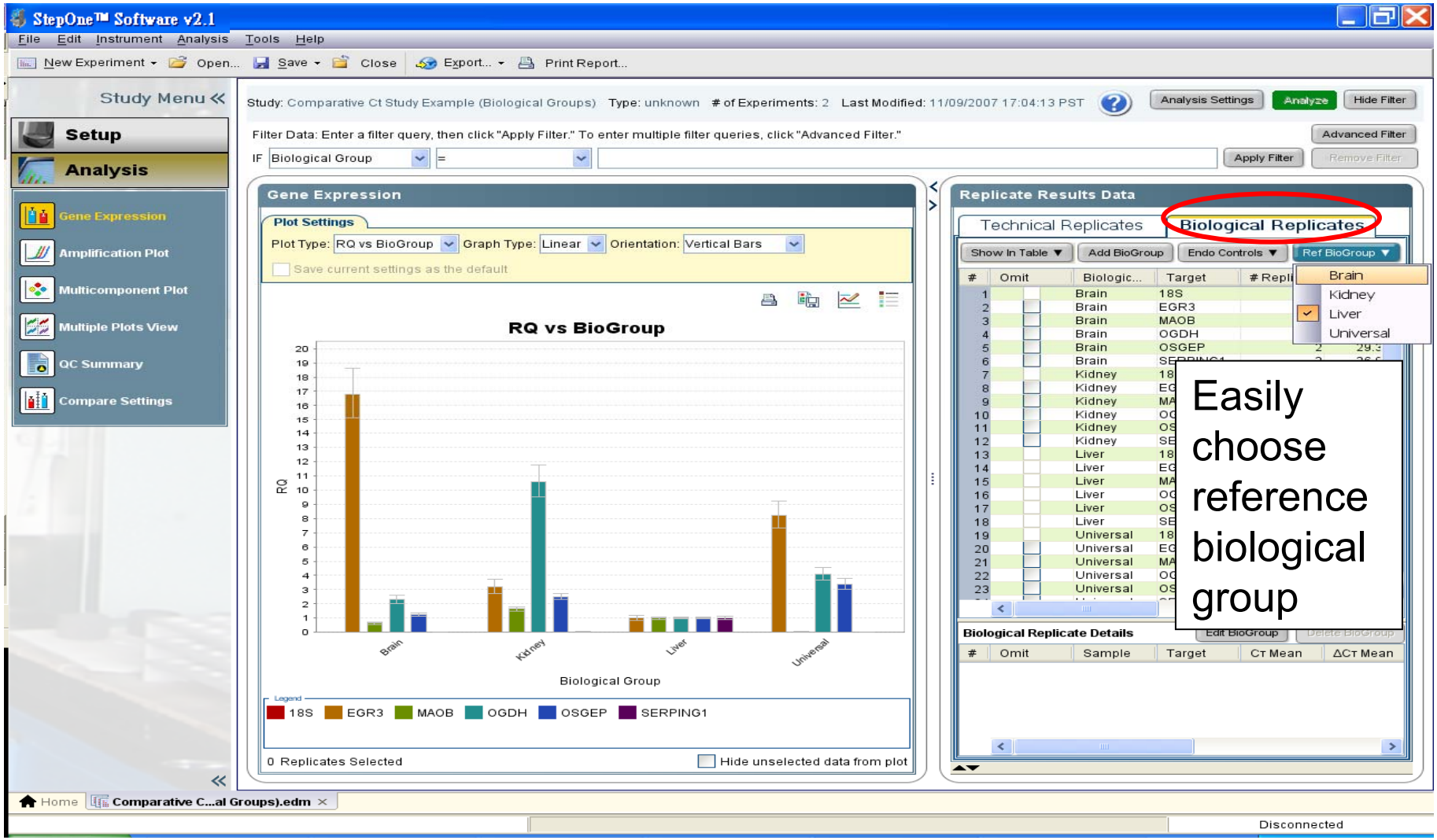
5. View Gene Expression Plot by Technical Replicates

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



6. View Gene Expression Plot by Biological Replicates

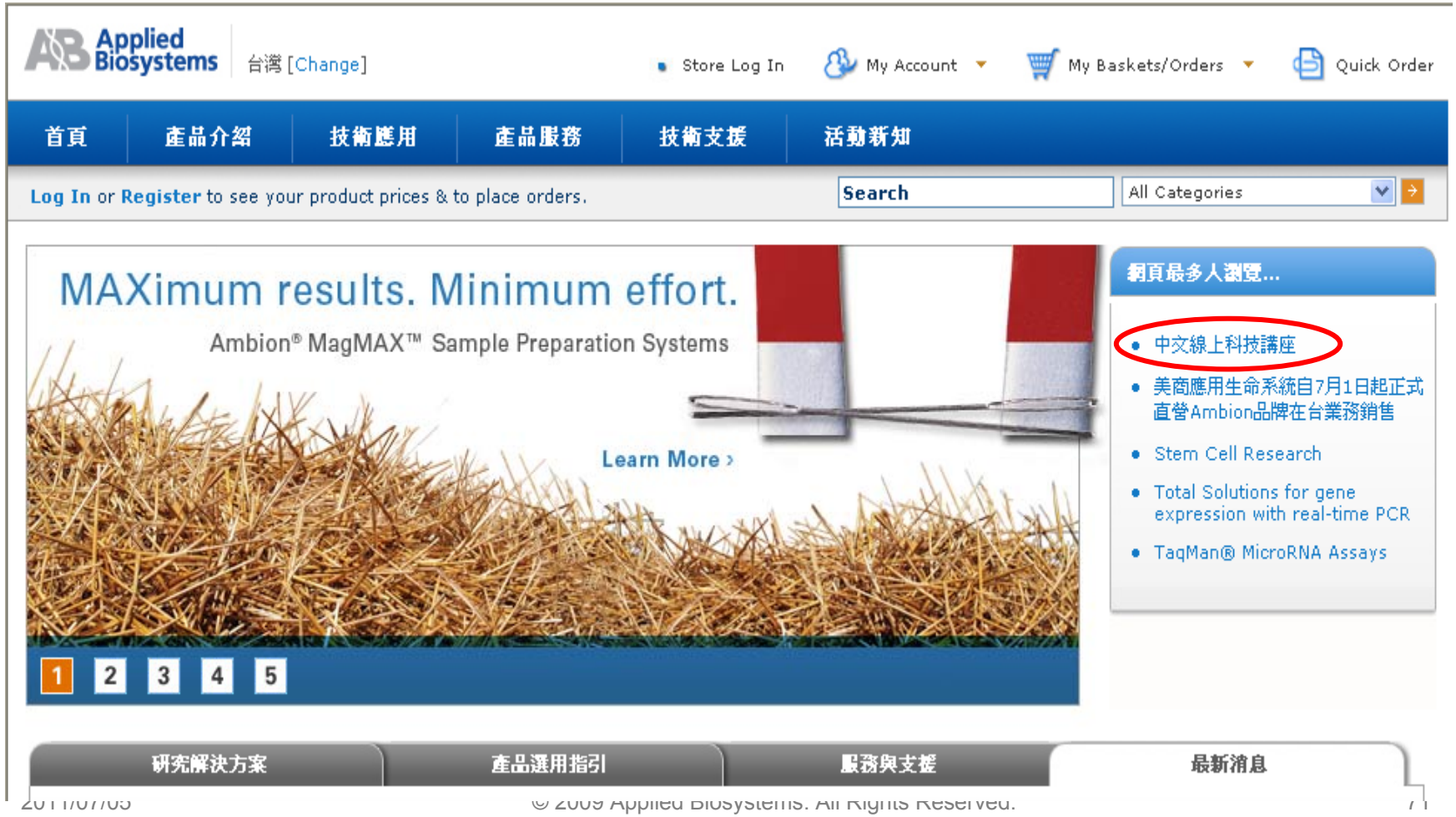
檢視 **biological replicate group** 的 **2^{-ddCt}** 結果



免費中文線上課程-

隨時複習real-time PCR相關知識與軟體操作

Http://www.appliedbiosystems.com.tw



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