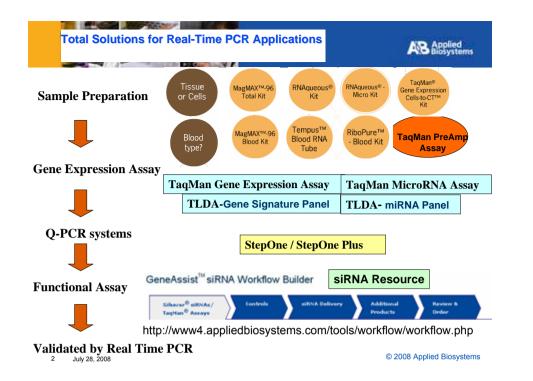


StepOne Plus ™ Real-Time PCR Solution Introduction

劉俐伶 Lily Liu 市場行銷及技術服務專員 Field Application Specialist





AB Applied Biosystem

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
- -10-30µl reaction volume



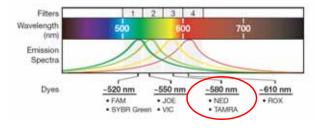


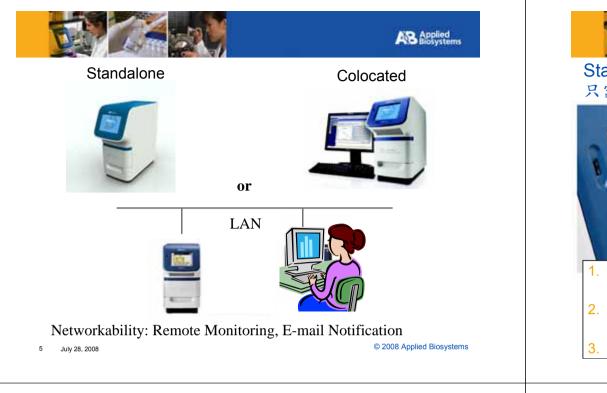


StepOnePlus[™] Real-Time PCR System: The Basics

- 4-color instrument
 - FAM[™]/SYBR[®] Green dyes
 - VIC[®]/JOE[™] dyes
 - ROX™ dye

– NED™/TAMRA™ dye







3. Analyze your data



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Colocated Direct control of instrument via computer



- 1. Setup: You can design the experiment on the PC connected to the StepOneplus System
- 2. Run: Start the run from the PC connected to the StepOneplus System and Real-Time data collection
- 3. Analyze the results on the PC connected to the StepOnePlus System



Computer minimum requirements

- Windows XP sp2
- Intel processor 1 GHz
- 512 MB of RAM
- One 20-GB hard drive
- CD Rom

Minimum Monitor Resolution

1280x1024 pixel resolution



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Hardware specifications



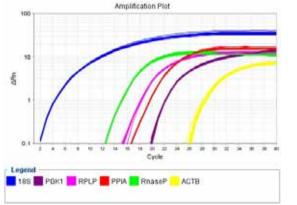
- Power switch in the back. The instrument automatically enters a standby mode after 4h. No need to power off the instrument after a run.
- During the standby mode, the touchscreen becomes gray, but is activated with a simple touch.
- The entire system becomes activated after pressing the blue soft button
- 8-10 min for the heated cover to warm up.

| 9 | July 28, 2008 | |
|---|---------------|--|
| | | |
| | | |
| | | |

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



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

16 replicates for each target

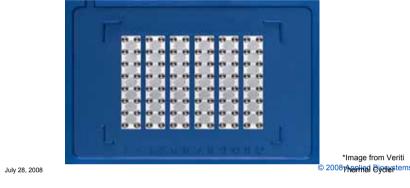


Step**One**Plus[™] Real-Time PCR System: The Basics

Veriflex[™] Block

-One block, Six Zones

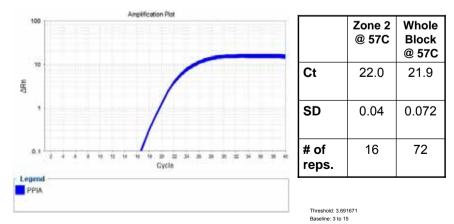
-The same "Better than gradient" feature from Veriti™ 96-well Thermal Cycler



10

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Veriflex[™] Block: Good uniformity across the block or the zone



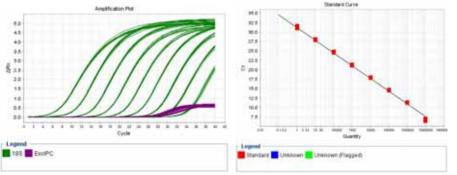
Data courtesy of Bill Kiffmeyer and Birgit Suppe

Data courtesy of Bill Kiffmeyer and Birgit Suppe



AS Applied

Strong Performance: Broad dynamic range, even when multiplexing

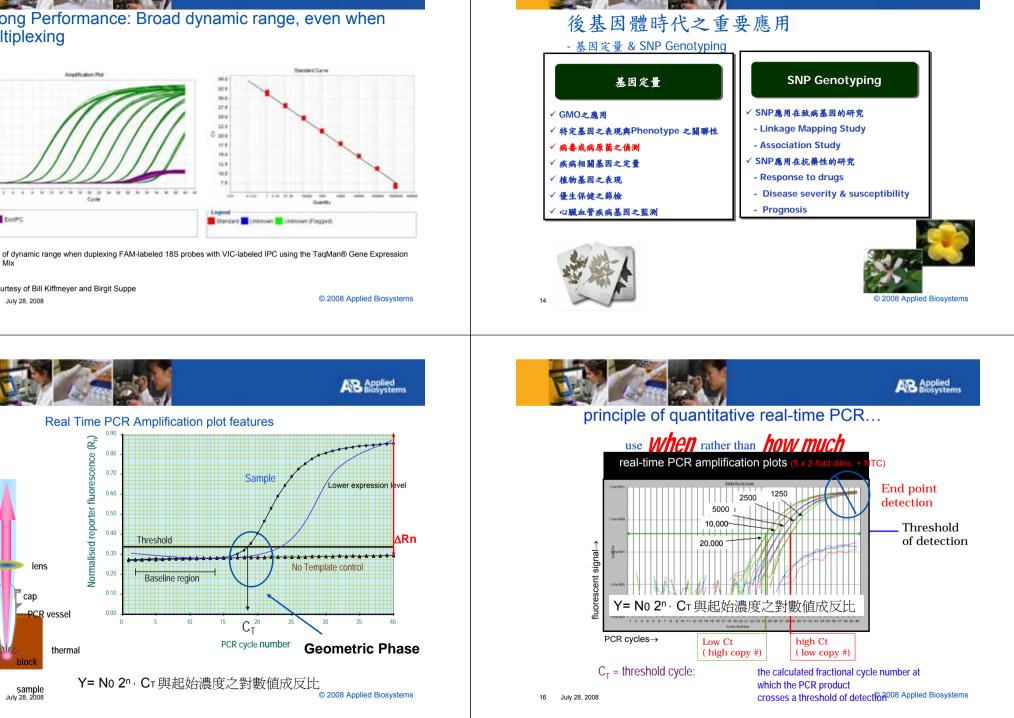


*8 logs of dynamic range when duplexing FAM-labeled 18S probes with VIC-labeled IPC using the TaqMan® Gene Expression Master Mix

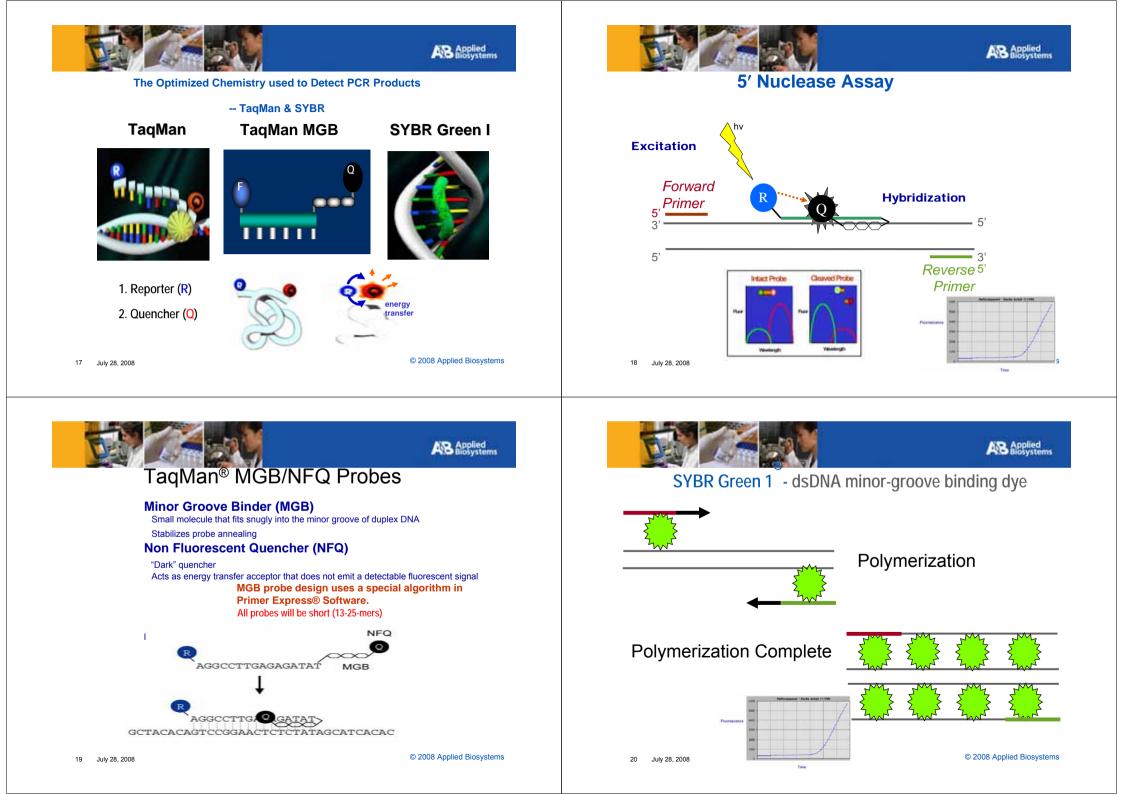
Data courtesy of Bill Kiffmeyer and Birgit Suppe

13 July 28, 2008

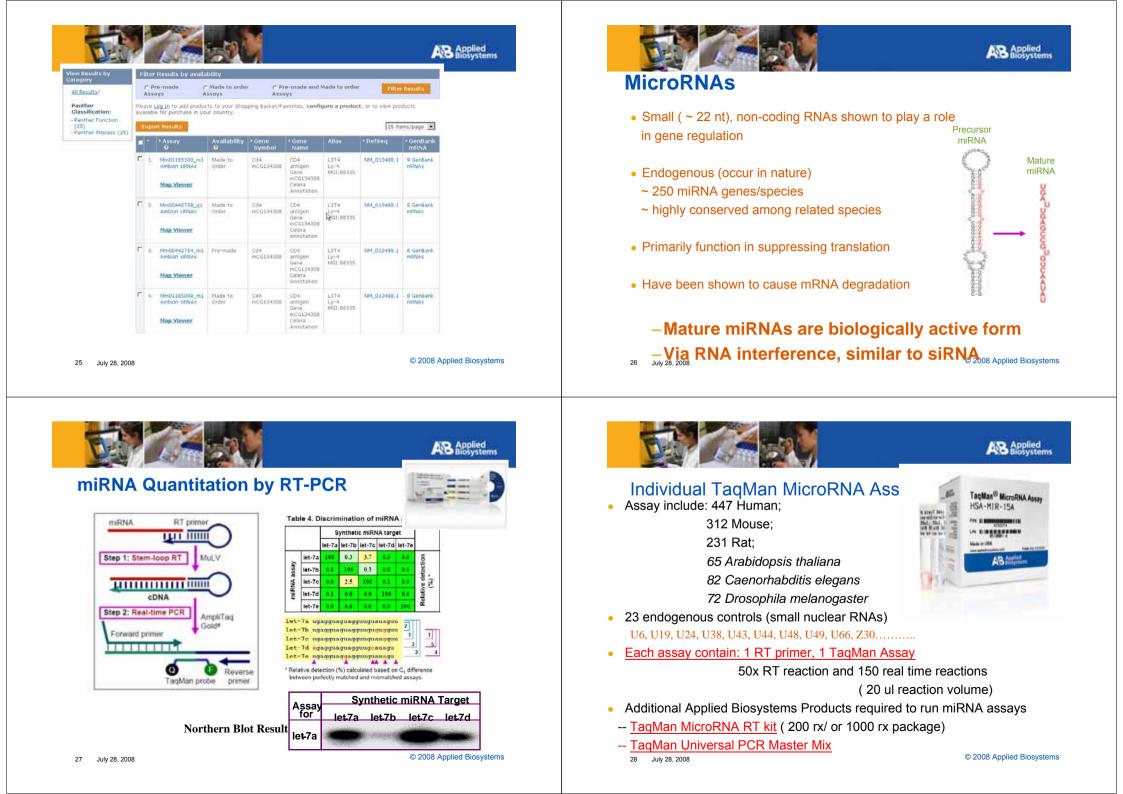
15



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miRNA search website

TagMan® MicroRNA Assays

http://www.appliedbiosystems.com/products/miRNA.html

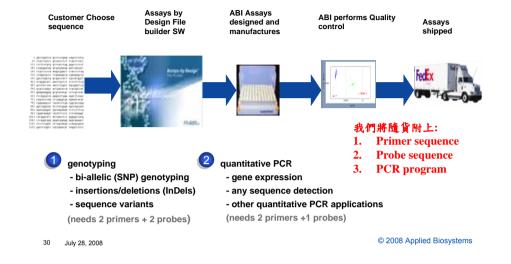
Click a tab below to learn more about TagNan miRNA assays. To find and order an miRNA, dick the miRNA Search tab.

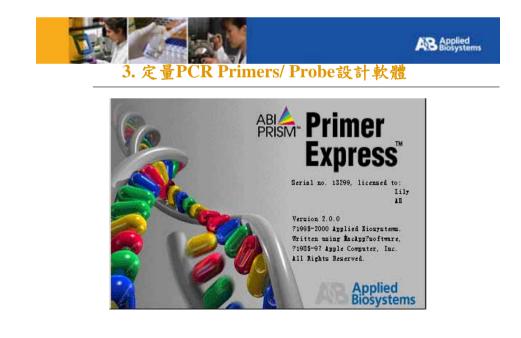
| To begin, select a sear Keyword: Search by s | Annuel Ander Description Literature/Resources ch mathod balow: pedies, array name, target sequence, militare name, or militare accession number. ploading a file containing multiple MicroRIA IDs, Accession numbers, Part Humbers, or MicroRIA | 🛞 Search Help |
|---|---|--------------------------|
| Keyword Search | in All Text | |
| E Advanced Keyvord | Search | |
| Control Rat Drosophila Human | Authaliana Moure C. eleganz | |
| 29 July 28, 200 | 80 | © 2008 Applied Biosystem |



2: Custom TagMan Assays Service

The Assays-by-Design Process







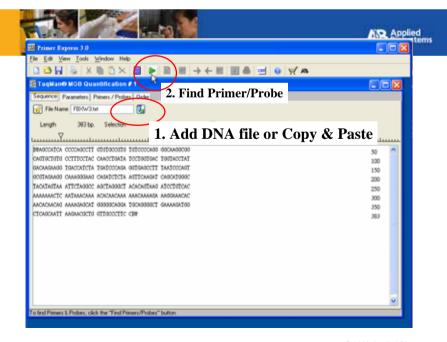
清楚明確的 TaqMan Probe & Primer 設計規範

| • | | |
|--|--|---|
| 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 値: 68-70℃ (Quantification assay) | Tm 值: 58-60℃ | |
| 65-67℃ (Allelic Discrimination assay) | | |
| Probe 長度: | Primer 長度: | |
| 13~25 bases (<u>TaqMan</u> MGB probe) | 20 bases (Optimal) | |
| 13~30 bases (TaqMan probe) | | |
| 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 | 200 bp amplicon | 500 bp amplicon |
| (GGG-MGB-3' or GGAG-MGB-3')* | 2.402 | |
| 避免 probe 的中間區域含有 2 個或以上的 CC di-nucleotides * | 1005 | |
| a: 針對 TagMan MGB probe | 1.622 | |
| b: 参数可選擇設定 | 1003 0369 0729 0.372 0.372 | - 1111 |
| July 28, 2008 | 0.005 0.301 1 3 5 7 9 11 15 15 17 19 21 25 25 27 29 81 85 87 29 41 40 1 3 5 | 7 9 11 12 15 17 19 21 28 25 27 29 11 12 18 18 17 19 |

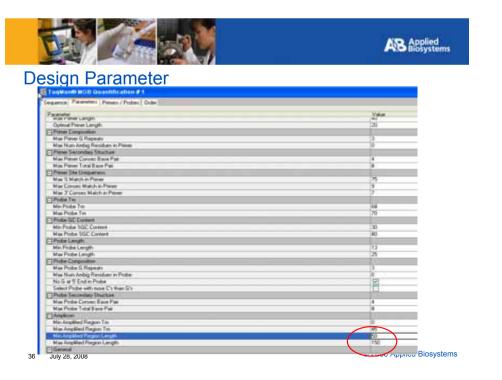


33 July 28, 2008

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| | | AB Biosystems |
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| Trimer Express 3.0 File Edit View Look V | | |
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| 122 | 22 | 54 | 50 | 167 | X X X | 59 | 54 | 145 | 15 | 10 | 60 | 79 | 10 40 | 40 58 59 | 66 |
| 51 95 | 21 | 59 | 44 | 119 | 11 | 750 750 | 44 | 28 UH | 0 | 10 | 53 | 10 | 0 | 50 | 0 |
| 95 | 3 | 56 | 44 | 101 | | 30 | 60 | 123 | 16 | 6.8 | 6) | 10 | 43 | 10 | 67 |
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| 95 | 2 | 14 | 2 | 161 | 12 | 20 | 50 | 123 | 16 16 | 00 00 | 0 | 10 | 4 | 50 50 50 | 0 |
| 121 | 22 | 60 | 50 | 167 | 28 | 399 | 38 | 344 | 16 | 60 | 56 | 75 | 40 | 58 | 17 |
| 40 | 1 | 80 | 61 | 160 | 38 | 100 | 414 | 145 | 11 | 10 | 43 | - (ii) | 60 | 60 60 | 44 |
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Reagents

- Optical Consumables
- RNA Isolation Kit
- RT kit

- AB Applied
- Real Time PCR Reagent



Step**One**Plus[™] Real-Time PCR System: The Basics

• Supported consumables:

- P/N 4346907

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

- P/N 4360954

Optical Adhesive Film - 25 films

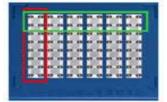
- P/N 4358293

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

-P/N 4323032

Optical 8-Cap Strip - 300 strips

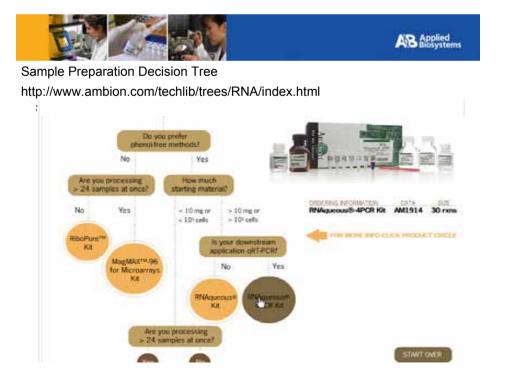


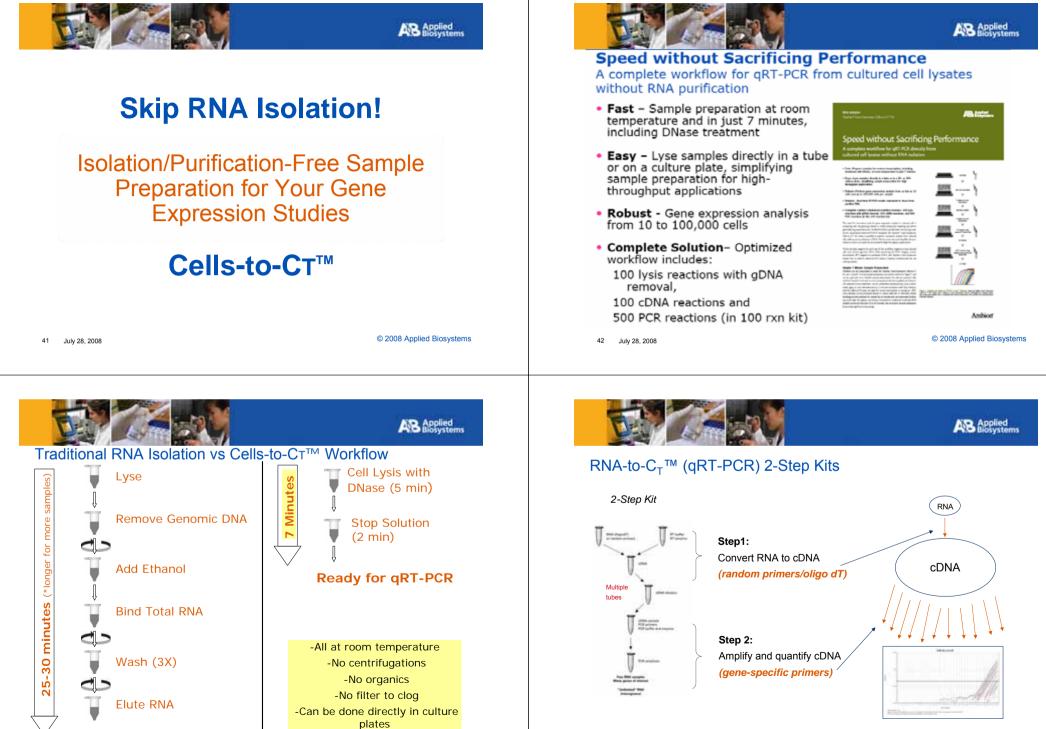


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THE REAL PROPERTY.

Place the tray containing the tube, Load at least 16 tube ^{© 2008 Applied Biosystems}





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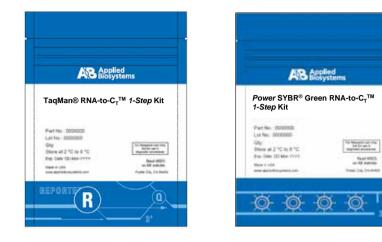
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New: RNA-to-C_T[™] (qRT-PCR) 1-Step Kits



49 July 28, 2008



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Crucial points for Success Total Solutions Optimization and Variety of qPCR Master Mixes http://www.ambion.com/techlib/trees/qpcr/





MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode (0.1 mL) - 4346906

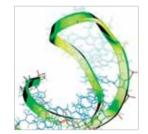
MicroAmp™ Optical Adhesive Film - 4360954

MicroAmp™ 96-Well Support Base - 4379590

MicroAmp™ Adhesive Film Applicator - 4333183

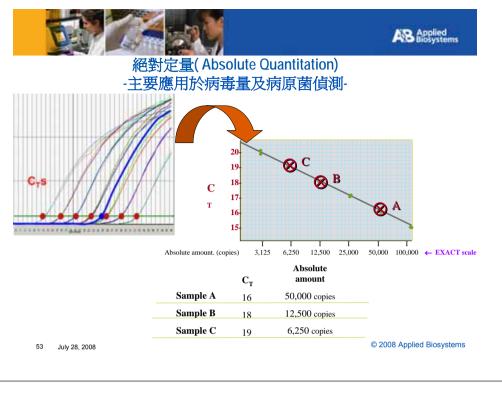


實驗設計 絕對定量 & 相對定量









Comparative Ct Method

t=24

cDNA

 \checkmark

total RNA

C-myc GAPDH

Comparison of the c-myc expression level

in T=0, T=12, T=24, T=48 time course study

t=48

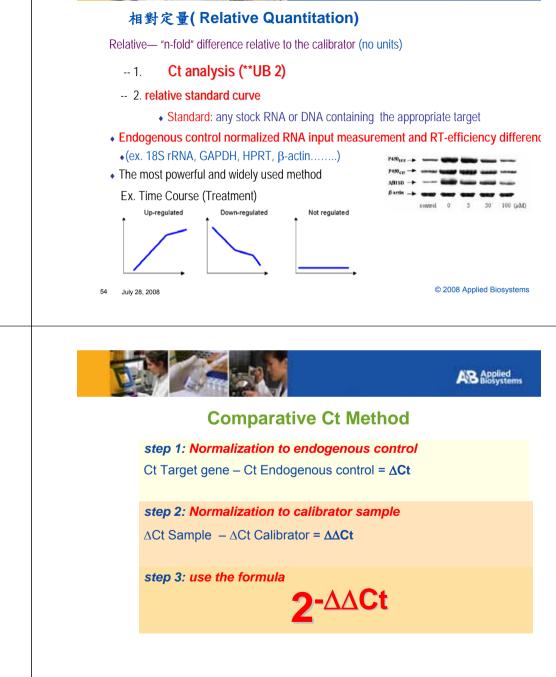
total RNA

cDNA

C-myc GAPDH

 \checkmark

time



t=12

total RNA

cDNA

 \checkmark

C-myc GAPDH

AB Applied

Spectrophotometer measure RNA quantity

Reverse Transcription; Ex. 5 ug RNA/ 50 uL =100 ng/uL

Real Time PCR Unknown samples(50 ng): T=0, T=12, T=24, T=48 AB Applied

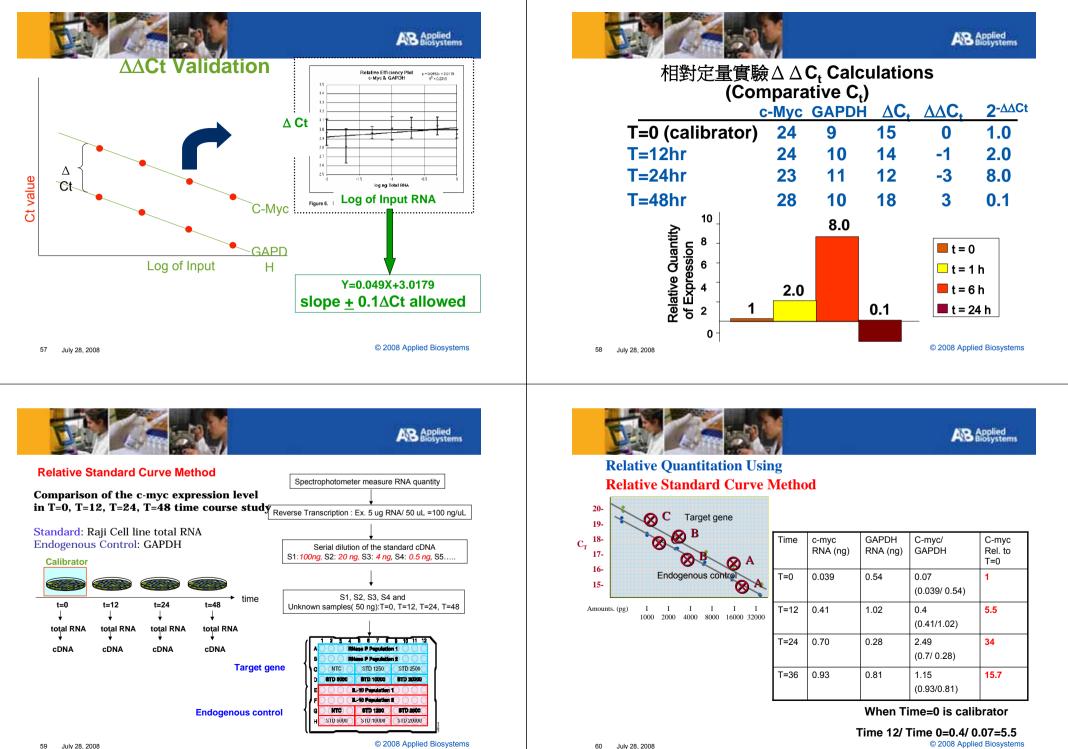
Calibrator

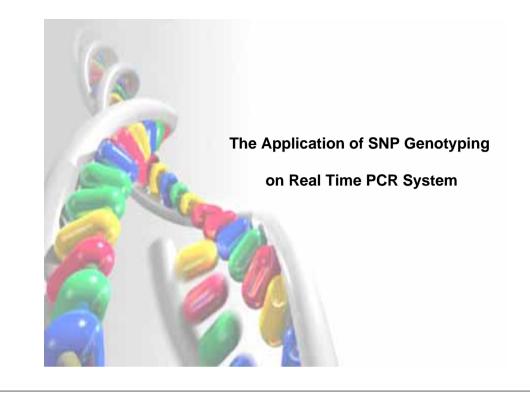
t=0

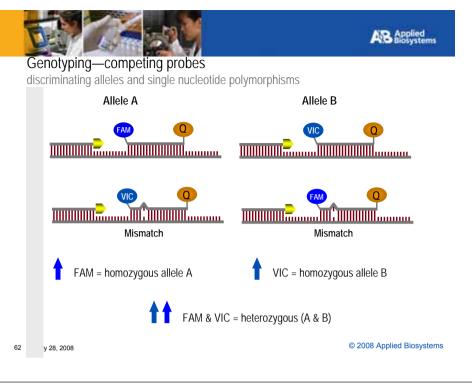
cDNA

C-mvc GAPDH

total RNA

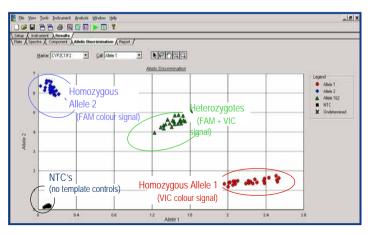






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allelic discrimination (SNP) data





Software Operation





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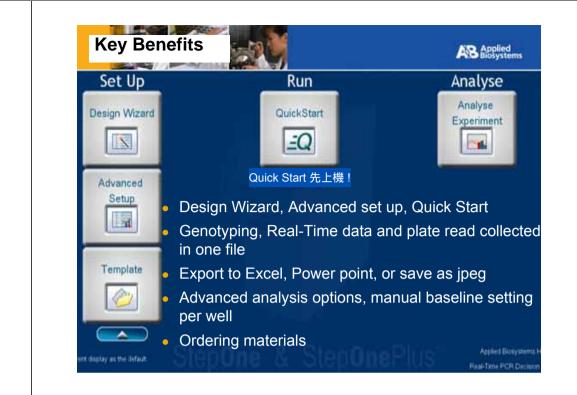
All in One Features 麻雀雖小五臟俱全!! 一套軟體可以符合全方位的應用

New

65

July 28, 2008

- Quantitation-Standard curve
- Quantitation-Comparative CT
- Quantitation-Relative standard curve
- Melt curve
- SNP Genotyping
- Presence/absence

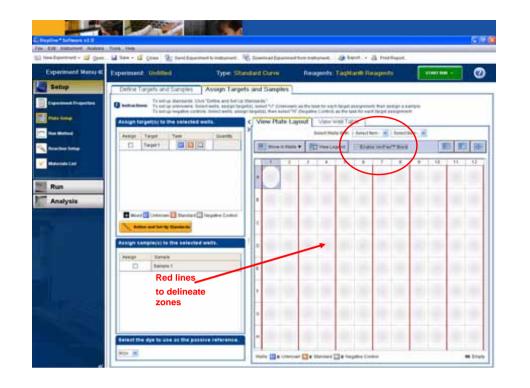


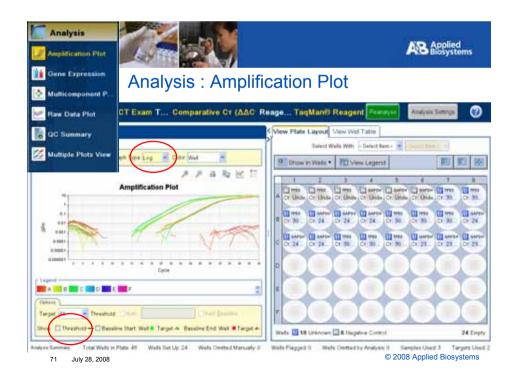
| kperiment wenu % | 下用擔心設定錯誤造成分析上的困 | 新編輯實驗型態,樣品位 擾 |
|--------------------------------------|--|---|
| Seperiment Properties | | |
| ✓ StepOnePlus™ Instrument (96 | Webs) ShipOne ^{na} essenament (43 Webs) | |
| Set up, run, and analyze an experime | ent using a 4-color, 96-well system. | |
| What type of experiment do you | want to set up? | |
| Quantitation - Standard Cu | Ouerblain - Retrive Stante & Cove | Quantitation - Comparative Cr (AAC) |
| Mett Curve . | Genitypina | Presencer&baance |
| Use standards to determine the aboo | lute quantity of target nucleic acid sequence in samples. | |
| Which reagents do you want to i | use to detect the target sequence? | |
| ✓ TaqMan® Reagents | SYER® Grein Progents | Other |
| PL DATE | designed to amplify the target sequence and a TagMan® probe of | festioned to detect amplification of the target |

| Automatic plate layout in the Design wizard | AB AB |
|---|-------|

 Automatic Standard Curve Setup Define Targets and Samples Assign Targets and Samples To set up standards: Instructions: To set up unknowns: bion to have the bet of targets in the reaction state. Define the standard time, belief wells for the standards, that the "Apple," Repeat for each standard curve in the reaction plate, then cikils "Close" to return to plate setup To set up negative co sign target(s) to the selected wells Colort a target for the standards 8.5 Task Assign Target L 11.5 Address 1 S Reimminieded #urRepkater. 1 3 Partamended Mixed 🛄 Unknown 🔛 Sta Lanting Quantity: 195.0 Erms the highest in lawest standard quarmy for the standard curve Define and Set Up Standard • Renal Factor, 1.5 Select a value from 1:10 to 10+ 5. Points X.3. Replicates = ... Required Wells issign sample(s) to the selected well it and attacks w Assign Sample he Welts: (I) Automatically Select Welts for Me. O Let Me Select Welts Sample 1 15 Repared Wells / 15 Selected Wells BLCI,C2C3C4,C5,O5,C7,C8,D3,D2,D3,D4,D5,D4 elect the dye to use as the passive. ROX 🖻 Vranje standards ov O Columns (B Rokes

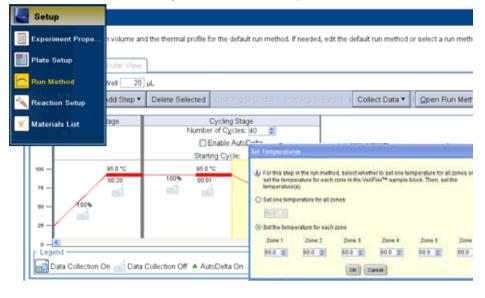
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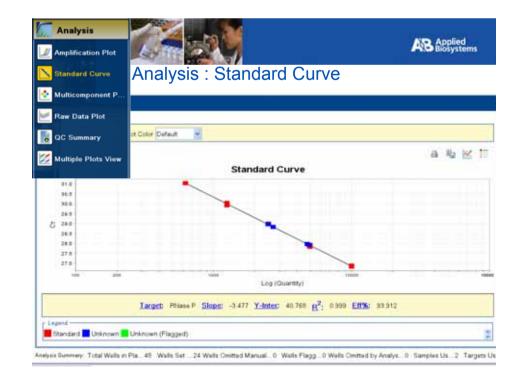






Enable VeriFlex [™] Block, you could edit the temperature for each zone







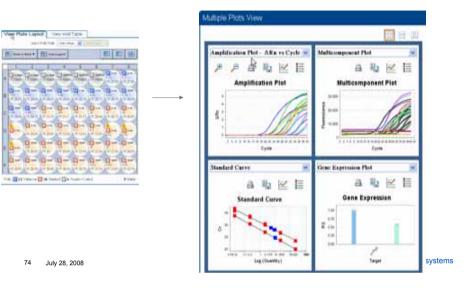
AR Applied

Analysis Report

| | | | | | | | | | | | | | _ |
|-----|----------|---------|-------|-----------|----------|---------------|-----------|---|-------|-----------|-----------|---------|----|
| Sh | ow in 1 | Table * | Group | By • | | | | | | Bim | 117 | 6 | |
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| 1 | A1 | | | Rhèse F | NTC | FAM-NEG-MGB | Undefer | | | | | | |
| 2 | A2 | 0 | | Phiase P | NTC | FAM-NFG-MG8 | Undeter | | | | | | |
| 2 | :A3 | | | Phase P. | NTC | FAM-NFQ-MGB | Undeter. | | | | | | |
| 4 | A4 A5 | | pop1 | Phiane P. | UNROWNE | FAM-NFQ-MOB | 28.96287 | 28.923790 | 0.074 | | 2,551.470 | 126.2 | |
| 5 | A5 | | pop1 | PINADA P. | UNKNOWN | FAM-NEG-MGB | | 28.929796 | 0.074 | 2,697.054 | 2,851.476 | 126.2 | |
| 6 | AB A7 | | pop1 | RName P | UNKNOWN | FAM-NEQ-MGB | 28.96972 | 28.923796 | 0.074 | 2,473.064 | 2,551.476 | 126.2 | |
| 7 | | | pop2 | | UNROVOWN | FAM-NEO-MOB | 27.576233 | | 0.024 | 4,774.927 | 4,830.585 | 70.119 | |
| - 8 | AB | | pop2 | | UNKNOWN | FAM-I/FQ-MG8 | | 27.958857 | 0.024 | 4,799.501 | 4,830.585 | 76,115 | |
| . 9 | 81 | | pop2 | | UNKNOWN | FAM-NFQ-MG8 | | 27.958857 | 0.024 | 4,917.327 | 4,830.585 | 76.118 | |
| 10 | 82 | | | Phase P. | STANDARD | FAM-NEG-MOB | 26.874498 | and the second se | 0.022 | 10,000 | | | |
| 11 | 83 | | | HIGHLE P. | STANDARD | FAM-NEG-MOB | 26.834158 | | 0.022 | 10,000 | | | |
| 12 | B4 | | | Phiase P. | STANDARD | FAM-HEQ-MGB | 20.007296 | | 0.022 | 10,000 | | | |
| 13 | 85 | | | Filese P | STANDARD | FAM-NED-MOB | | 27.894386 | 0.045 | 5,000 | | | |
| 14 | 00 | | | FITANN F. | STANDARD | FAM-NEQ-MG8 | | 27.894386 | 0.045 | 5,000 | | | |
| 15 | 87 | | | FITVASA F | STANDARD | FAM-NEG-MGB | | 27.034306 | 0.045 | 5,000 | | | |
| 16 | BB | | | FINate P | STANDARD | FAM-NFO-MGB | | 28.581377 | 0.021 | 2,500 | | | |
| 11 | C1 | 0 | | PNase P | STANDARD | FAM-NEQ-MG8 | | 28.381377 | 0.021 | 2,500 | | | |
| 18 | 0.2 | 12 | | Fitese F | STANDARD | FAM-NEQ-MGB | | 28.981377 | 0.021 | 2,500 | | | |
| 19 | C3 | | | Filiane P | STANDARD | FAM-NEQ-MOB | | 29.985449 | 0.059 | 1,250 | | | |
| 20 | C4 | | | RNase P | STANDARD | FAM-NEG-MGB | 29.9701 | 29.985449 | 0.059 | 1,200 | | | |
| 21 | 105 | _0 | | RNana F | STANDARD | FAMILIED MIGH | 30.050293 | 29.985449 | 0.059 | 1.260 | | | |



Result: Export to Excel, PowerPoint, or save as jpeg

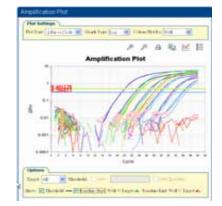






Absolute & Relative Standard Curve

Show the different gene standard curve in the same time





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74



Accelerate the Pace of Discovery, Enable Sciences for Life

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技術服務E-mail: liull@appliedbiosystems.com 訂貨及維修服務專線: 0800-251-326/ 02-23582838-8313

業務經理 吳文凱先生 ext: 8205