



ThermoFisher
S C I E N T I F I C

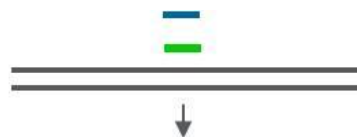
Applied Biosystems QuantStudio™ 3 Real-Time PCR System之原理與應用介紹

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

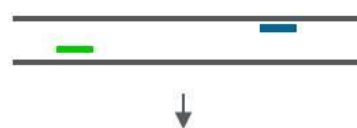
The world leader in serving science

Polymerase Chain Reaction (PCR)

Primers and DNA



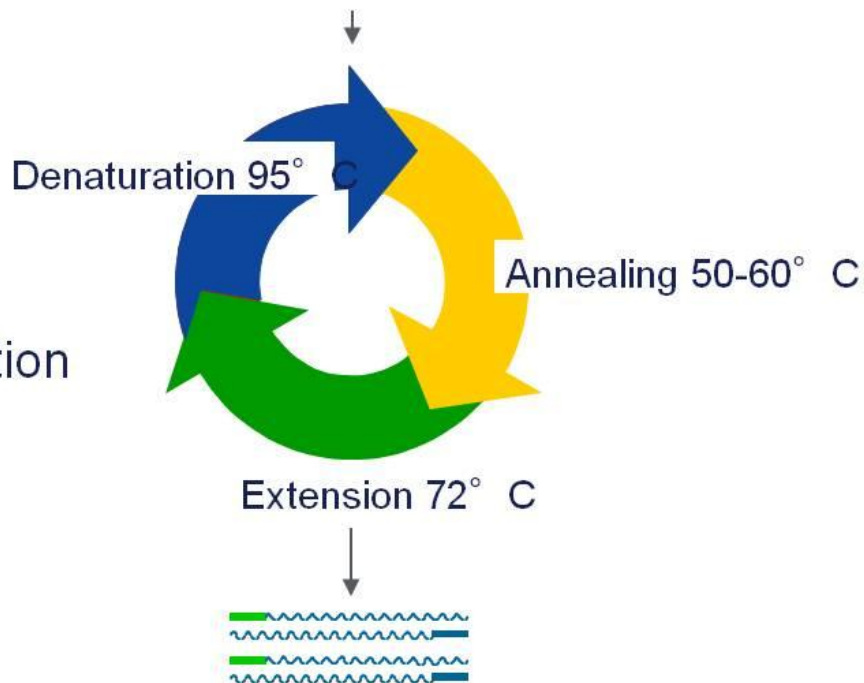
Strand denaturation and primer annealing



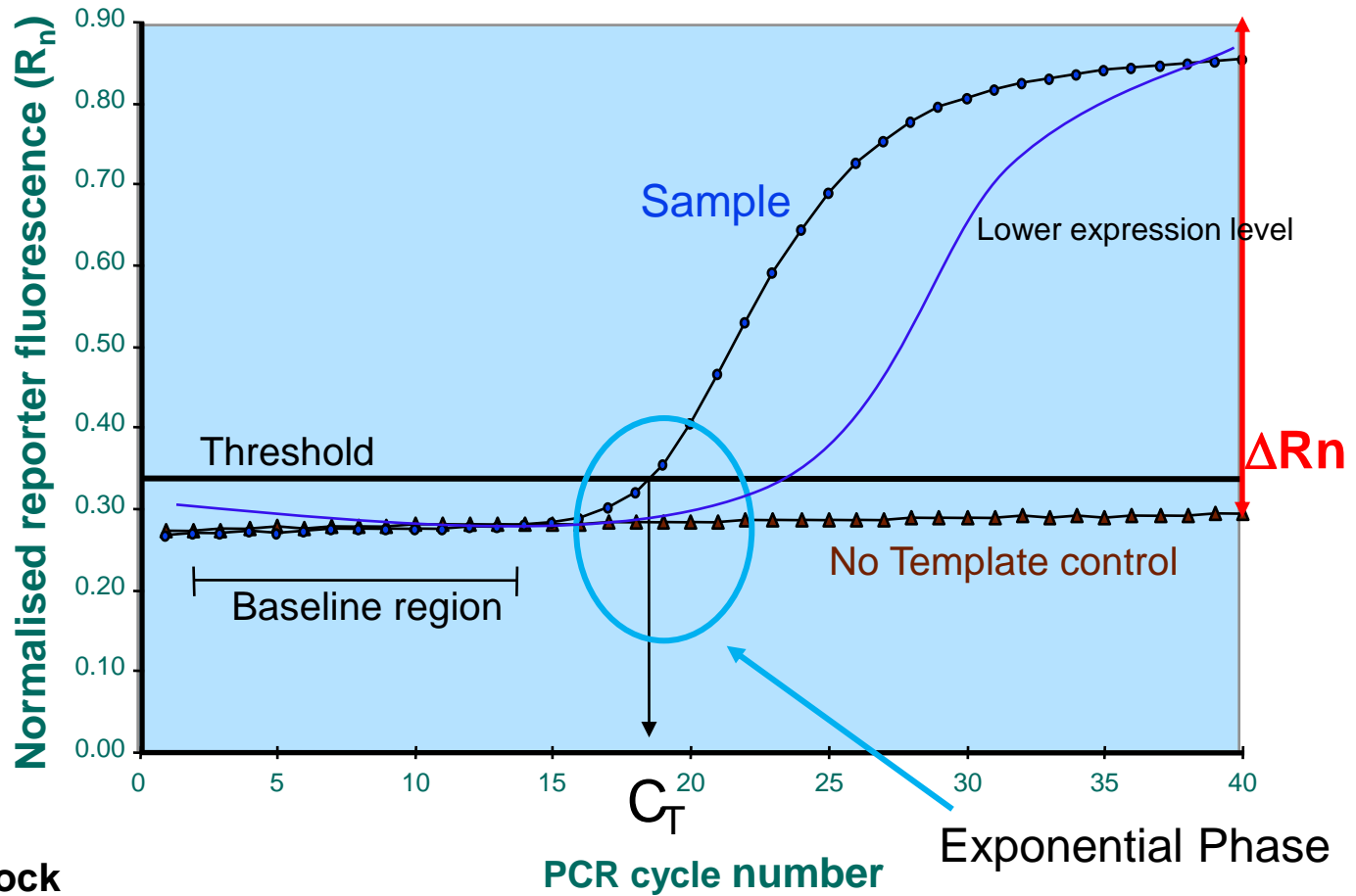
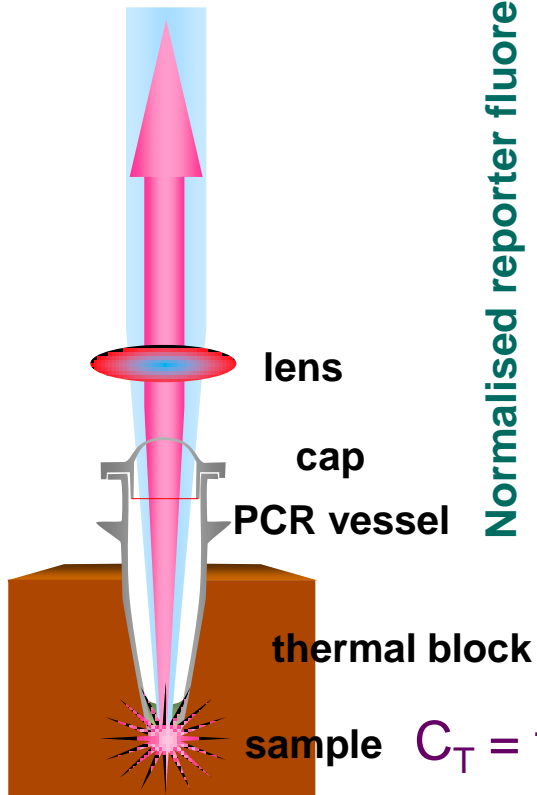
Primer extension



Cycling
Exponential amplification
of PCR products

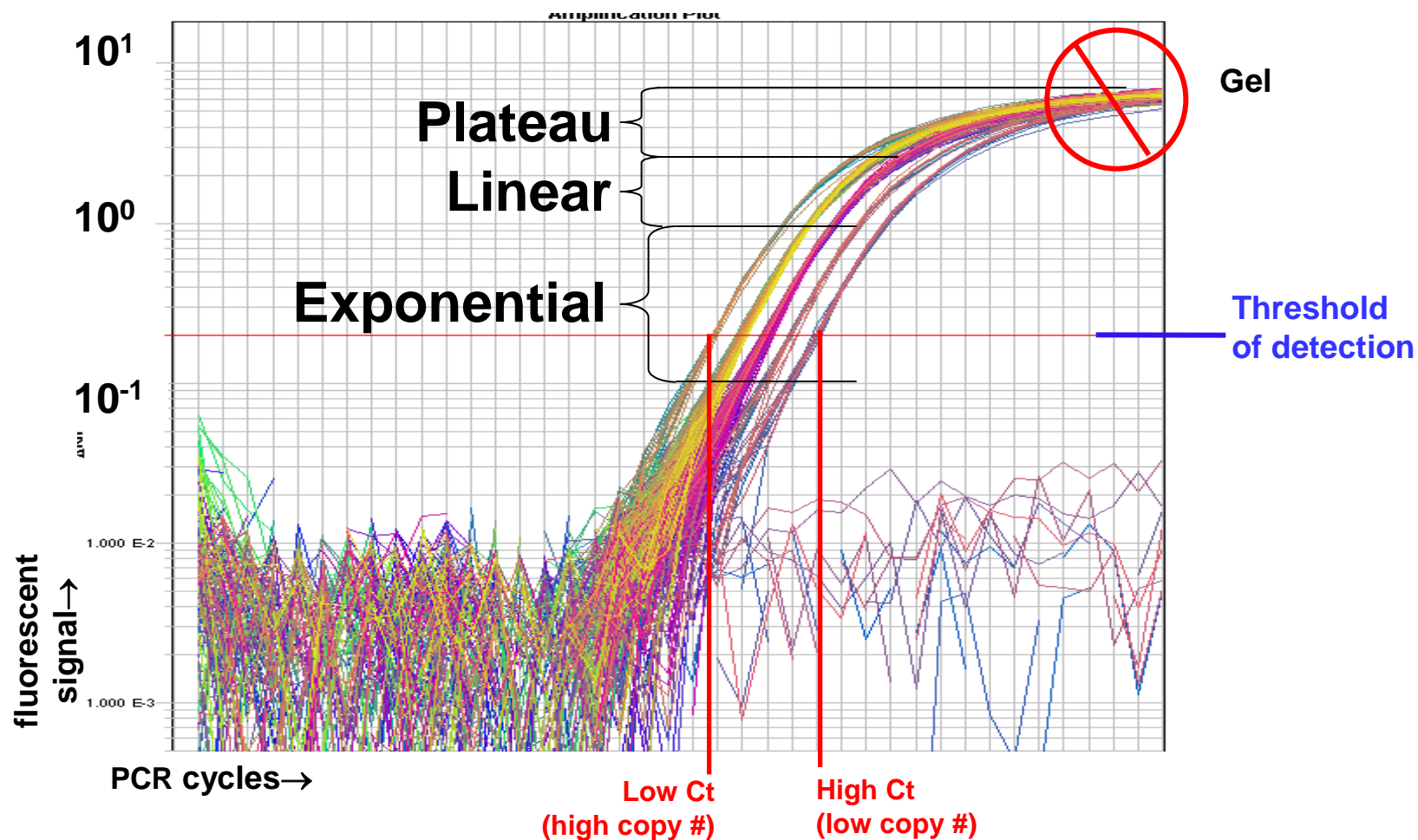


Principle of Real-time PCR



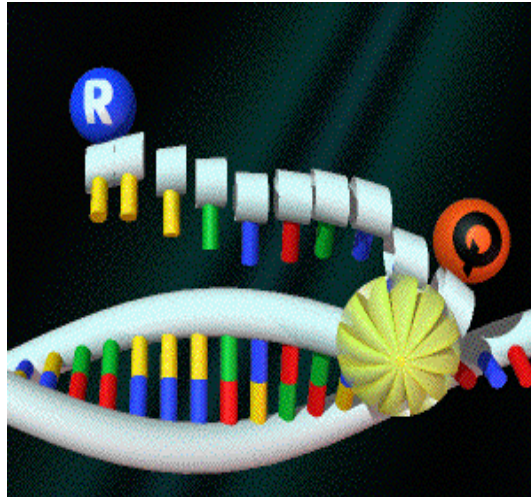
C_T = threshold cycle: calculated fractional cycle number at which PCR amplification curve crosses the threshold of detection

Real-time PCR Signal Detection: Exponential Phase



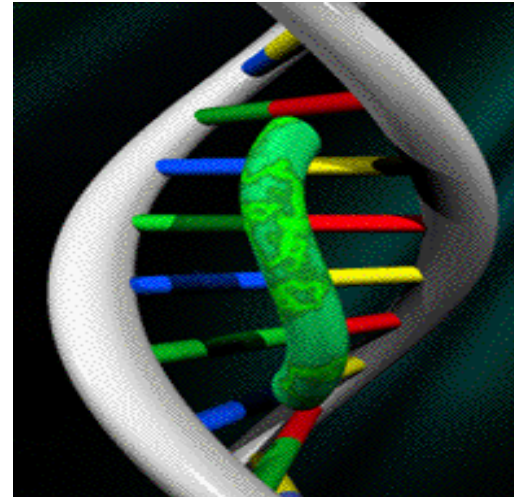
$Y = N_0 2^n$, C_T 與起始濃度之對數值成反比

TaqMan[®] and TaqMan[®] MGB



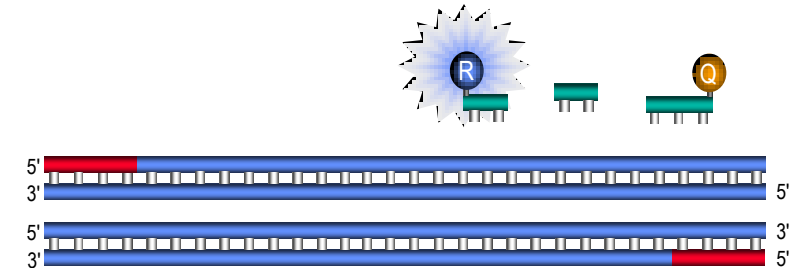
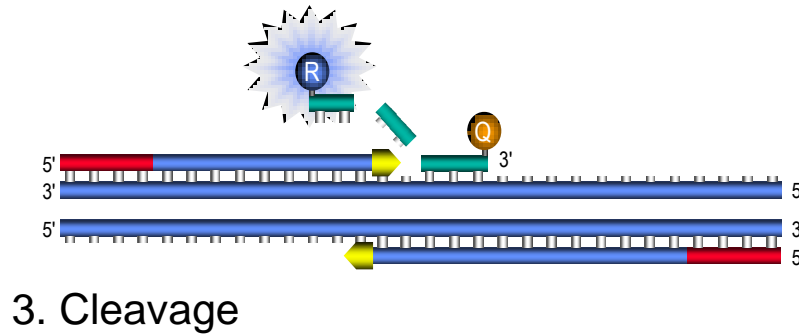
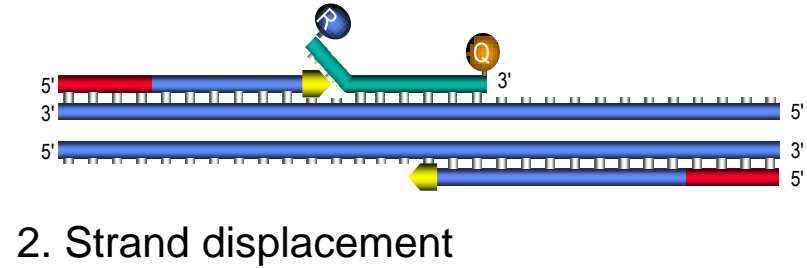
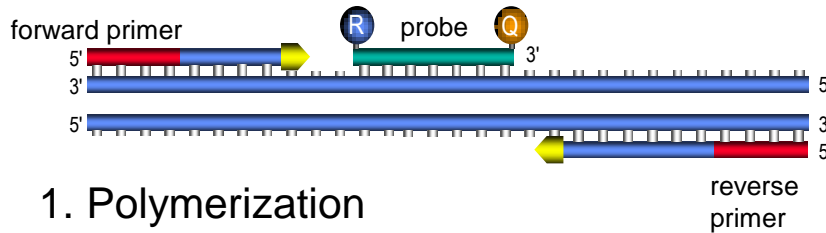
Fluorogenic 5' Nuclease Assay

SYBR[®] Green I dye

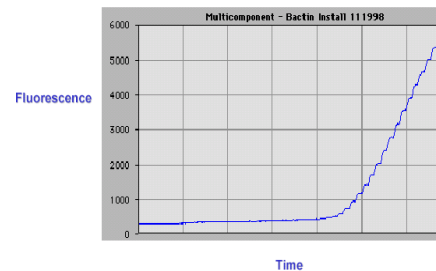


Binds Double-stranded DNA

TaqMan[®] Assay: Fluorogenic 5'-nuclease Assay



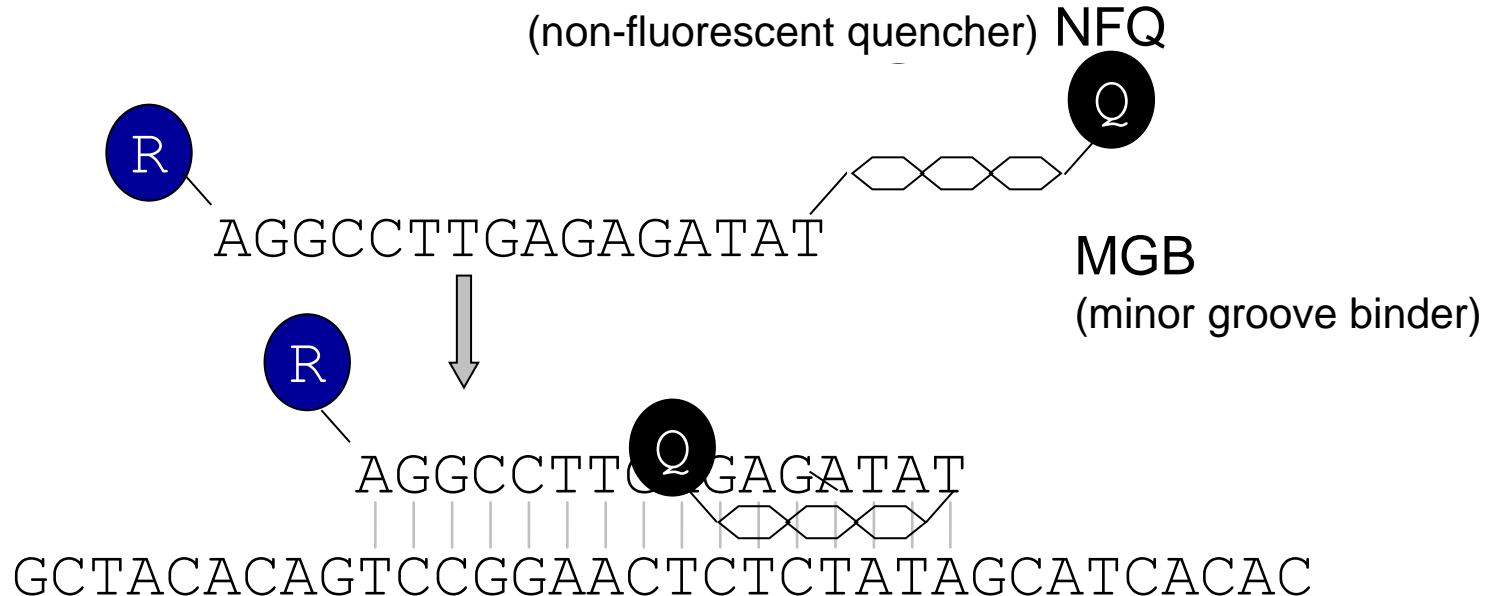
R = Reporter (FAM, VIC, etc.)
Q = Quencher (NFQ/MGB, etc.)



4. Polymerization completed

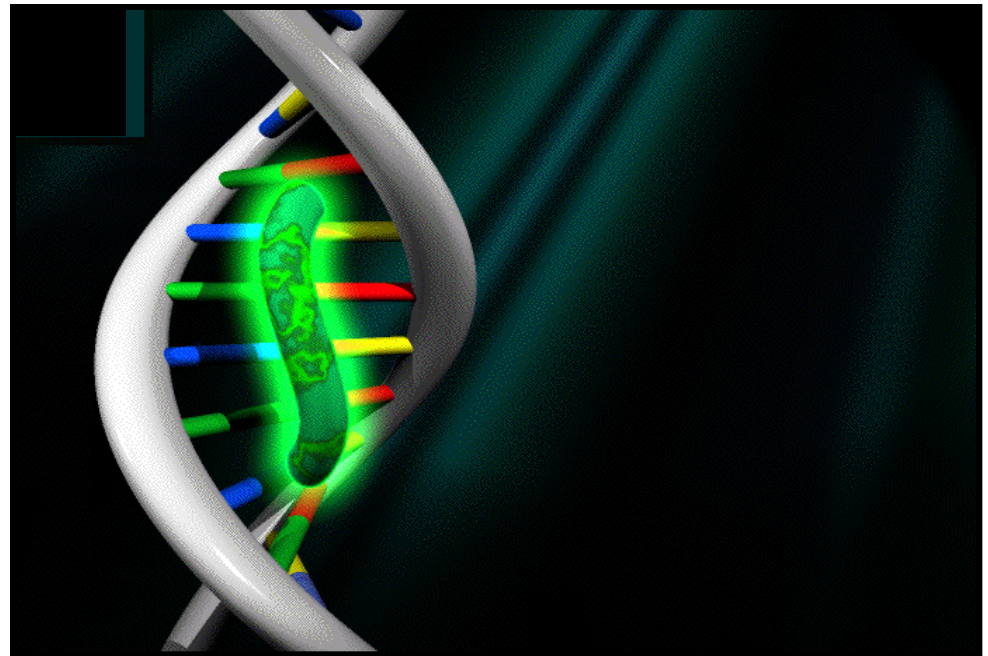
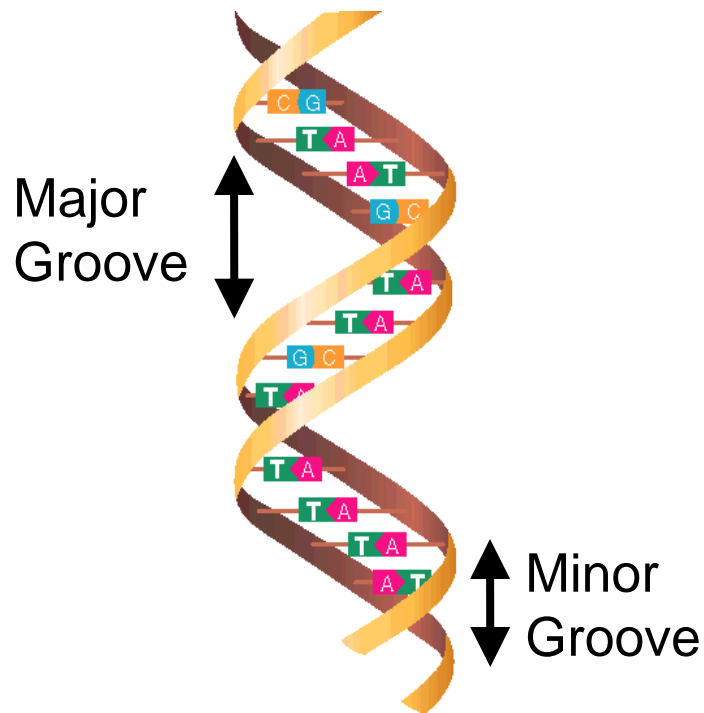
TaqMan® Probe: TaqMan® MGB/NFQ Probes

- Minor Groove Binder (MGB)
 - Small molecule that fits snugly into minor groove of duplex DNA
 - Stabilizes probe annealing
- Non-fluorescent Quencher (NFQ)
 - “Dark” quencher acts as energy transfer acceptor that doesn’t emit a detectable fluorescent signal
 - MGB probe design uses a special algorithm in Primer Express® Software
- Shorter probe length (13-25-mers)

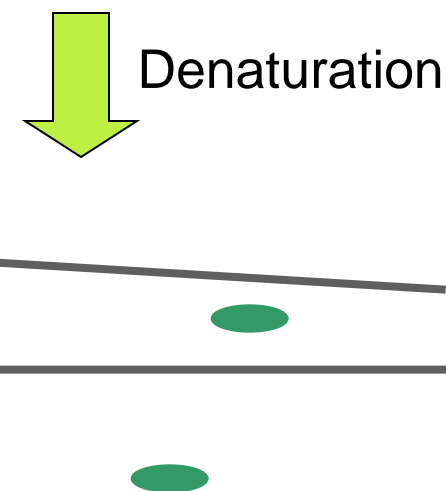
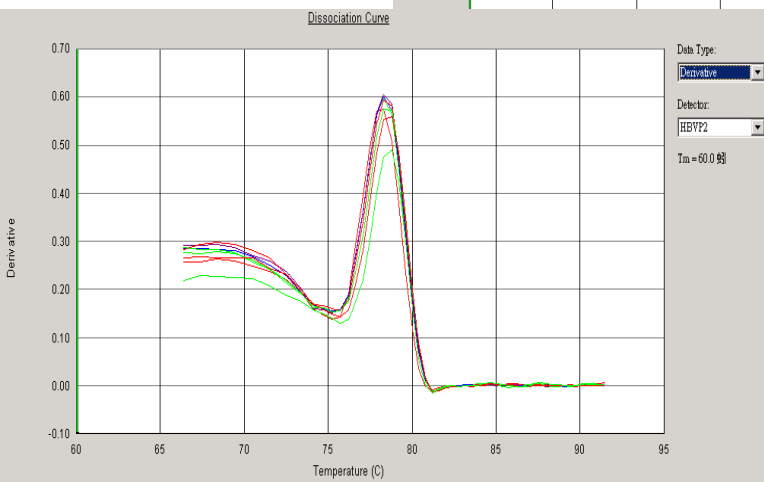
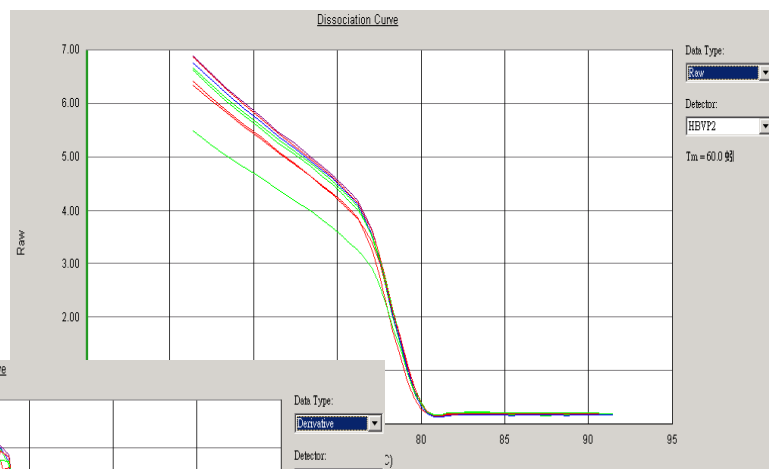
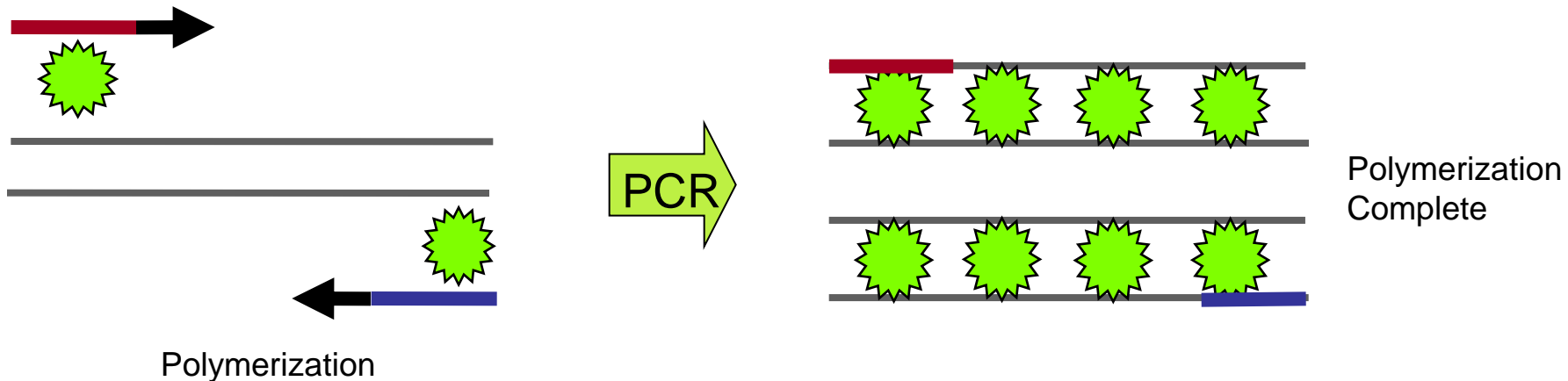


Real-time PCR Chemistries: SYBR® Green I Dye

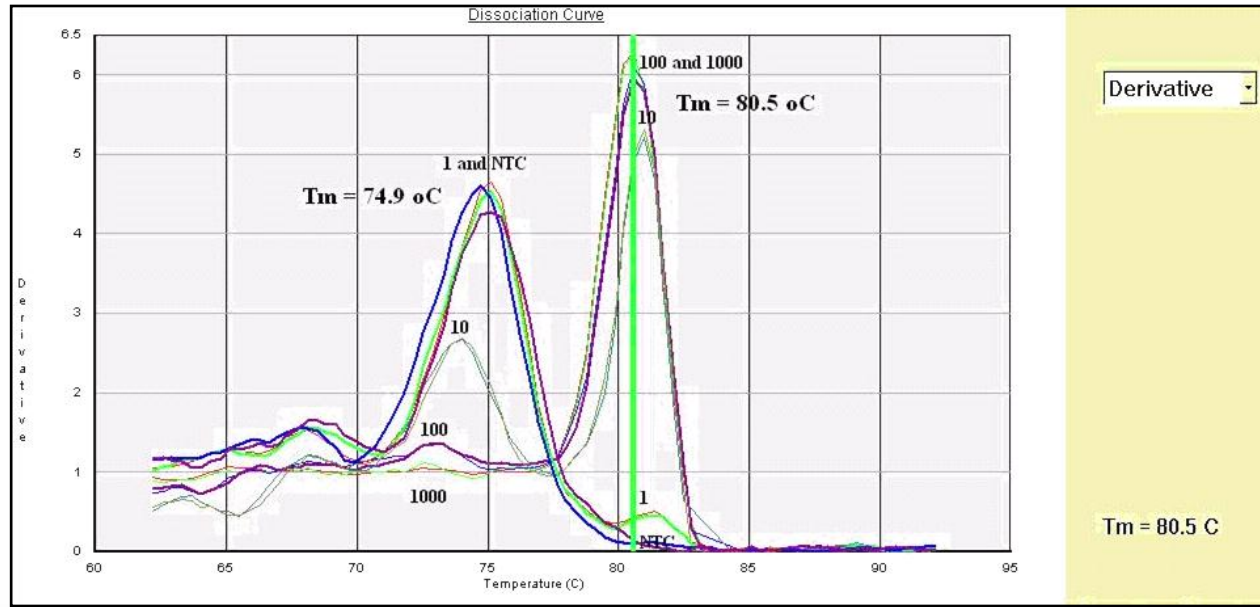
- A 'minor groove'-binding molecule specific to the minor groove of double-stranded DNA
- Fluoresces at an increased intensity when bound



SYBR® Green I Dye: Melting Curve Analysis



SYBR® Green I Dye: Melting Curve Analysis



- Use NTC to check whether non-specific product is primer dimer
- If the non-specific product is primer dimer:
 - Optimize primer concentration
 - Re-design primer pair

Real-time PCR Chemistries

	TaqMan® Assay	SYBR® Green I Dye
Specificity	More specific	Less specific
	Probe hybridization	
Sensitivity	Very high	Very high
Flexibility	Multiplex PCR	No probe required
	SNP detection	Screening tool
	+/- application	
Optimization	Ready to use 20x primer/probe mix - no need to optimize	Need to optimize PCR program
	Gold standard for MAQC	Need to check primer-dimer info
	PCR efficiency 100±10%	Need to check PCR efficiency

Reverse Transcription and Real-time PCR Reaction

One-step vs Two-step Workflows

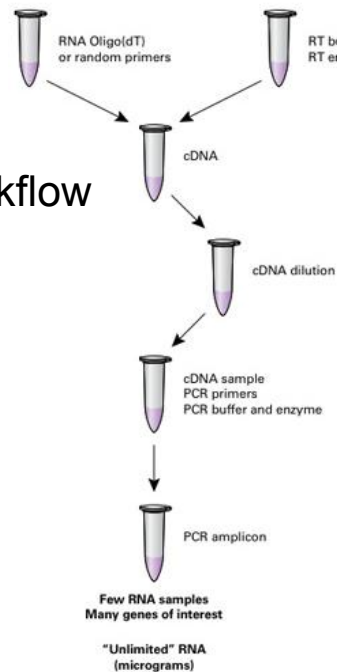
• One-step Technology

- RT and PCR are performed in single buffer system
 - ✓ One tube, one step
 - ✓ Reduce chance of cross-contamination
 - ✓ Easy for high throughput workflow
 - ✓ Cost effective when few targets/sample analyzed
 - ✓ Uses gene-specific primers
 - X cDNA can not be stored



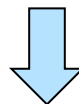
• Two-step Technology

- RT and PCR are performed in two separate reactions
 - ✓ Cost advantaged when interrogating multiple targets
 - ✓ cDNA can be stored and used for further experiments
 - ✓ Best choice if RNA is limiting
 - X Multiple steps, longer time to result



1-step qRT-PCR: Real-time PCR Reactions

Component	Volume for one reaction	Notes
4X TaqMan® Fast Virus 1-Step Master Mix	5 µL	—
TaqMan® Gene Expression Assay (20X)	1 µL	If you are not using pre-formulated TaqMan® Gene Expression Assays, Applied Biosystems recommends primer concentrations of 400 to 900 nM and a probe concentration of 100 to 250 nM.
Sample	Variable	Use as much sample as needed, up to the maximum allowed by the reaction volume.
RT-PCR Grade Water	Variable	Fill to the total reaction volume.
Total volume per reaction	20 µL	—



For sample volumes ≤30 µL

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

[†] Use the default run mode for your system and sample block module (that is, Fast mode on Fast instruments and standard mode on standard instruments).

[‡] Reverse transcription works best between 48 °C and 55 °C.

1-step qRT-PCR: Master Mixes

- TaqMan® Fast Virus 1-Step Master Mix (PN 4444434)
 - 4X master mix to amplify both RNA and DNA
 - Formulated to handle common RT-PCR inhibitors found in blood, stool, and other difficult samples
 - Up to triplex (ROX as passive reference)

- TaqPath™ 1-Step Multiplex Master Mix (PN A28522)
 - 4X master mix to amplify both RNA and DNA
 - Tolerant to common RT-PCR inhibitors
 - Manufactured in an ISO 13485 certified facility
 - Up to quadruplex (does not include passive reference)

2-step qRT-PCR: Real-time PCR Reactions

Reverse Transcription : High Capacity RNA-to-cDNA Kit

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



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

Real-time PCR:

TaqMan Chemistry

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

20µl

SYBR Chemistry

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

20µl



Standard mode

PCR condition:
 50°C, 2min
 95 °C, 10 min
 95 °C, 15 sec } 40 cycles
 60 °C, 1min

Fast mode

PCR condition:
 95 °C, 20 sec
 95 °C, 1 sec } 40 cycles
 60 °C, 20 sec

SYBR Green:

- Check Primer Concentration
- Add Melt Curve Program

2-step qRT-PCR: Master Mixes

Standard Mode

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



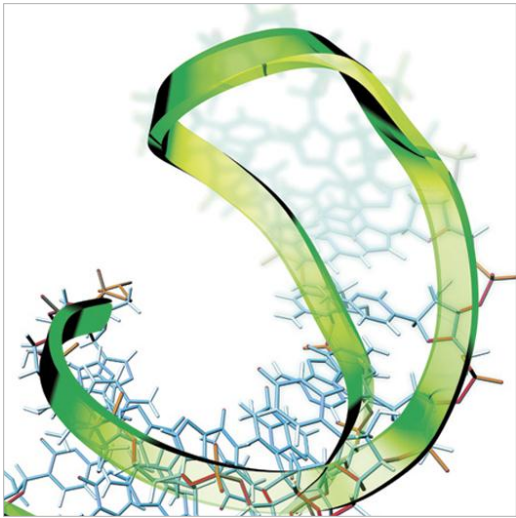
Fast Mode

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



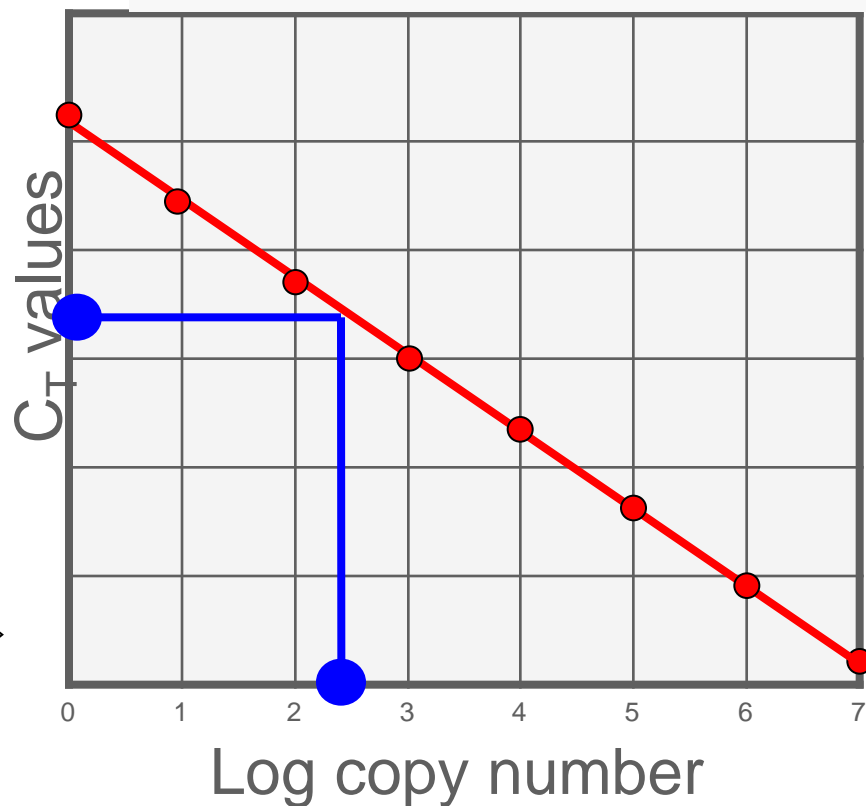
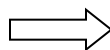
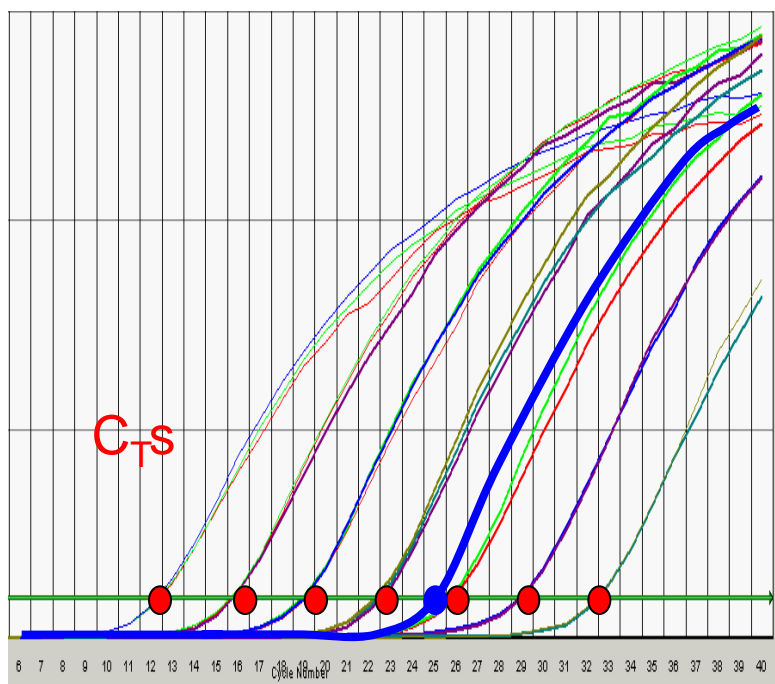
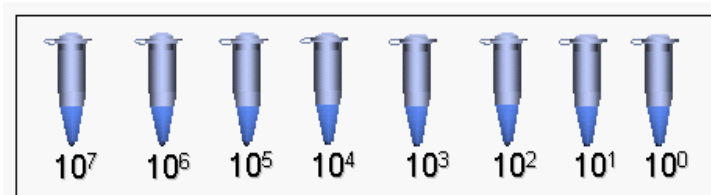
Real-time PCR Quantification Methods

- Absolute Quantification vs. Relative Quantification



絕對定量 (Absolute Quantification)

- 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.



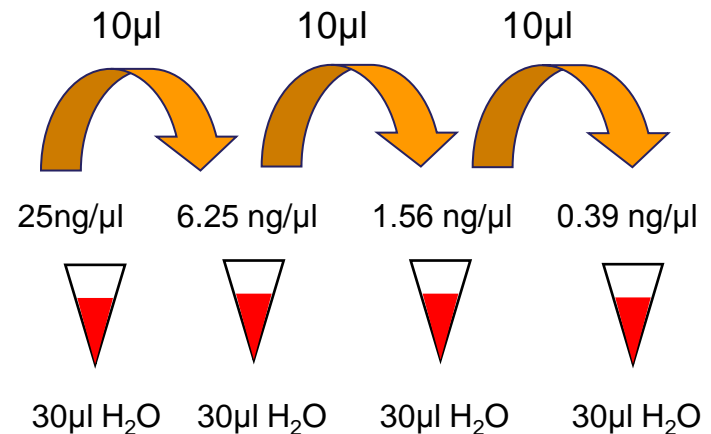
C_T is directly proportional to log of amount of input template

相對定量 (Relative Quantification)

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

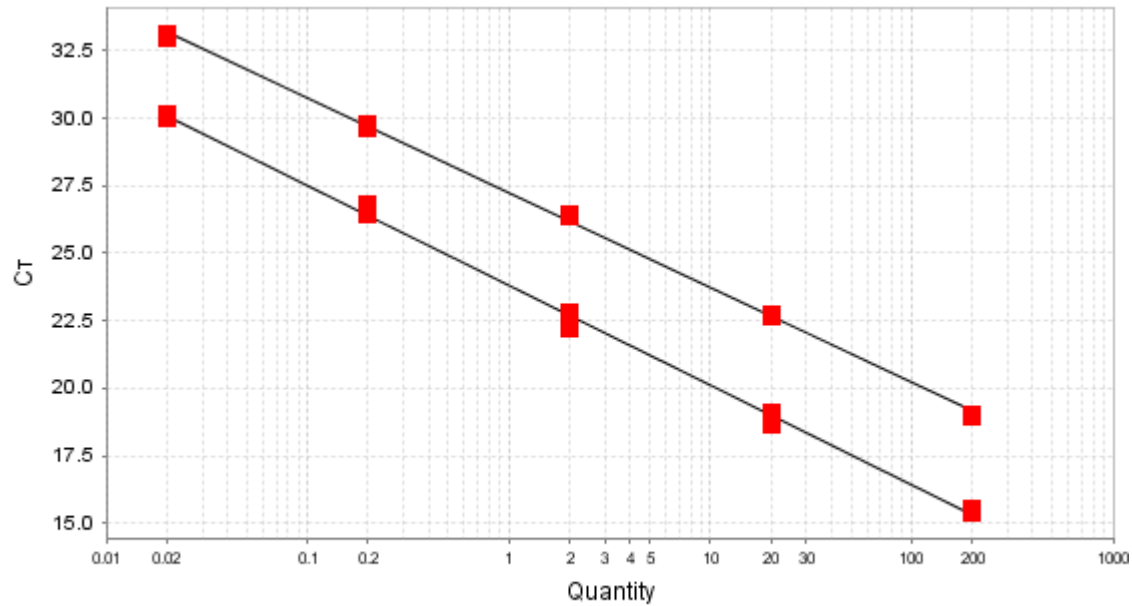
相對定量 (Relative Quantification): PCR Efficiency Validation

- 2 μ g RNA in 20 μ l RT = 100ng cDNA/ μ l
- Gene name: C-Myc and GAPDH
- cDNA 4-fold serial dilution: 10 μ l cDNA + 30 μ l H₂O (25ng/ μ l)
 - 1. 25ng/ μ l
 - 2. 6.25 ng/ μ l
 - 3. 1.56 ng/ μ l
 - 4. 0.39ng/ μ l
 - 5. NTC (duplicate for each sample)
 - 每個濃度點各做二重複



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

相對定量 (Relative Quantification): PCR Efficiency Validation



Target: GAPDH **Slope:** -3.506 **Y-Inter:** 27.226 **R²:** 0.999 **Eff%:** 92.853

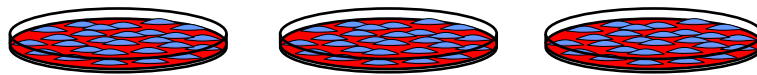
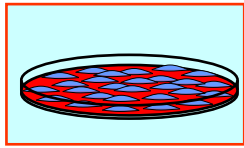
Target: c-myc **Slope:** -3.696 **Y-Inter:** 23.82 **R²:** 0.998 **Eff%:** 86.438

$90 \leq \text{Eff\%} \leq 110 \rightarrow \Delta\Delta \text{ Ct}$
 $\text{Eff\%} < 90 \rightarrow \text{Relative standard curve}$

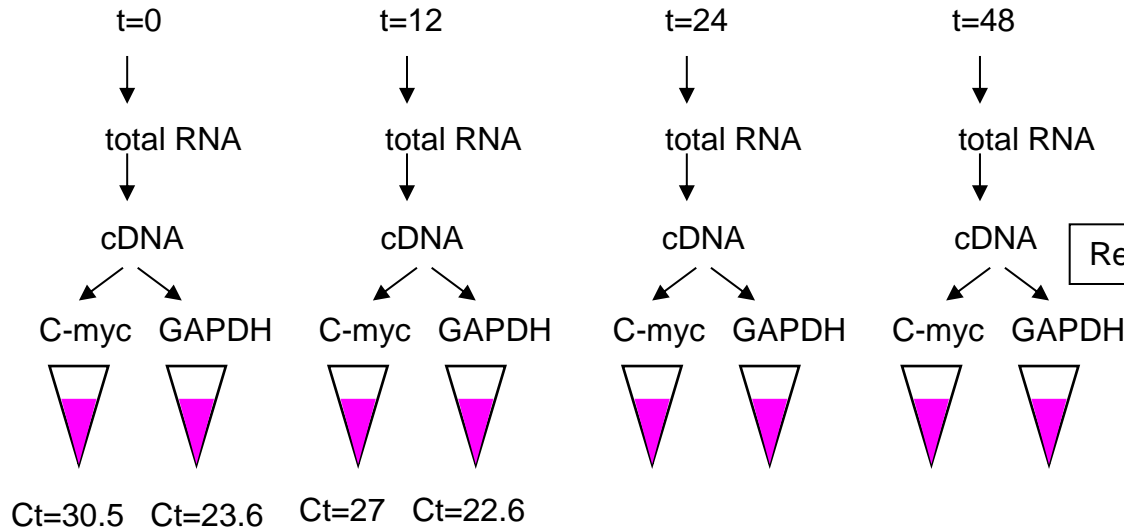
相對定量 (Relative Quantification): Comparative Ct ($\Delta\Delta Ct$)

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

Reference Sample



time



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

相對定量 (Relative Quantification): Comparative Ct ($\Delta\Delta Ct$)

step 1: Normalization to endogenous control

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

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

step 2: Normalization to reference sample

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

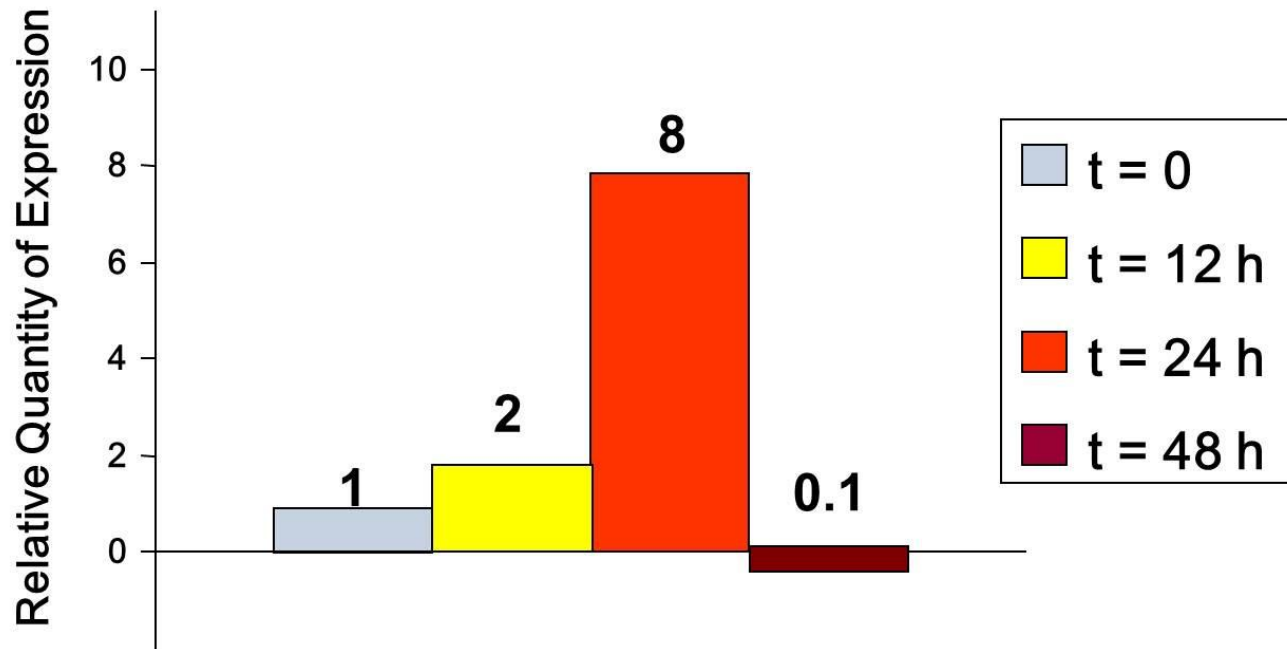
step 3: use the formula

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

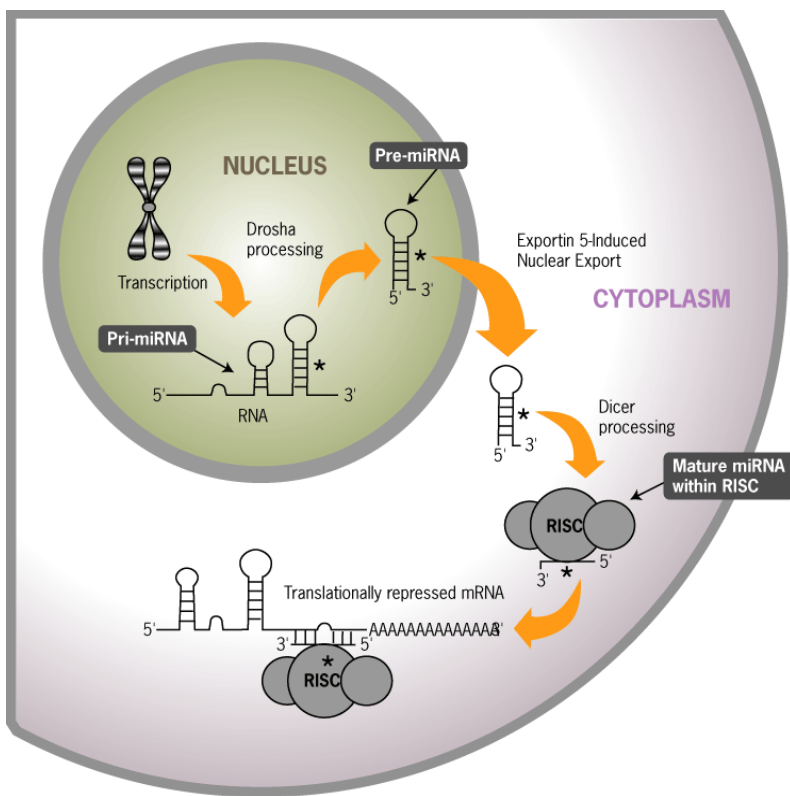
A reference sample is a sample to which unknown samples are compared (e.g. untreated sample or control).

相對定量 (Relative Quantification): Comparative Ct ($\Delta\Delta C_t$)

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



Introducing TaqMan™ Advanced miRNA Assays



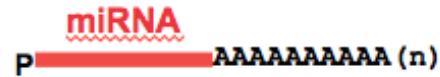
Mature
miRNA

UGAUUGAGCCGUGUCAUAU

- Excellent sensitivity in biological samples (serum/plasma, tissue)
- Low input amount (2µl) saves precious samples; no need to run multiple RTs and split sample
- Universal cDNA can be used for any miRNA assay
- cDNA can be archived for future miRNA studies
- TaqMan™ advanced miRNA assays
 - SKU A25576, Size S, 250 reactions
- TaqMan™ advanced miRNA cDNA synthesis kit

TaqMan™ Advanced miRNA Assays: How it Works

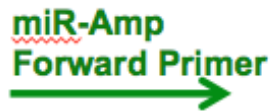
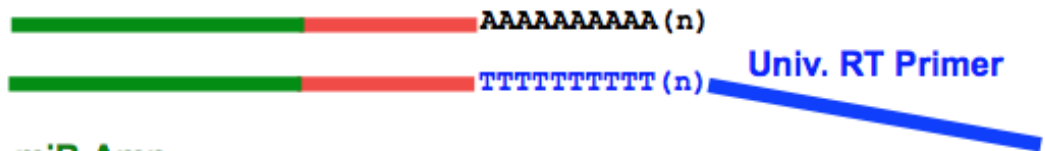
Step 1. Poly A Tailing



Step 2. Ligation



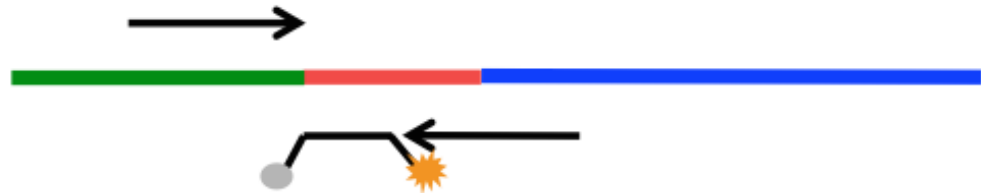
Step 3. Universal RT



Step 4. miR-Amp



Step 5. TaqMan qPCR



TaqMan™ Advanced miRNA Assays: High Specificity

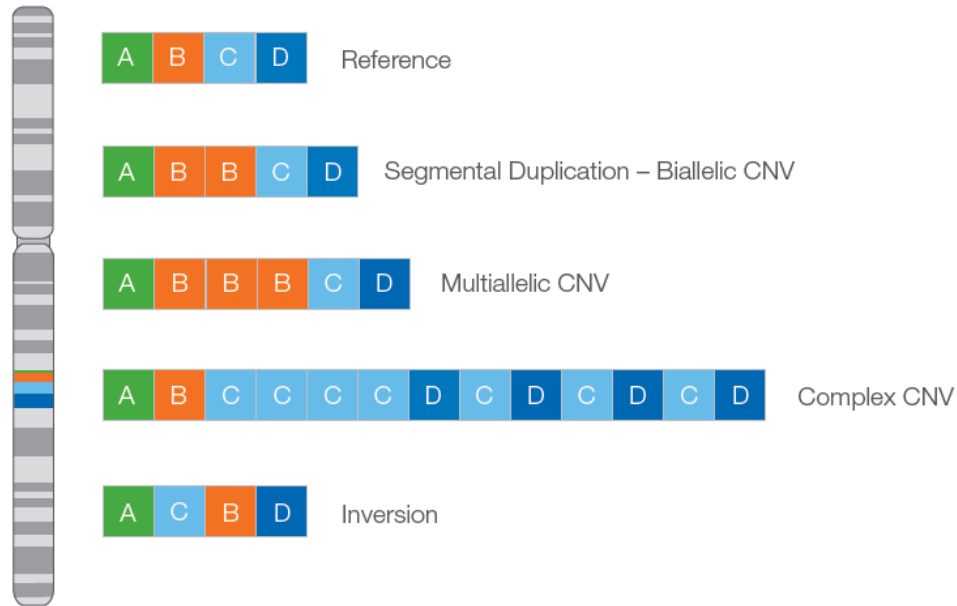
miRNA Name	miRNA Sequence
hsa-let-7a-5p	UGA GGU AGU AGG UUG UAU AGU U
hsa-let-7b-5p	UGA GGU AGU AGG UUG UGU GGU U
hsa-let-7c-5p	UGA GGU AGU AGG UUG UAU GGU U
hsa-let-7d-5p	AGA GGU AGU AGG UUG CAU AGU U
hsa-let-7e-5p	UGA GGU AGG AGG UUG UAU AGU U
hsa-let-7f-5p	UGA GGU AGU AGA UUG UAU AGU U
hsa-let-7g-5p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

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

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

Extremely low
cross-reactivity,
usually 1% or lower

Copy Number Variation (CNV)



Chromosome

Figure adapted from Estivill et al. (2007) *PLoS Genet.* October; 3(10):e190.

Copy Number Variation (CNV)

- A structural genomic variant involving copy number changes in comparison to a reference genome
- **Deletion** or **duplication** events involving **>1 kb** of DNA. Most are <10 Kb; some rare CNVs >1 Mb

CNVs are found in normal individuals and have also been associated with disease and other phenotypes

- **Pre-designed human and mouse assays for copy number analysis**

Human

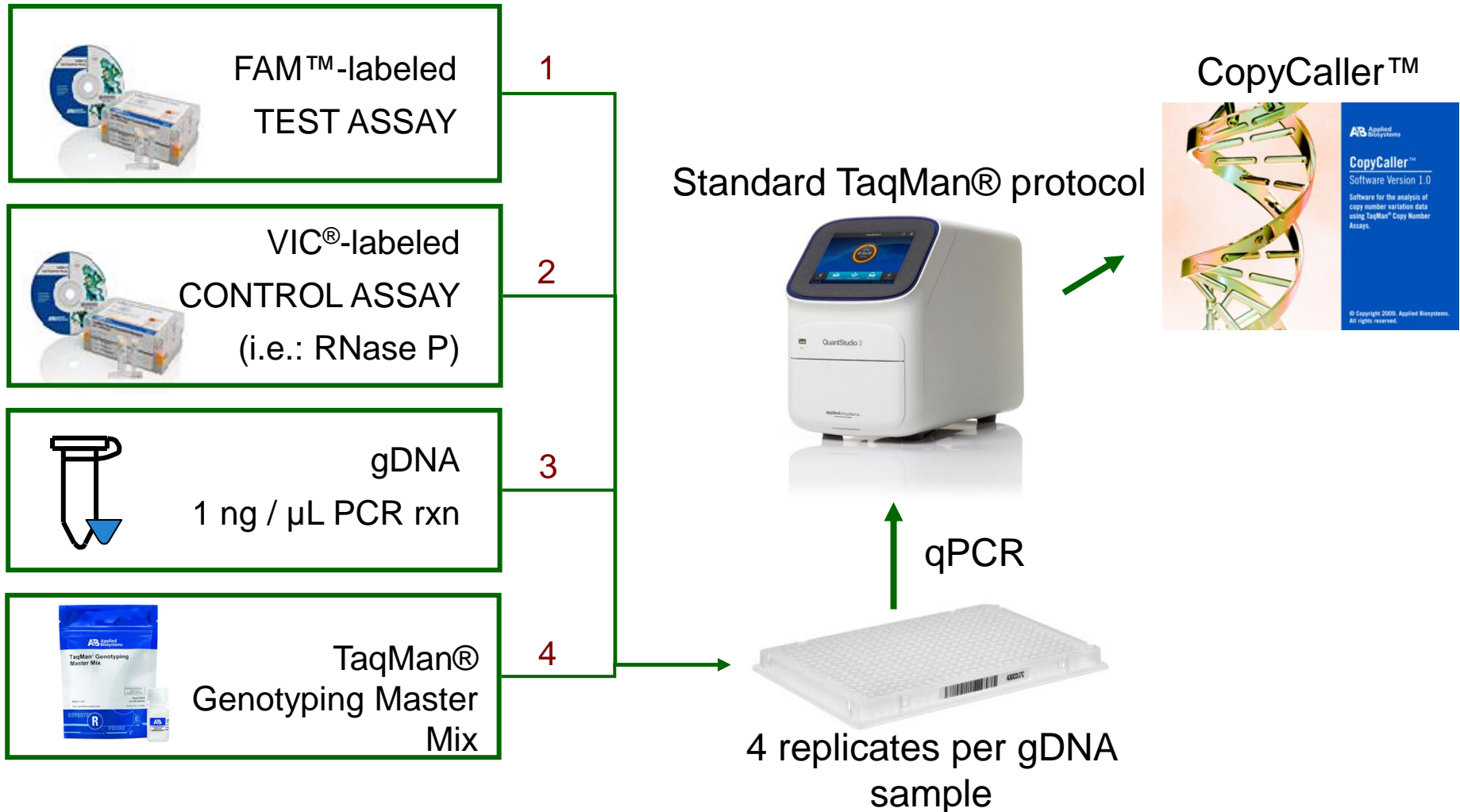
- Over 1.6 million pre-designed assays available for genome-wide coverage
- Genes (exons, introns, and junctions)
- Known copy number variations (CNVs)
- Extragenic/non-gene regions

Mouse

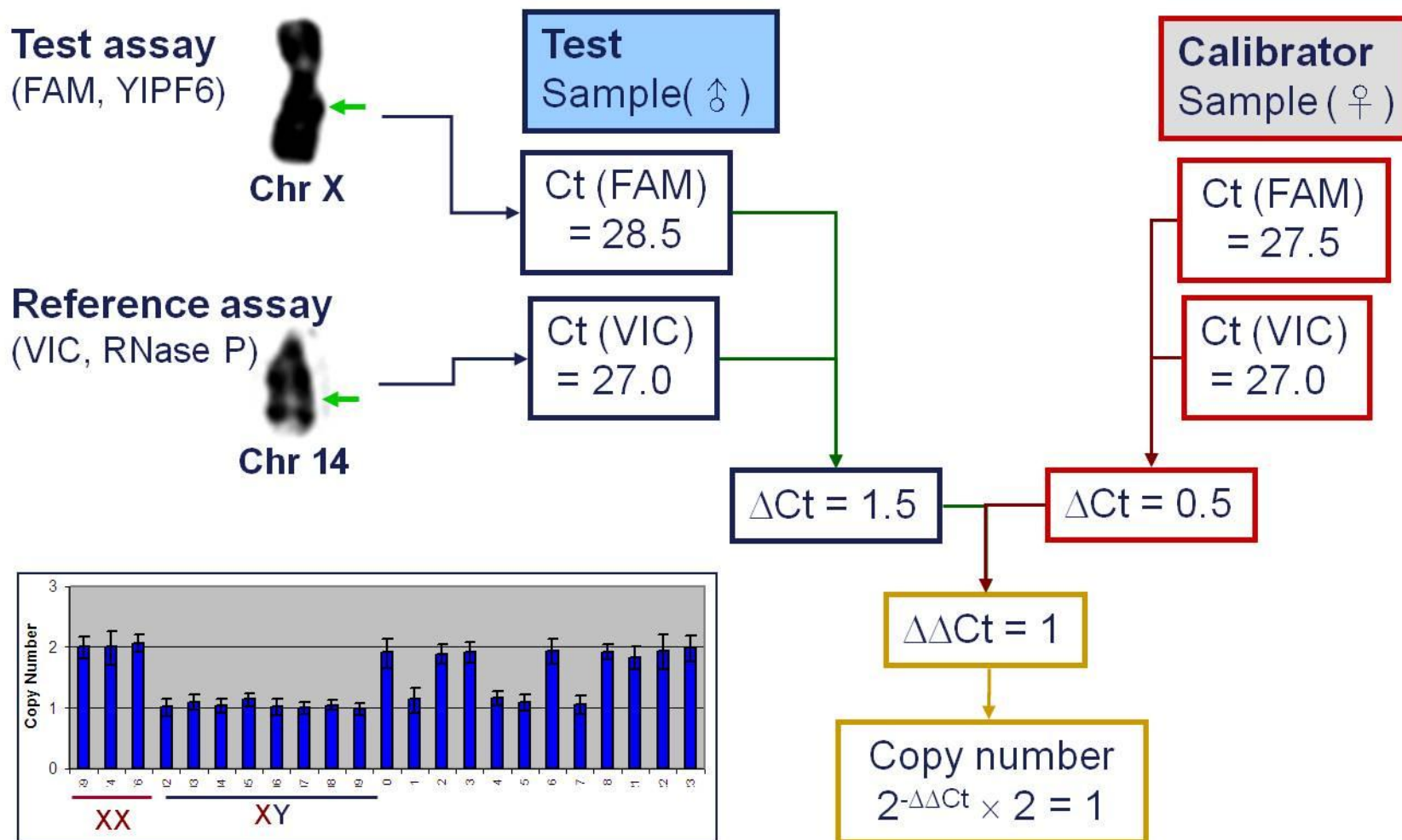
- Over 180,000 pre-designed assays available
- Gene exon coverage

Workflow of TaqMan® Copy Number Variation Assays

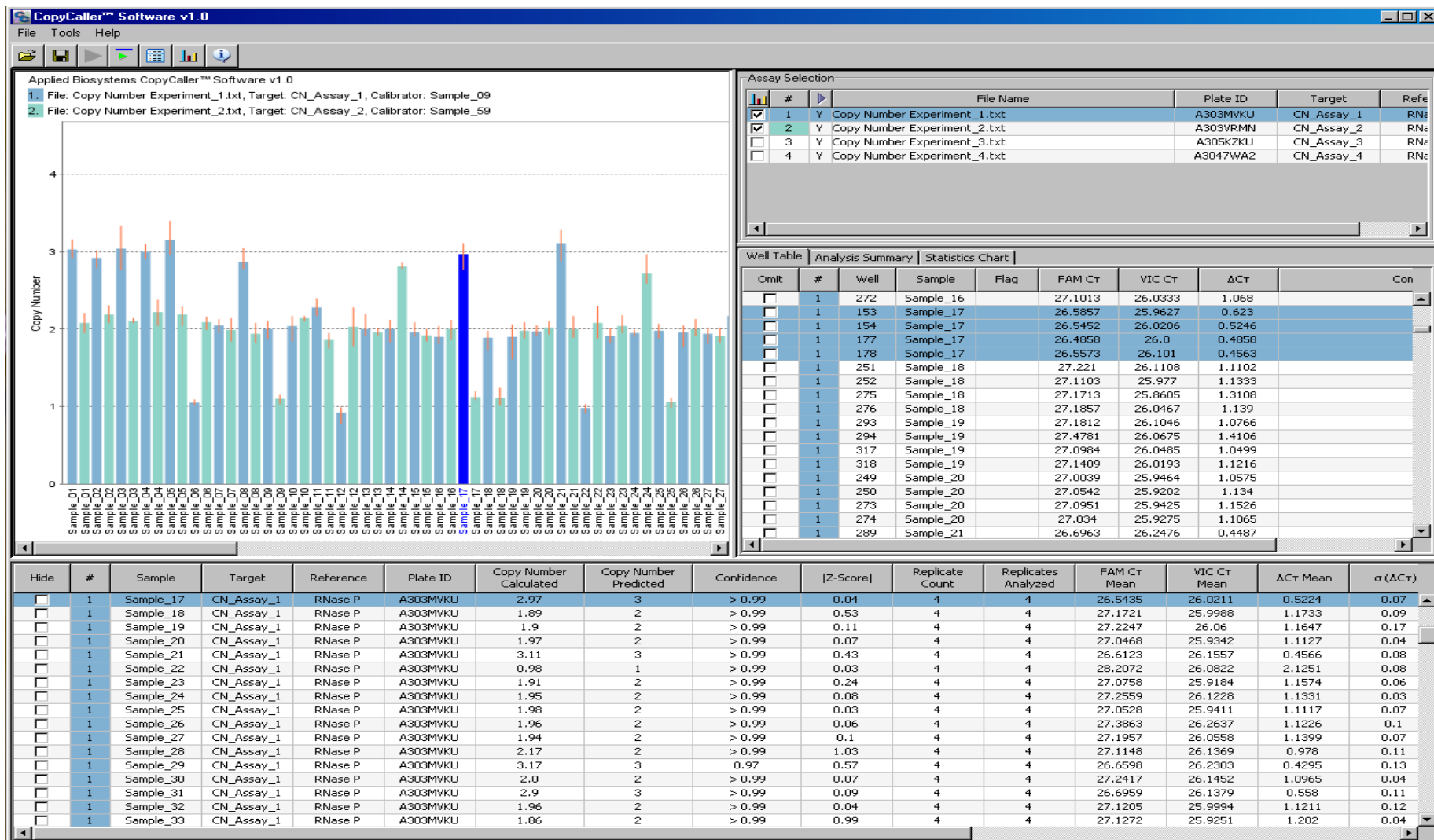
★ > 1.6M Pre-Designed TaqMan Copy Number Assays available



Determination of DNA Copy Number



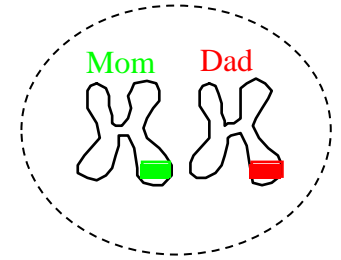
CopyCaller™ Software-輕鬆獲得CNV結果



- **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

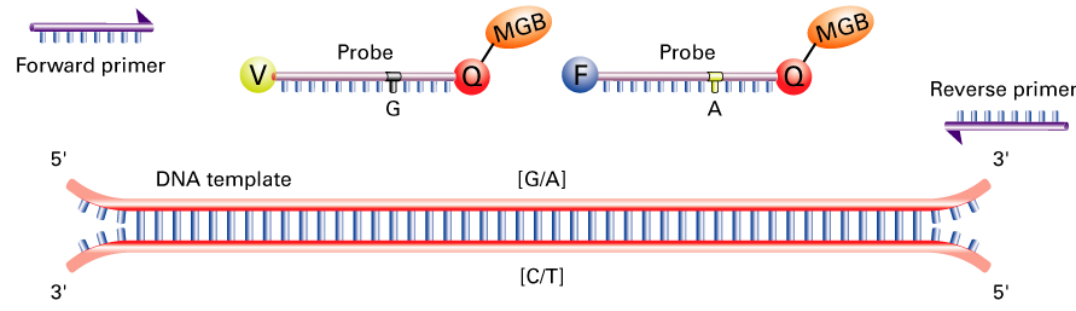
What are SNPs?

- **Diploid** organisms
 - 2 sets of chromosomes
- Each person has 2 copies or **2 alleles** of each gene – *1 allele on each chromosome.*
- Each person receives 1 allele from each parent.
- If both alleles are the same, the person is **homozygous** for that gene.
- If the alleles differ, then the person is **heterozygous** for that gene.

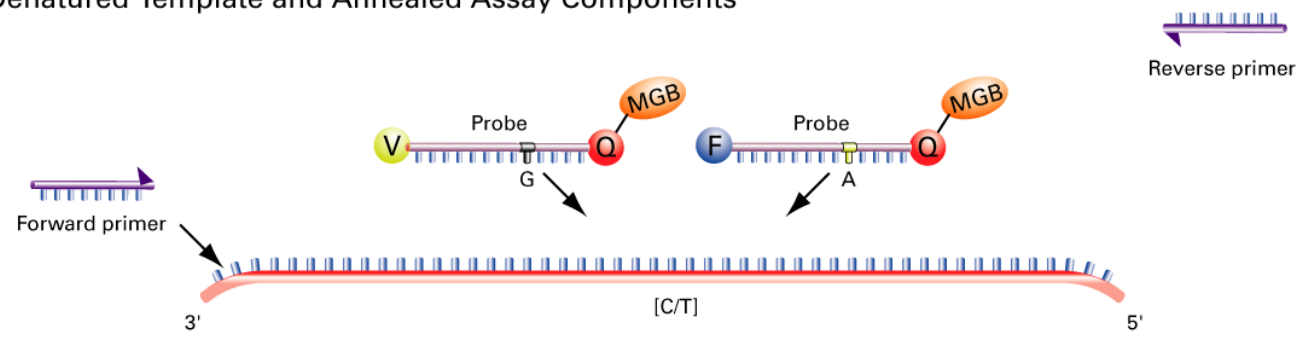


TaqMan® SNP Genotyping Assay Overview

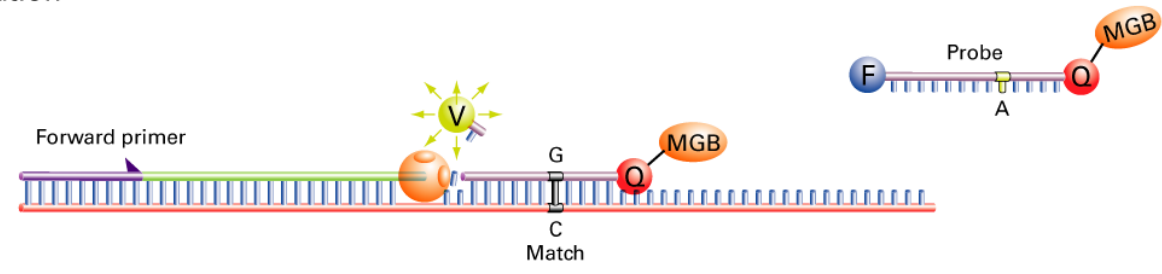
1. Assay Components and DNA Template



2. Denatured Template and Annealed Assay Components



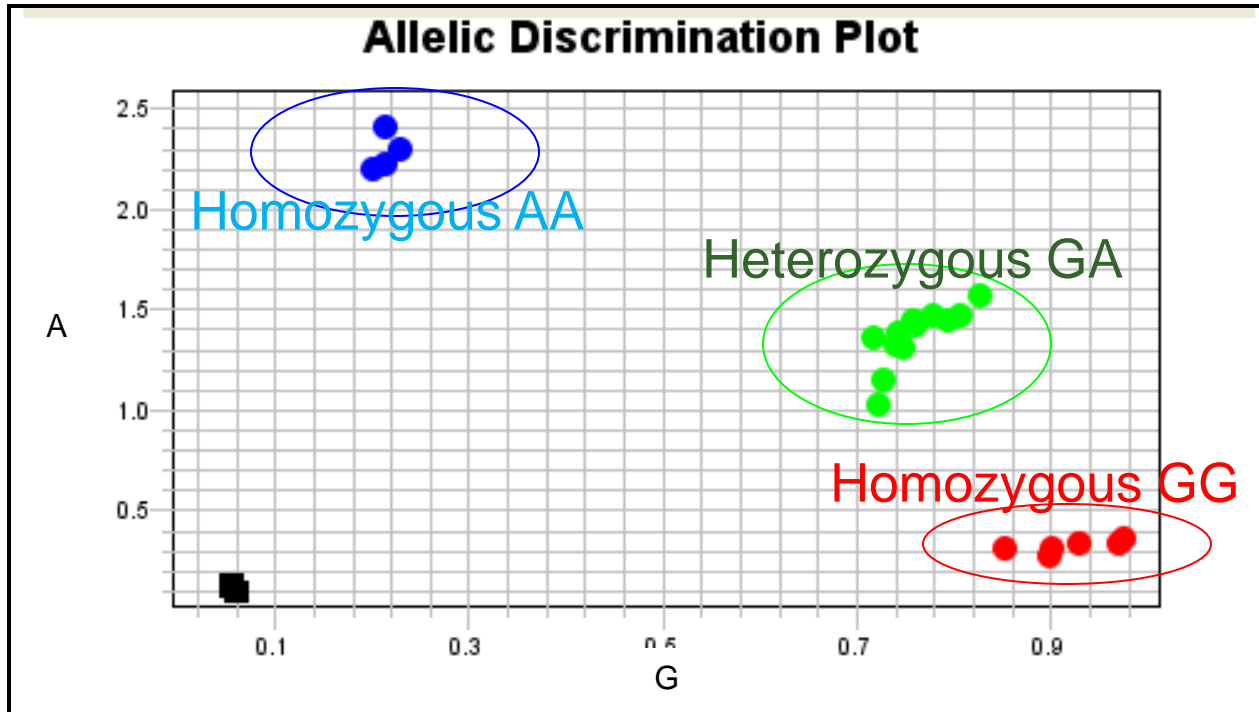
3. Signal Generation



LEGEND

- VIC® dye
- FAM™ dye
- Quencher
- Minor Groove Binder
- AmpliTaq Gold® DNA Polymerase
- Probe
- Primer
- Template
- Extended Primer

Allelic Discrimination (SNP) Data



Allele 2 Δ...	Pass.Ref	Call
0.152	3,617.946	■ Negative Control (NC)
0.186	3,784.869	■ Negative Control (NC)
0.334	4,068.745	● Homozygous 1/1
2.282	3,774.144	● Homozygous 2/2
0.392	4,004.767	● Homozygous 1/1

Sample 12	C_11711420_30	... A	C	VIC-NFQ...	FAM-NF...	0.782	1.5	3,991.875	● Heterozygous 1/2
Sample 13	C_11711420_30	... A	C	VIC-NFQ...	FAM-NF...	0.716	1.206	3,942.024	● Heterozygous 1/2
Sample 14	C_11711420_30	... A	C	VIC-NFQ...	FAM-NF...	0.815	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

Types of Cancer Mutations

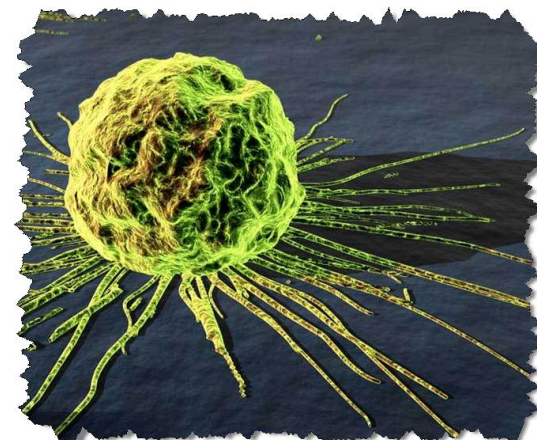
- Germline mutations
 - Inherited mutations
 - Present in all cells
 - Heterozygote (50%) or homozygote (100%) profile
 - Single gene to multi-genes
 - SNP to large chromosome rearrangement
- Somatic mutations
 - Mutations associated with the cancer itself
 - Present in some somatic cells (*i.e.* CTC)
 - Require sensitive methods to detect minor allelic frequency
 - Single gene to multi-gene
 - SNP to large chromosome rearrangement

Example: BRCA1
Breast Cancer

Example: PI3K
Breast, Colon Cancer

TaqMan® Mutation Detection Assays (TMDA)

- Somatic Mutation Detection by castPCR™ Technology
- TMDA Product Line Summary
 - Assays for 778 key mutations from 46 cancer genes
 - Corresponding gene reference assays
 - Wild-type assays for a subset of mutation targets
 - Internal Positive Control Reagents (IPC kit)
 - Mutation Detector™ Software
- Somatic mutations reported in the important genes related to biological pathways such as EGFR, Ras-Raf, KIT, FLT3, and PDGFRA
- High sensitivity (0.1-1%) for use with FFPE samples and biopsies
- High specificity for generating accurate results



■ Gene List

- | | |
|----------|----------|
| ■ AKT1 | ■ JAK2 |
| ■ ALK | ■ KRAS |
| ■ APC | ■ KIT |
| ■ BRAF | ■ MPL |
| ■ CDKN2A | ■ NPM1 |
| ■ CTNNB1 | ■ NRAS |
| ■ EGFR | ■ PDGFRA |
| ■ FGFR3 | ■ PIK3CA |
| ■ FLT3 | ■ PTEN |
| ■ GNAS | ■ TP53 |
| ■ HRAS | ■ VHL |
| ■ IDH1 | |

TaqMan® Mutation Detection Assays (TMDA)

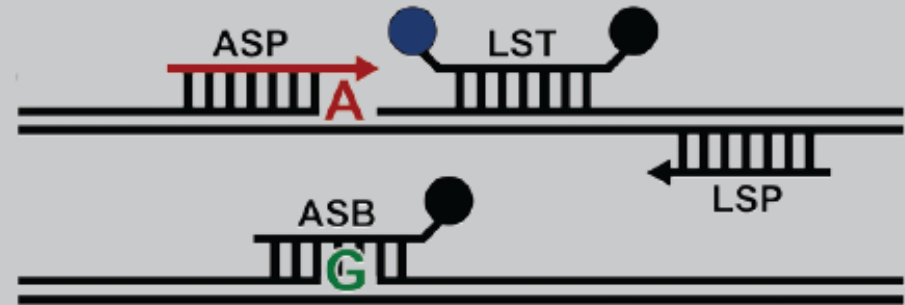
- Superior Sensitivity – 0.1 %
- High Specificity
- Simple and scalable workflow – 3 hrs from sample to results

Competitive Allele-Specific TaqMan® PCR - castPCR

Description

- Detects specific or multiple mutant alleles
- An allele-specific primer detects the mutant allele
- An MGB blocker oligonucleotide suppresses the wild type allele

Schematic



ASP = Allele-specific primer
ASB = Allele-specific blocker (MGB)
LST = Locus-specific TaqMan® probe
LSP = Locus-specific primer

Mutation Detector Software



Mutation Detector™ Software

File Help

Current Study: EXAMPLE_CellLine_dCt_cutoff

Show % Mutation

Perform Analysis

Well Data Replicates Average Assay Attributes

	Plate	Well	Assay	Sample	Control	Sample Ct	Quantity	Omitted	Well Flag
1	KRAS_gDNA_Titration_Run1_plate1_quant...	74	KRAS_516_mu	G12C_1		22.81	20.0	<input type="checkbox"/>	
2	KRAS_gDNA_Titration_Run1_plate1_quant...	73	KRAS_516_mu	G12C_1		22.82	20.0	<input type="checkbox"/>	
3	KRAS_gDNA_Titration_Run1_plate1_quant...	50	KRAS_516_mu	G12C_1		22.8	20.0	<input type="checkbox"/>	
4	KRAS_gDNA_Titration_Run1_plate1_quant...	49	KRAS_516_mu	G12C_1		22.93	20.0	<input type="checkbox"/>	
5	KRAS_gDNA_Titration_Run1_plate1_quant...	76	KRAS_516_mu	G12C_2		23.96	20.0	<input type="checkbox"/>	
6	KRAS_gDNA_Titration_Run1_plate1_quant...	75	KRAS_516_mu	G12C_2		24	20.0	<input type="checkbox"/>	
7	KRAS_gDNA_Titration_Run1_plate1_quant...	52	KRAS_516_mu	G12C_2		24.03	20.0	<input type="checkbox"/>	
8	KRAS_gDNA_Titration_Run1_plate1_quant...	51	KRAS_516_mu	G12C_2		24.02	20.0	<input type="checkbox"/>	
9	KRAS_gDNA_Titration_Run1_plate1_quant...	78	KRAS_516_mu	G12C_3		24.84	20.0	<input type="checkbox"/>	
10	KRAS_gDNA_Titration_Run1_plate1_quant...	77	KRAS_516_mu	G12C_3		24.98	20.0	<input type="checkbox"/>	
11	KRAS_gDNA_Titration_Run1_plate1_quant...	54	KRAS_516_mu	G12C_3		24.9	20.0	<input type="checkbox"/>	
12	KRAS_gDNA_Titration_Run1_plate1_quant...	53	KRAS_516_mu	G12C_3		24.95	20.0	<input type="checkbox"/>	
13	KRAS_gDNA_Titration_Run1_plate1_quant...	80	KRAS_516_mu	G12C_4		25.98	20.0	<input type="checkbox"/>	
14	KRAS_gDNA_Titration_Run1_plate1_quant...	79	KRAS_516_mu	G12C_4		26.08	20.0	<input type="checkbox"/>	
15	KRAS_gDNA_Titration_Run1_plate1_quant...	56	KRAS_516_mu	G12C_4		26.26	20.0	<input type="checkbox"/>	
16	KRAS_gDNA_Titration_Run1_plate1_quant...	55	KRAS_516_mu	G12C_4		26.04	20.0	<input type="checkbox"/>	
17	KRAS_gDNA_Titration_Run1_plate1_quant...	82	KRAS_516_mu	G12C_5		26.96	20.0	<input type="checkbox"/>	
18	KRAS_gDNA_Titration_Run1_plate1_quant...	81	KRAS_516_mu	G12C_5		27.07	20.0	<input type="checkbox"/>	
19	KRAS_gDNA_Titration_Run1_plate1_quant...	58	KRAS_516_mu	G12C_5		27.15	20.0	<input type="checkbox"/>	
20	KRAS_gDNA_Titration_Run1_plate1_quant...	57	KRAS_516_mu	G12C_5		27.1	20.0	<input type="checkbox"/>	
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24	KRAS_gDNA_Titration_Run1_plate1_quant...	59	KRAS_516_mu	G12C_6		28.03	20.0	<input type="checkbox"/>	

General Current Study Settings

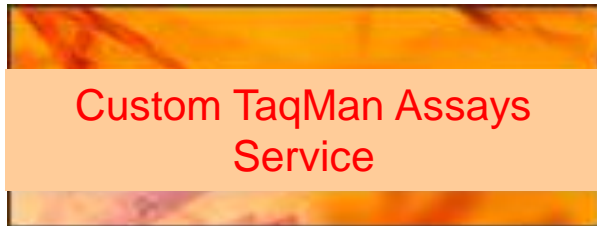
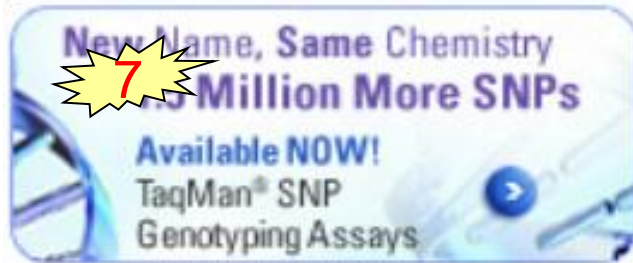
Study name: EXAMPLE_CellLine_dCt_cutoff
 Operator:
 Date: 6/24/11
 Enter comments:

Results

	Plate	Sample	Quantity	Assay	Detect...	Detecte...	#...	Avg C...	Std D...	Ref Assay	#...	Avg C...	Std D...	ΔCt	Calibr...	ΔCt _{norm}	Flag
1	KRAS_gDNA_Titration_R...	G12S_4	20.0	KRAS_517_mu	0.1%	6.41%	4	28.53	0.26	KRAS_517_wt	4	23.45	0.04	5.08	1.21	3.87	
2	KRAS_gDNA_Titration_R...	G12V_7	20.0	KRAS_520_mu	0.1%	1.16%	4	30.48	0.42	KRAS_520_wt	4	22.76	0.03	7.73	1.315	6.41	
3	KRAS_gDNA_Titration_R...	G12C_4	20.0	KRAS_516_mu	0.1%	14.6%	4	26.09	0.12	KRAS_516_wt	4	23.41	0.09	2.68	0.135	2.54	
4	KRAS_gDNA_Titration_R...	KRAS_wt	20.0	KRAS_517_mu	0.1%	0%	4	40	0	KRAS_517_wt	4	22.94	0.02	17.06	1.21	15.85	MUNEG
5	KRAS_gDNA_Titration_R...	KRAS_wt	20.0	KRAS_516_mu	0.1%	0%	4	40	0	KRAS_516_wt	4	22.77	0.16	17.23	0.135	17.09	MUNEG
6	KRAS_gDNA_Titration_R...	G12C_8	20.0	KRAS_516_mu	0.1%	0.67%	4	30.81	0.8	KRAS_516_wt	3	23.47	0.07	7.34	0.135	7.21	
7	KRAS_gDNA_Titration_R...	G12V_8	20.0	KRAS_520_mu	0.1%	0.59%	3	31.56	0.08	KRAS_520_wt	4	22.84	0.05	8.72	1.315	7.4	
8	KRAS_gDNA_Titration_R...	G12R_6	20.0	KRAS_518_mu	0.1%	6.23%	4	27.54	0.11	KRAS_518_wt	4	23.15	0.03	4.39	0.473	3.91	
9	KRAS_gDNA_Titration_R...	G12S_6	20.0	KRAS_517_mu	0.1%	1.17%	4	30.91	0.26	KRAS_517_wt	4	23.3	0.06	7.61	1.21	6.4	
10	KRAS_gDNA_Titration_R...	G12C_5	20.0	KRAS_516_mu	0.1%	7.39%	4	27.07	0.08	KRAS_516_wt	4	23.29	0.02	3.78	0.135	3.65	
11	KRAS_gDNA_Titration_R...	G12V_6	20.0	KRAS_520_mu	0.1%	3.93%	4	28.73	0.26	KRAS_520_wt	4	22.8	0.05	5.93	1.315	4.61	
12	KRAS_gDNA_Titration_R...	G12R_8	20.0	KRAS_518_mu	0.1%	1.05%	4	30.07	0.49	KRAS_518_wt	4	23.03	0.05	7.04	0.473	6.56	
13	KRAS_gDNA_Titration_R...	G12R_4	20.0	KRAS_518_mu	0.1%	23.8%	4	25.4	0.13	KRAS_518_wt	4	23.24	0.05	2.16	0.473	1.68	
14	KRAS_gDNA_Titration_R...	G12V_9	20.0	KRAS_520_mu	0.1%	0.14%	4	33.52	0.64	KRAS_520_wt	4	22.68	0.05	10.84	1.315	9.52	
15	KRAS_gDNA_Titration_R...	G12S_9	20.0	KRAS_517_mu	0.1%	0%	4	37.04	1.42	KRAS_517_wt	4	23.26	0.04	13.78	1.21	12.57	
16	KRAS_gDNA_Titration_R...	G12C_9	20.0	KRAS_516_mu	0.1%	0%	4	33.22	0.22	KRAS_516_wt	4	22.85	0.06	10.36	0.135	10.23	
17	KRAS_gDNA_Titration_R...	G12R_9	20.0	KRAS_518_mu	0.1%	0.23%	4	32.33	0.57	KRAS_518_wt	4	23.08	0.06	9.25	0.473	8.77	
18	KRAS_gDNA_Titration_R...	G12S_1	20.0	KRAS_517_mu	0.1%	>99.9%	4	25.48	0.06	KRAS_517_wt	2	37.42	0.49	-11.94	1.21	-13.15	
19	KRAS_gDNA_Titration_R...	G12C_2	20.0	KRAS_516_mu	0.1%	53.6%	4	24	0.03	KRAS_516_wt	4	24.08	0.1	-0.07	0.135	-0.21	

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

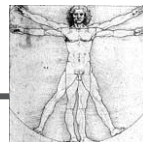
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<input type="checkbox"/> R. norvegicus	<input type="checkbox"/> D. melanogaster
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<input type="checkbox"/> M. mulatta (Rhesus)	<input type="checkbox"/> C. familiaris (Canine)
<input type="checkbox"/> D. rerio (Zebrafish)	<input type="checkbox"/> B. taurus (Cow)
<input type="checkbox"/> G. gallus (Chicken)	<input type="checkbox"/> O. cuniculus (Rabbit)
<input type="checkbox"/> S. scrofa (Pig)	<input type="checkbox"/> E. caballus (Horse)
<input type="checkbox"/> O. sativa (Rice)	<input type="checkbox"/> Pathogens



- *TaqMan Gene Expression Assays*
 - > 1,300,000 個已設計及測試過的基因定量試劑組
 - 提供所有相關生物資訊 (23 species)
- *TaqMan microRNA and primary microRNA Assays*
- *TaqMan SNP Genotyping Assays*
- *TaqMan Copy Number Assays*
- *TaqMan Mutation Detection Assays*

- **Custom** TaqMan Assays
 - All-in One tube TaqMan-based Assay

- Primer Express Software
- 上機條件皆相同~~不用再花時間測試primer溫度了



Finding the Right Assay for Your Research

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

What type of experiment are you conducting?

Gene Expression SNP Genotyping Copy Number siRNA

MicroRNA Mutation Detection Antibodies

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

TaqMan® Advanced miRNA Assays ^{NEW} TaqMan® MicroRNA Assays Controls

TaqMan® Pri-MicroRNA Assays Mimic/Inhibitors

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

[Hs] Human [Mm] Mouse [Rn] Rat More (221) All

Enter target information

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

Enter Single Sequence

Select a single species to search by sequence

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

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

TaqMan® Gene Expression Array Plates

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

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

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

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

By disease

Alzheimer's, cancer, diabetes, more >

By pathway

ABC transporters, CREB, more >

By biological process

Angiogenesis, DNA repair, more >

Endogenous controls

Human, mouse, eukaryotic, more >

Search for pre-configured TaqMan® Array cards and plates

What Array format do you want?

All Arrays


96-well standard

96-well Fast

384-well

OpenArray

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

 Human

 Mouse

 Rat

Enter target information

Enter Pathway, Disease, Gene Target

 Enter / Upload Multiple Targets

Search

ThermoFisher
SCIENTIFIC

Targets and Pathway Information

Narrow Your Results

Species

Human

Mouse

Array Format

384-well card

96-well Fast

96-well standard

[Reset Filters](#)

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

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

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

[My Price](#)

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

Panel Description

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

[View Less...](#)

Targets

ABCA1, ACHE, ADAM10, ADAM17, ADAM9, AGER, APBA1, APBA2, APBA3, APBB1, APBB2, APBB3, APCS, APH1A, APH1B, APLP1, APLP2, APOE, APP, BACE1, BACE2, BCHE, BPTF, CAPN1, CAPNS1, CAPNS2, CASP3, CASP6, CDC2, CDK5, CDK5R1, CHRM1, CHRM3, CHRNA4, CHRNA7, CSNK1A1, CSNK1D, CTSC, CTSD, CTSG, CYP46A1, GAL, GAP43, GJB1, GLS, GRIN1, GRIN2A, GRIN2B, GRIN2C, GRIN2D, GSK3B, HSD17B10, IDE, IFNG, IL1A, IL1B, IL6, INS, INSR, LRP1, LRP2, LRPAP1, MAPK1, MAPK3, MAPT, MME, NAE1, NCSTN, PDE8B, PKN1, PLD1, PPP2CA, PRKACB, PRKCA, PRKCB1, PRKCE, PRKCG, PSEN1, PSEN2, PSENE1, SERPINA3, SLC18A3, SLC30A3, SNCA, SOAT1, SOD2, ST6GAL1, TNF, UBQLN1, UCHL1, VSNL1

[View Less...](#)

Controls

18S, GAPDH, GUSB, HPRT1

Pathway Information

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

[View Less...](#)

Plate Layout and Assay ID

Narrow Your Results

Species

- Human
- Mouse

Array Format

- 384-well card
- 96-well Fast
- 96-well standard

[Reset Filters](#)

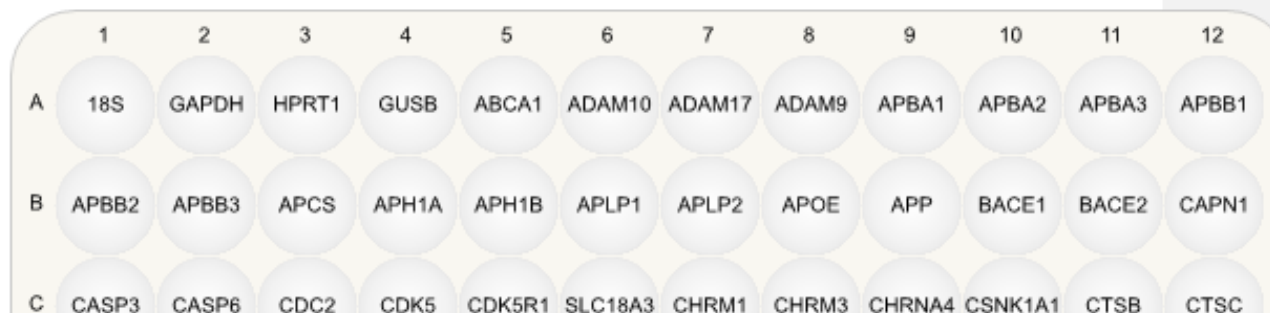
TaqMan® Array Human Alzheimer's Disease 96-well plate, Fast

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

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

[My Price](#) [新增到购物车](#)

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

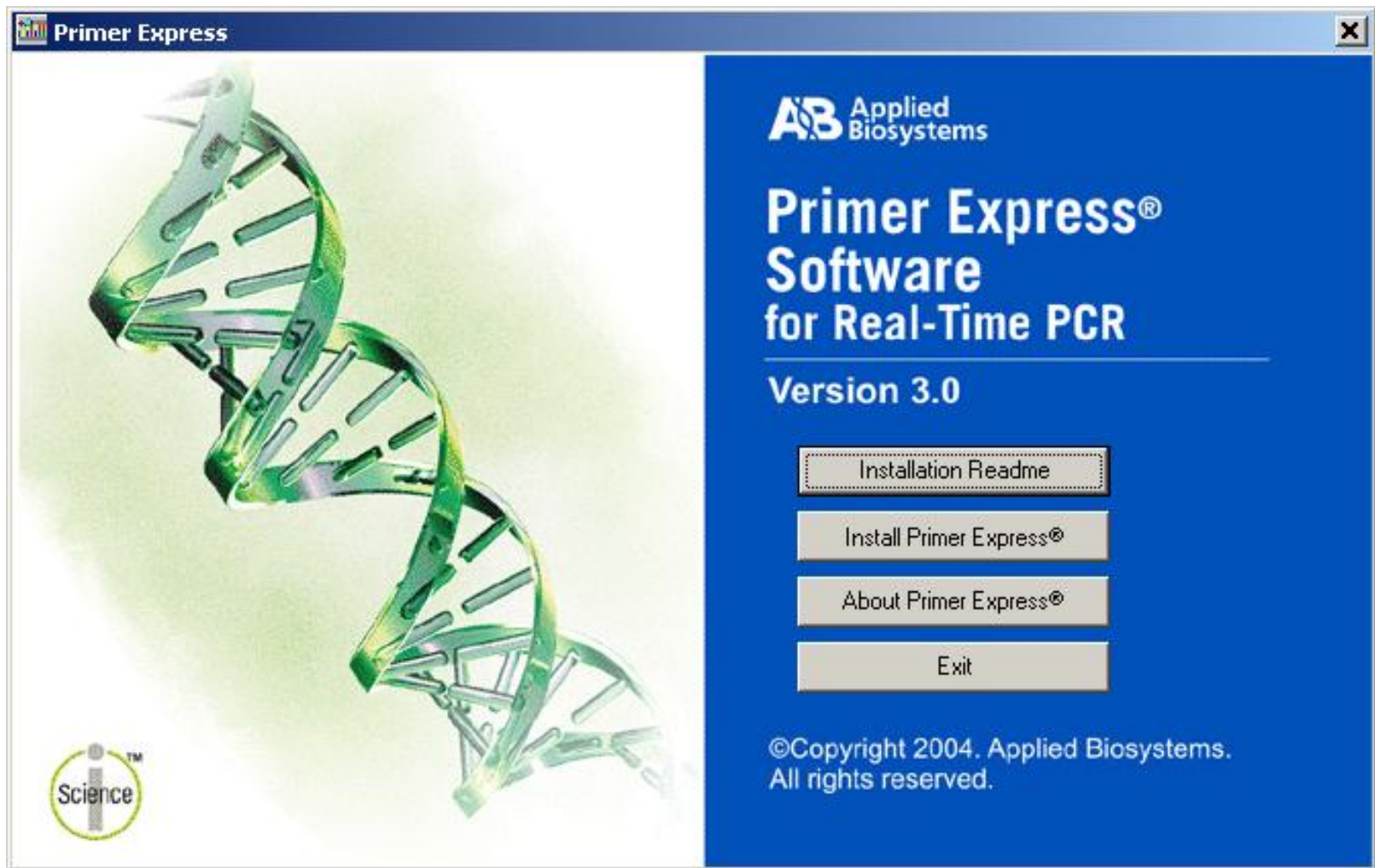


[Export to Excel](#)



Assay ID	1	2	3	4	5	6	7	8	9	10	11	12
A	Hs99999901_s1	Hs99999905_m1	Hs99999909_m1	Hs99999908_m1	Hs00194045_m1	Hs00153853_m1	Hs00234224_m1	Hs00177638_m1	Hs00154104_m1	Hs00194072_m1	Hs00191660_m1	Hs00377427_m1
B	Hs00300268_m1	Hs00195923_m1	Hs00356632_g1	Hs00211268_m1	Hs00229911_m1	Hs00193069_m1	Hs00155778_m1	Hs00171168_m1	Hs00169098_m1	Hs00201573_m1	Hs00273238_m1	Hs00559804_m1
C	Hs00263337_m1	Hs00154250_m1	Hs00364293_m1	Hs00358991_g1	Hs00243655_s1	Hs00268179_s1	Hs00265195_s1	Hs00181247_m1	Hs00793391_m1	Hs00157194_m1	Hs00175188_m1	Hs00175188_m1
D	Hs00157205_m1	Hs00175195_m1	Hs00189461_m1	Hs00702141_s1	Hs00248163_m1	Hs00609557_m1	Hs00168219_m1	Hs00168230_m1	Hs00181352_m1	Hs00275656_m1	Hs00189576_m1	Hs00610438_m1
E	Hs00174143_m1	Hs00174092_m1	Hs00174097_m1	Hs00174131_m1	Hs00355773_m1	Hs00169631_m1	Hs00233856_m1	Hs00189742_m1	Hs00158875_m1	Hs00177066_m1	Hs00385075_m1	Hs00213491_m1
F	Hs00153519_m1	Hs00299716_m1	Hs00405493_m1	Hs00708570_s1	Hs00160118_m1	Hs00427259_m1	Hs00176944_m1	Hs00176973_m1	Hs00176998_m1	Hs00178455_m1	Hs00177010_m1	Hs00177028_m1
G	Hs00997789_m1	Hs01577197_m1	Hs00153674_m1	Hs00240906_m1	Hs00162077_m1	Hs00167309_m1	Hs00260517_s1	Hs00174128_m1	Hs00188233_m1	Hs00374305_m1	Hs00544355_m1	Hs01085739_g1
H	Hs00542592_g1	Hs01000370_m1	Hs00992319_m1	Hs00998426_m1	Hs01063373_m1	Hs01017895_m1	Hs01042347_m1	Hs00967138_m1	Hs01016626_m1	Hs00900696_m1	Hs00949382_m1	Hs00923840_m1

Gene Symbol	1	2	3	4	5	6	7	8	9	10	11	12
A	18S	GAPDH	HPRT1	GUSB	ABCA1	ADAM10	ADAM17	ADAM9	APBA1	APBA2	APBA3	APBB1
B	APBB2	APBB3	APCS	APH1A	APH1B	APLP1	APLP2	APOE	APP	BACE1	BACE2	CAPN1
C	CASP3	CASP6	CDC2	CDK5	CDK5R1	SLC18A3	CHRM1	CHRM3	CHRNA4	CSNK1A1	CTSB	CTSC
D	CTSD	CTSG	BPTF	GJB1	GLS	GRIN1	GRIN2A	GRIN2B	GRIN2D	GSK3B	HSD17B10	IDE
E	FNG	IL1A	IL1B	IL6	INS	INSR	LRP1	LRP2	LRPAP1	MAPK1	MAPK3	MAPT
F	ORC3L	NCSTN	PDE8B	PSENEN	PLD1	PPP2CA	PRKACB	PRKCA	PRKCB1	PRKCE	PRKCG	PKN1
G	PSEN1	PSEN2	SERPINA3	SNCA	SOAT1	SOD2	CAPNS2	TNF	UCHL1	VSNL1	GAL	ACHE
H	AGER	NAE1	BCHE	CAPNS1	CHRNA7	CSNK1D	CYP4A1	GAP43	GRN2C	SLC30A3	ST8GAL1	UBQLN1



清楚明確的 TaqMan Probe and Primer 設計規範

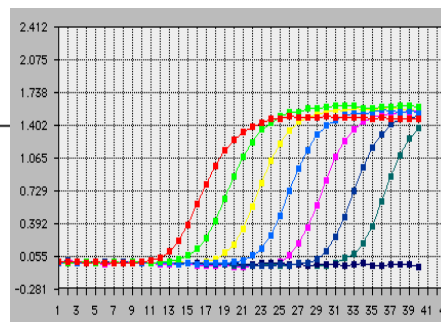
TaqMan Probe	Primer
Probe 與 Primer 的距離愈近愈好, PCR 產物大小建議在 50-150 bp 為最佳	
G/C % 為 30-80 %	
避免有重複序列的出現, 尤其避免 4 個以上 G 的出現	
T _m 值: 68-70°C (Quantification assay) 65-67°C (Allelic Discrimination assay)	T _m 值: 58-60°C
Probe 長度: 13~25 bases (TaqMan MGB probe) 13~30 bases (TaqMan probe)	Primer 長度: 20 bases (Optimal)
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

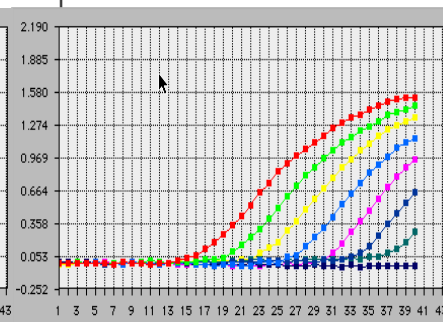
b: 參數可選擇設定

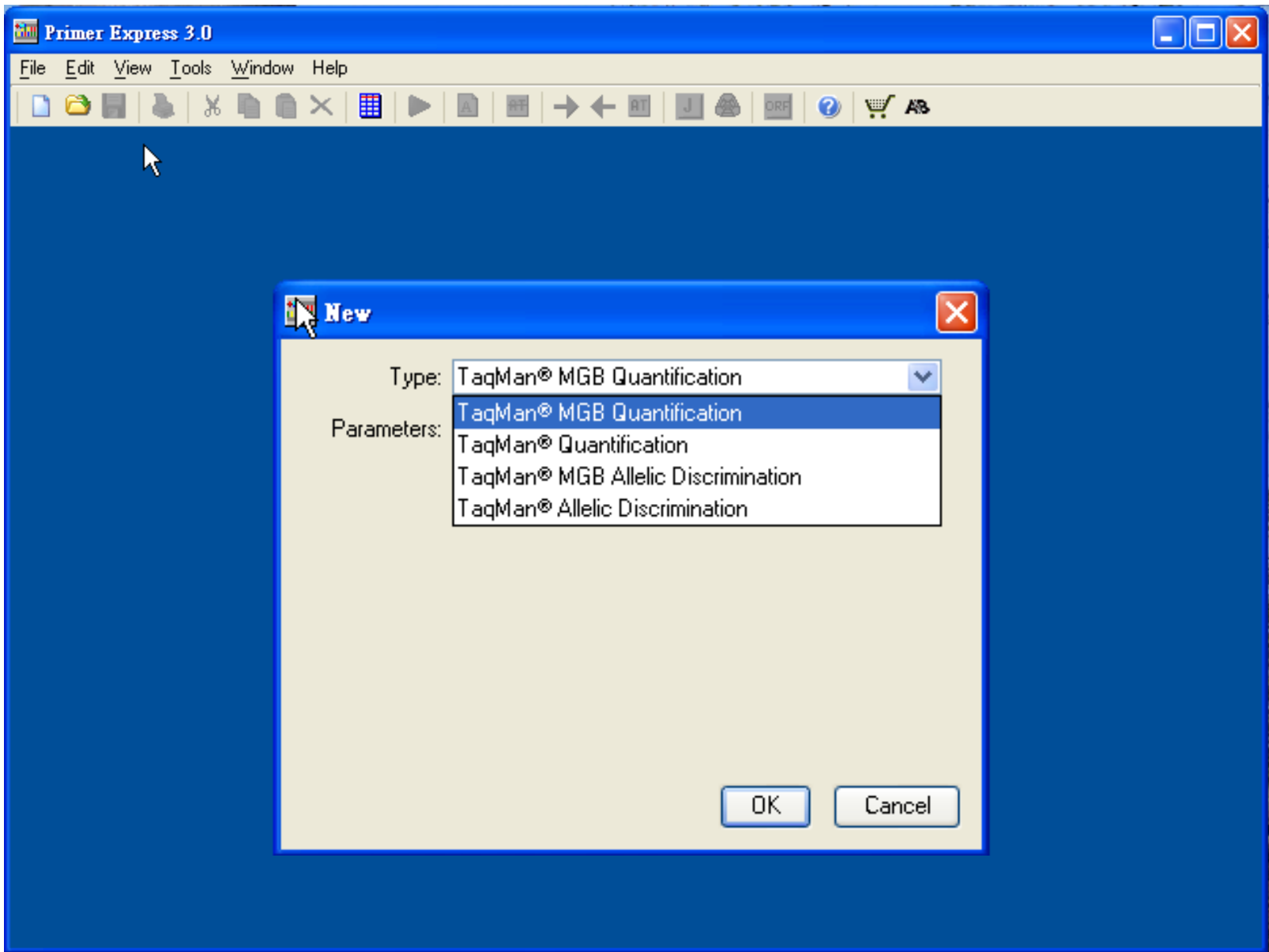


200 bp amplicon



500 bp amplicon





Sequence

The screenshot displays the Primer Express 3.0 software interface. The main window is titled "TaqMan® MGB Quantification # 1". The "Sequence" tab is active, showing a DNA sequence with a length of 383 bp. The sequence is displayed in a text area with a vertical scrollbar on the right. The sequence is as follows:

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

Two red circles highlight the "Find Primers/Probes" button in the toolbar and the "File Name" input field. Two white text boxes with black borders provide instructions:

- 1. Add DNA file or Copy & Paste
- 2. Find Primer/Probe

At the bottom of the window, a status bar reads: "To find Primers & Probes, click the 'Find Primers/Probes' button".

Design Parameter

TaqMan® MGB Quantification # 1			
Sequence	Parameters	Primers / Probes	Order
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			

Results

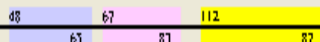
TaqMan® MGB Quantification # 1

Sequence Parameters **Primers / Probes** Order

Candidate Primers & Probes

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

Location

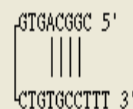


Secondary Structure

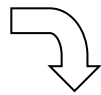
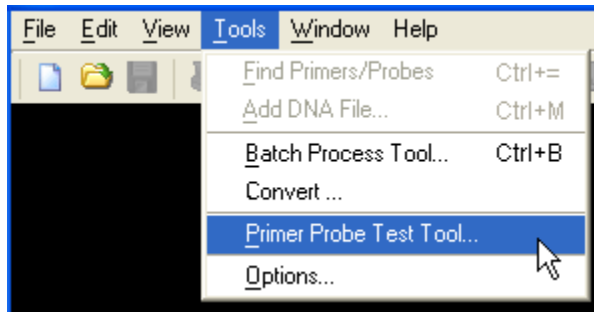
Oligo	Length
<input checked="" type="radio"/> Forward Primer	18
<input type="radio"/> Reverse Primer	26
<input type="radio"/> Probe	17
Forward Primer	
CGGCAGTGCTGTGCCTTT	
Reverse Primer	
CACCTTCTTGTCATAGGTACCGTCA	
Probe	
CTACCAACCTGATATCC	

Hairpin Self Dimers Cross Dimers

Most Stable Structure Found



Check Tm of Primers



The 'Primer Probe Test Tool' window displays the following information:

Parameters
Document Type: TaqMan® MGB Quantification
Parameter: Default

Primers and Probes

Primer/Probe	Tm	%GC	Length
Fwd Primer: ACTGATCGATCAGCTACGCATC	58.1	50	22
Rev Primer: TCGATCGATCGATCGATGC	59.2	53	19
Probe 1	0.0	0	0
Probe 2	0.0	0	0

The Tm values for the Fwd and Rev primers (58.1 and 59.2) are circled in red in the original image.

1. Primer Concentration Optimization

- Primer final concentration
- No primer dimer or non-specific product involved

2. PCR Primer Efficiency Validation

- Serially-diluted sample to generate standard curve for target gene and endogenous control gene

3. Test with samples that are comparable to real experiment for each gene



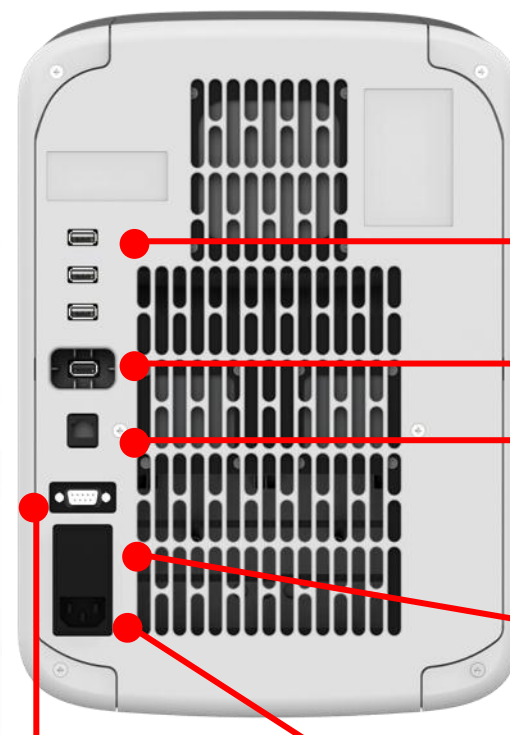
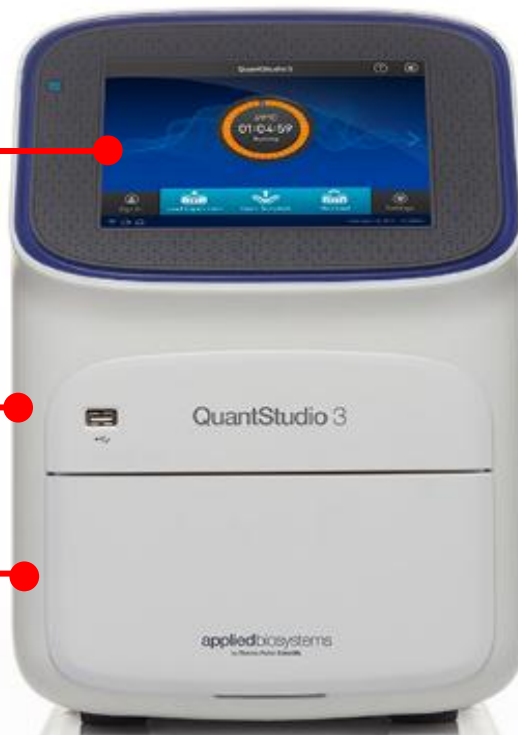
Applied Biosystems QuantStudio™ 3 Real-Time PCR System

QuantStudio™ 3 Real-Time PCR Systems: The Basics

Touchscreen (stand-alone capabilities, PIN-protected user accounts, and dye calibration/RNaseP functionality)

USB port for template upload and data download

Motorized block drawer (controlled by touchscreen)



USB ports

WiFi adapter port (optional use)

Ethernet port : RJ45 (10/100Mbps)

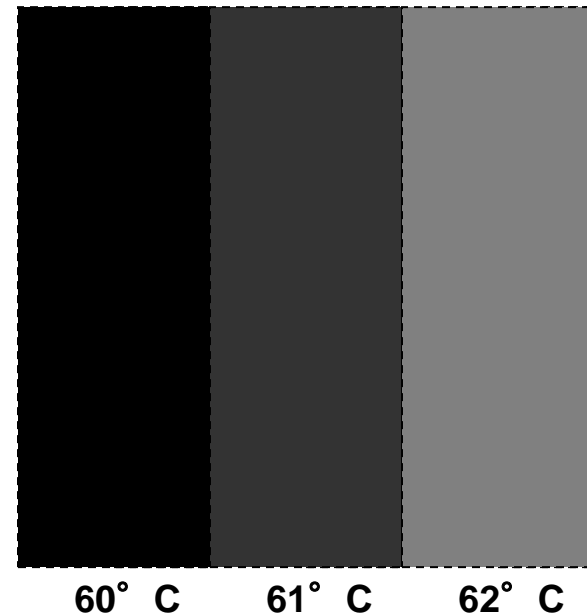
Fuse cover

Power port: 100/240 VAC

RS232 port (Service only)

QuantStudio™ 3 Real-Time PCR System: The Basics

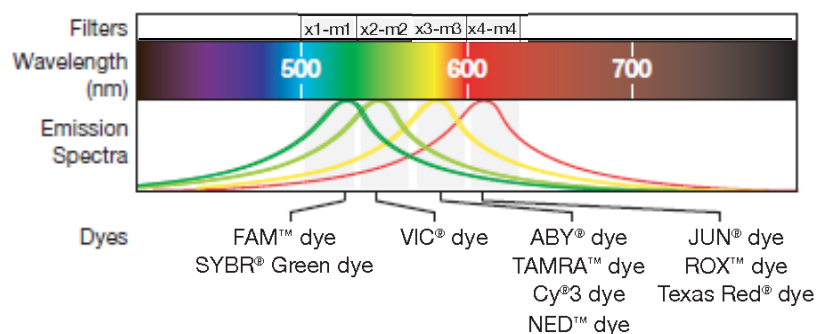
- VeriFlex™ Block with 3 programmable zones
 - Independent temperature control in each zone (more precise than gradient)
 - Can program at will, including multiple zones with same temp
 - Great for optimization and also running multiple assays at the same time



Multiplexing Capabilities

- OptiFlex™ System with Bright White LED
- Four color locked filter system
- Factory calibrated

Peak channel	Color	Filter wavelength (nm) ^[1]		Pre-calibrated dyes	Example custom dyes
		Excitation	Emission		
x1-m1	Blue	470 ± 15	520 ± 15	FAM™ and SYBR® Green	SYT09
x2-m2	Green	520 ± 10	558 ± 12	VIC®	HEX™, TET™, and JOE™ ^[2]
x3-m3	Yellow	550 ± 10	587 ± 10	ABY®, NED™, and TAMRA™	Cy®3
x4-m4	Orange	580 ± 10	623 ± 14	JUN® and ROX™	Texas Red®



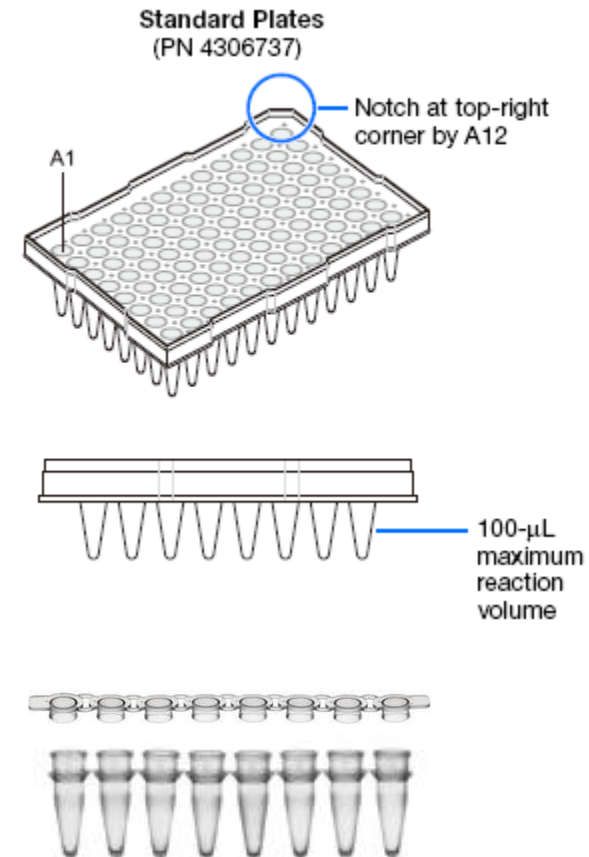
QuantStudio™ 3 Real-Time PCR System: Consumables

- 樣品量多時

- MicroAmp Optical 96-Well Reaction Plate (0.2ml) -10 plates (P/N N8010560)
- ABI PRISM™ Optical Adhesive Covers - 100 films (P/N 4311971)

- 樣品量少時

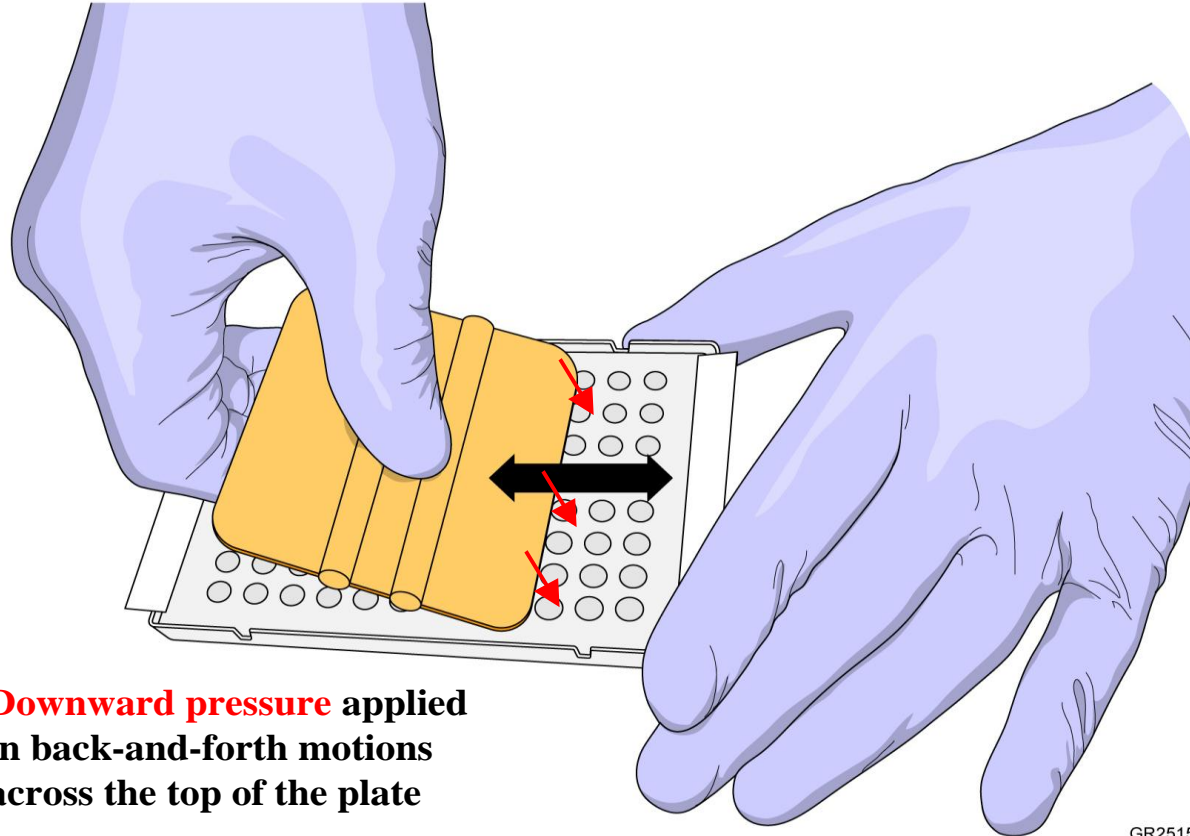
- ABI PRISM™ Optical 8 Tubes/Strip (0.2ml) - 125 strips (P/N 4316567)
- MicroAmp Optical 8 Caps/Strip - 300 strips (P/N 4323032)



★ Load at least 16 tubes with tray

Sealing the Plate

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



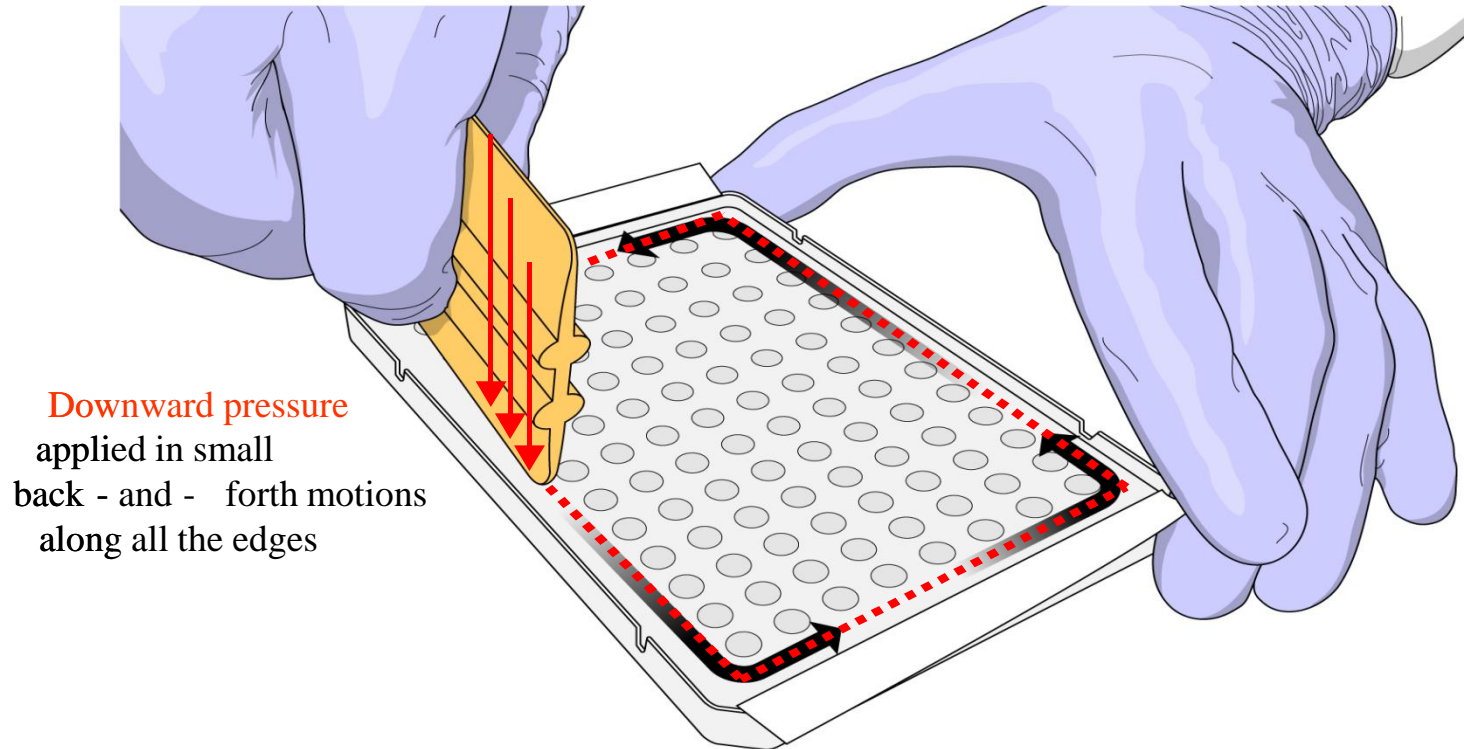
Downward pressure applied
in back-and-forth motions
across the top of the plate

GR2515

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

Sealing the Plate

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

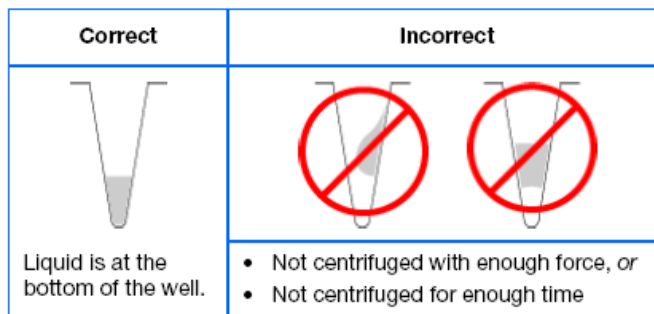


GR2516

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

QuantStudio™ 3 Real-Time PCR System: Operation Notes

- Use a tray for 8-tube strips
- Do not label on the consumables
 - This may increase the background signal
- Avoid bubbles when pipetting into each well
 - Centrifuge samples



Stand-alone, Desktop, or Online



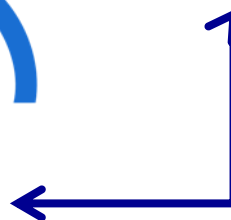
Connected Laptop with QuantStudio Design and Analysis desktop software



WiFi



LAN



USB



Connect to the Design and Analysis Cloud software using any device with a compatible web browser

Note: You can start an experiment run only from the instrument touchscreen or from the Desktop Software

QuantStudio™ 3 Touch Screen



Edit Run Protocol



Full method editing capabilities on the touch screen, including VeriFlex, Pause, and Melt

Monitor Progress During the Run

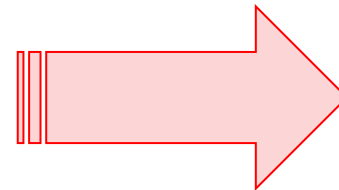
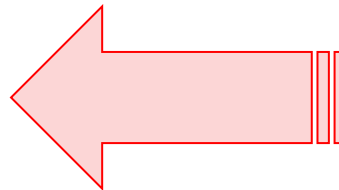
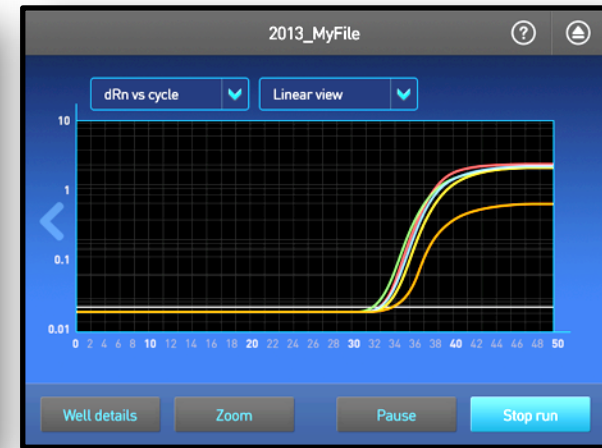
Time Remaining



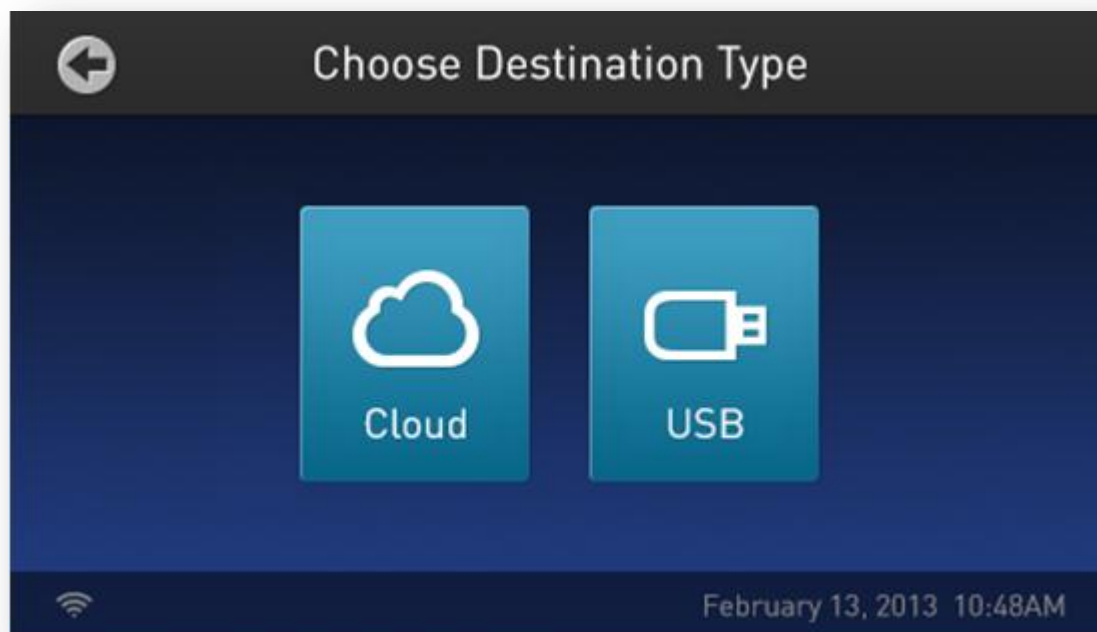
Thermal Protocol Status



Live Amplification Curves



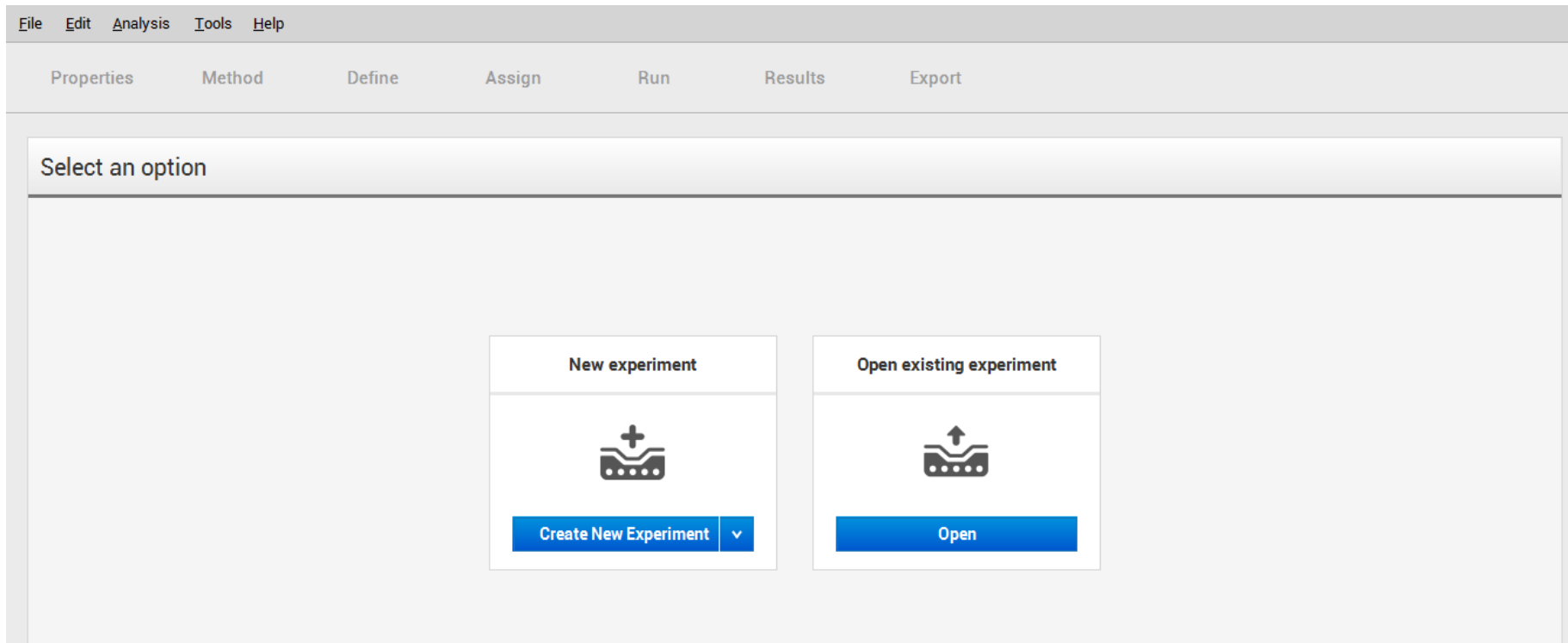
Options to Upload Data



- 1. Cloud = Data saved to user's online account**
- 2. USB = Data saved to attached USB drive**
- 3. Desktop = Data automatically saves back to desktop if run started from desktop**

- QuantStudio™ Design and Analysis Software supports a variety of analysis methods, including:
 - Absolute Quantitation
 - Standard Curve
 - Relative Quantitation
 - Relative Standard Curve
 - Comparative CT ($\Delta\Delta$ CT)
 - Presence/absence (Plus/Minus) assays with an internal positive control
 - Melt curve analysis
 - Genotyping (including real-time amplification)
- Multiplate GEx analysis available online on the QuantStudio Design and Analysis **Cloud** Software (<https://www.thermofisher.com/tw/en/home/cloud.html>)

QuantStudio™ Design and Analysis Software



- Similar look and feel as online software
- <http://www.thermofisher.com/tw/en/home/technical-resources/software-downloads/ab-quantstudio-3-and-5-real-time-pcr-system.html>


Experiment Properties

 Save 

Name

Barcode

User name

Instrument type 

Block type 

Experiment type 

Chemistry 

Run mode 

[Manage chemistry details](#)

Comments - optional

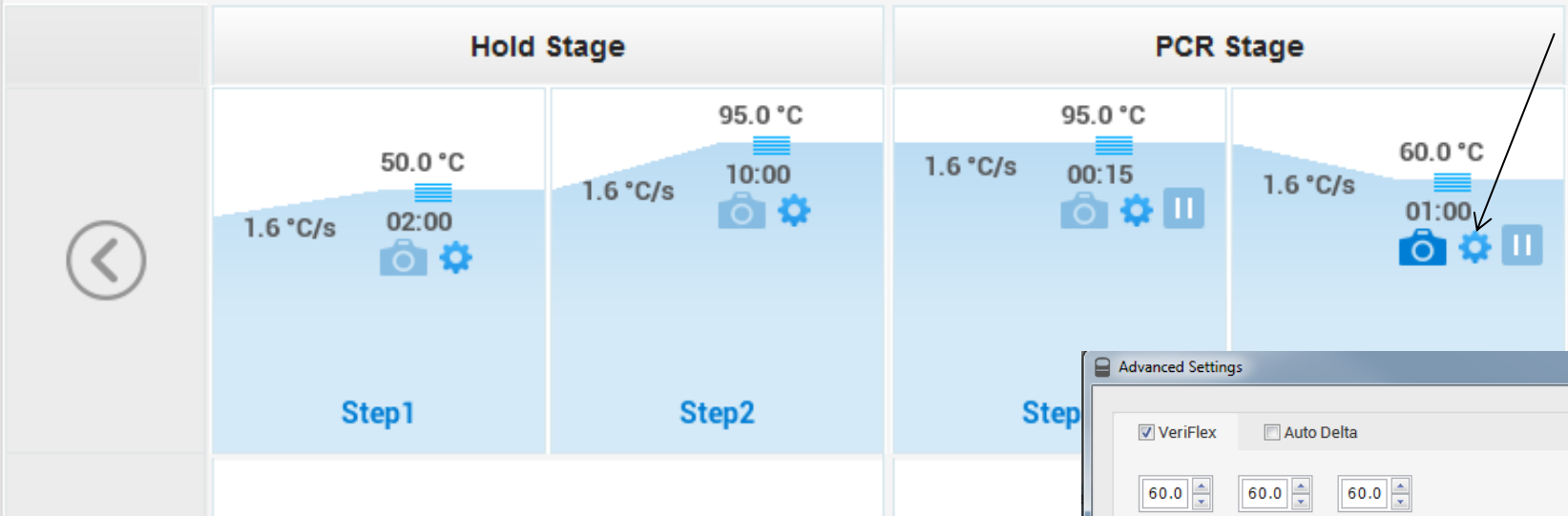
Next

Experiment Method

Volume	Cover
20 μ L	105.0 $^{\circ}$ C

Advanced Settings

- Veriflex™
- AutoDelta



Legends: Data Collection On Data Collection Off Pause On

Advanced Settings

VeriFlex Auto Delta

60.0 60.0 60.0
1-4 5-8 9-12

^Temperature difference between adjacent zones <= 5.0

Cancel Save

Assign Targets and Samples

Quick Setup | **Advanced Setup**

Well Attributes

Sample: ▼

Target: ▼

Well Comments:

Plate Attributes

< **View** ▼

	1	2	3
A			
B			
C			
D			
E			
F			
G			
H			


Select well and type sample names

Wells: **U** **O** **S** **O** **N** **O**


Assign Targets and Samples

Quick Setup **Advanced Setup**

























Targets Add Action ▼







	Name	Reporter	Quencher	Comments	Task	Quantity
<input type="checkbox"/>	 Target 1	FAM	NFQ-MGB		▼	<input type="button" value="X"/>

Samples Add Action ▼

	Sample Name	Comments	+
<input type="checkbox"/>	 Sample 1		<input type="button" value="X"/>

View ▼

	1	2	3
A			
B			
C			
D			
E			
F			
G			
H			

Wells:      

Run Control

START RUN



Save



Qt QuantStudio® 3 System

Run Started at: 01-07-2015 01:59:12 UTC

Run Complete at: 01-07-2015 02:32:15 UTC

Post-run summary

Experiment Name	DVT3_4Plex	Start Time	01-07-2015 01:59:12 UTC
Stop Time	01-07-2015 02:32:15 UTC	Run Duration	33 minutes and 2 seconds
User Name	DEFAULT	Instrument Name	QuantStudio® 3 System QuantStudio® 5 System
Firmware Version	0.11.1	Software Version	NA
Instrument Serial Number	dvt003	Sample Volume	10 QuantStudio® 3 System
Cover Temperature	105	Instrument Type	QuantStudio® 5 System
Block Type	96-Well 0.2-mL Block		
Errors Encountered			

Start Run from touchscreen or desktop

Results

View

Amplification Plot

Amplification Plot

Multicomponent Plot

Raw Data Plot

QC Summary

Standard Curve

Amplification Plot

ΔRn

Cycle

View

Group by

#	Well	Omit	Flag	Sample Na...	Target Na...	Task
1	A1	<input type="checkbox"/>	✓	NTC	KAZ	NTC
2	A2	<input type="checkbox"/>	✓			
3	A3	<input type="checkbox"/>	✓	1E1	KAZ	STANDA...
4	A4	<input type="checkbox"/>	✓	1E2	KAZ	STANDA...
5	A5	<input type="checkbox"/>	✓	1E3	KAZ	STANDA...
6	A6	<input type="checkbox"/>	✓	1E4	KAZ	STANDA...
7	A7	<input type="checkbox"/>	✓	1E5	KAZ	STANDA...
8	A8	<input type="checkbox"/>	✓	1E6	KAZ	STANDA...
9	A9	<input type="checkbox"/>	✓	1E7	KAZ	STANDA...
10	A10	<input type="checkbox"/>	✓	1E8	KAZ	STANDA...
11	A11	<input type="checkbox"/>	✓	1E9	KAZ	STANDA...
12	A12	<input type="checkbox"/>	✓	1E10	KAZ	STANDA...
13	B1	<input type="checkbox"/>	✓	NTC	KAZ	NTC

Wells: 0 80 8

Toggle between plate view and well details

Results

Action Save

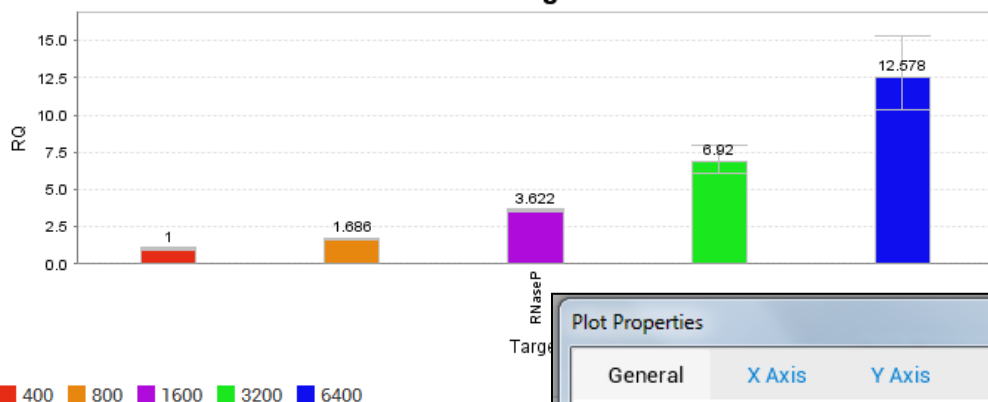
Icons for zoom, print, copy, paste, refresh, and view.

Gene Expression

View

Table icons for grid and list views.

RQ vs Target



#	Cr Mean	Δ Cr Mean	Δ Cr SD	$\Delta\Delta$ Cr	RQ	RQ Min	RQ Max
1	25.455	-4.47	0.103	-3.653	12.578	10.308	15.3
2	29.925						
3	29.354	-0.817	0.068	0	1	0.903	1.1
4	27.379	-2.674	0.018	-1.857	3.622	3.528	3.7
5	28.391	-1.57	0.018	-0.753	1.686	1.641	1.7
6	26.38	-3.608	0.091	-2.791	6.92	6.041	7.9

Plot Properties

General X Axis Y Axis

Title

Text: RQ vs Target

Font: SansSerif.bold, 18 **Select**

Color: 0, 0, 0

Show Title

Bar

Show Error Bar

Show Value Label

Show Target Label

Show Sample Label

20 Group Spacing in %

60 Bar Width in %

Cancel Save

Font Chooser

Roboto Regular	PLAIN	13
Tw Cen MT Condensed Extra Bold	Plain	13
Utsaah	Bold	14
Vani	Italic	15
Verdana	Bold/Italic	16
Vijaya		17
Viner Hand ITC		18
Vivaldi		19
Vladimir Script		20
Wrinda		22
Webdings		24
Wide Latin		27
Wingdings		30
Wingdings 2		34
Wingdings 3		39
Roboto Regular		45

The quick brown fox jumps over the lazy dog.

OK Cancel

Results

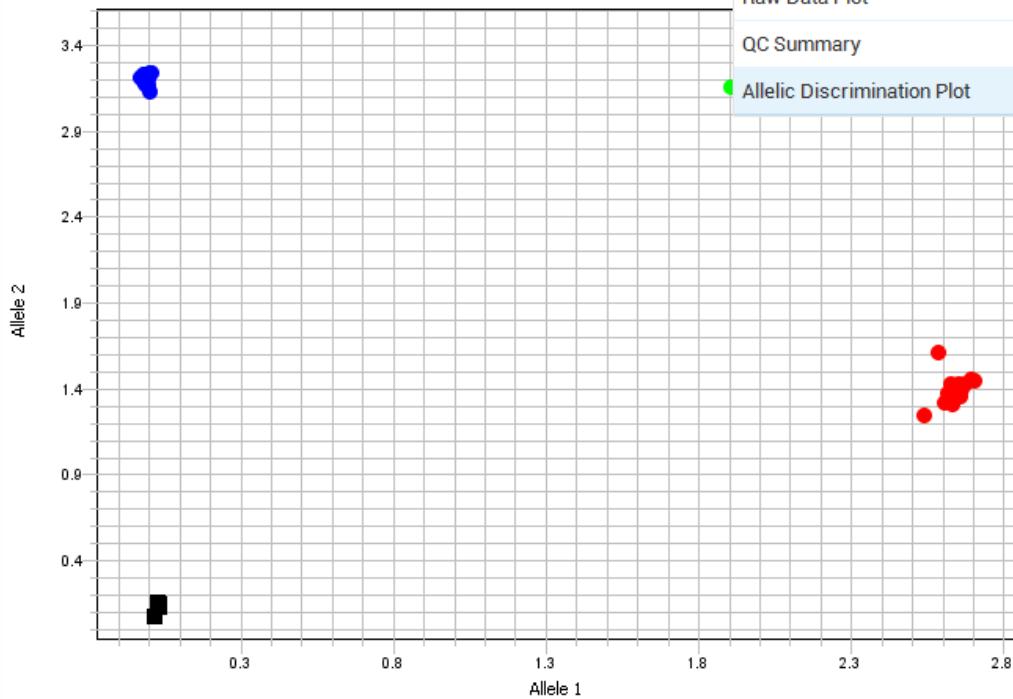
Action ▼ Save ▼

🔍 🔍 🖨️ 📄 📏 🗨️ 📄 👁️

Allelic Discrimination Pl... ▼

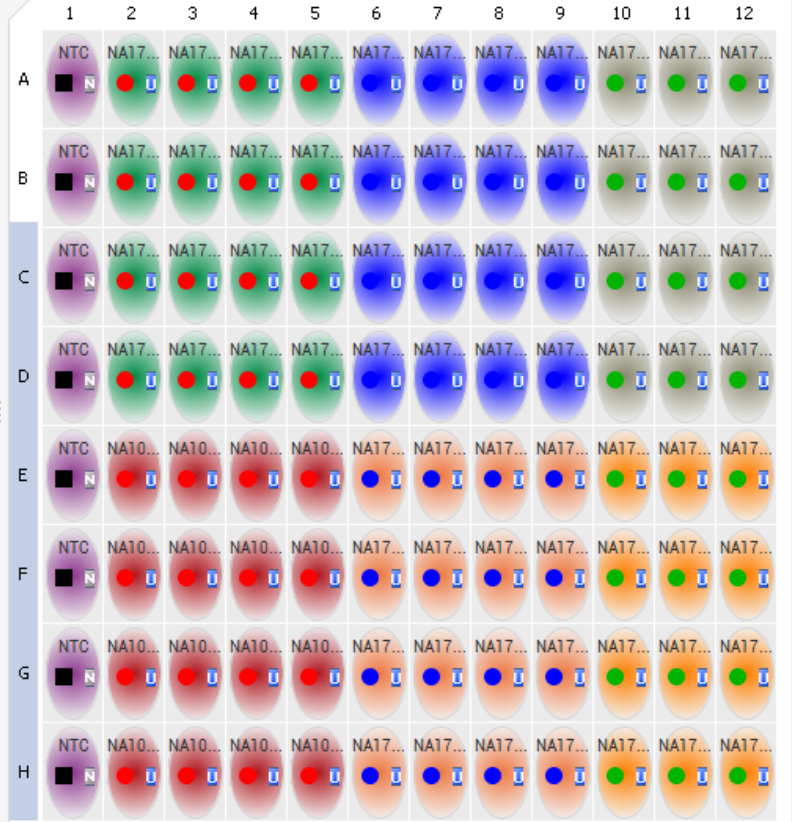
- Amplification Plot
- Multicomponent Plot
- Raw Data Plot
- QC Summary
- Allelic Discrimination Plot

Allelic Discrimination Plot



● Homozygous Allele 1/Allele 1 ● Homozygous Allele 2/Allele 2
 ● Heterozygous Allele 1/Allele 2 X Undetermined

View ▼ 🔍 🔍 ↻ 📄 📄



Wells: U 88 N 8 I 0 Z 2 0 P 2 0

0 Empty

Export

 Auto Export

Export

 Save

Name of export file

File Type

QuantStudio



(*.xls)



Location

C:\Applied Biosystems\QuantStudio Design & Ar

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Content

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Real-Time PCR 實驗配置說明



Real-Time PCR RT-PCR protocol

在這堂 15 分鐘的線上教學課程中，主講者將詳細介紹進行 Real-Time PCR 實驗配置，包含 Reverse Transcription 及 Real-Time PCR 反應配置流程。



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