



## iQ5 Software Training-Data analysis



**iQ5** Multicolor Real-Time PCR Detection System



**iQ5**<sup>TM</sup>  
Optical System Software  
Version 2.0

**BIO-RAD**

Bio-Rad Laboratories, Inc.  
© 2006. All rights reserved.

The image shows a Bio-Rad iQ5 Real-Time PCR Detection System. It is a white, compact, benchtop instrument with a green base. The top panel has a green indicator light and the text "iQ5 Multicolor Real-Time PCR Detection System". The front panel features a color LCD screen and a keypad with various colored buttons. The Bio-Rad logo is visible on the front panel.

# iQ5 Overview

The screenshot displays the Bio-Rad iQ5 software interface in the Setup tab. The interface is organized into several main sections:

- Left Panel:** Contains navigation icons for Workshop, Run-Time Central, Data Analysis, Calibration, and User Profile.
- Top Panel:** Shows tabs for Setup (active), Plate Summary, Protocol, Plate, Run Set, and Data File.
- File Browser:** A tree view on the left shows folders like Drivers, Images, Logs, Masks, etc. A list on the right shows files such as 'MylQ 2-fold Dilution Data' and 'MylQ 96well SYBR Uniformity Data'.
- Selected Data File:** Displays 'Sample 96well SYBR Uniformity Data.opd'. Below it, a 'Notes' field contains the text: 'SYBR uniformity test using IL1-beta plasmid in 96 replicates including a Melt Curve'. Buttons for 'Run', 'Run End Point', and 'Analyze' are present.
- Selected Protocol:** Shows '25StepStandard.meltcurve.tmo within Sample 96well SYB'. It includes a table for cycle steps and a thermal profile graph.
- Selected Plate Setup:** Shows 'SYBRUniformity25ul.pts within Sample 96well SYBR Unifor'. It includes fields for 'Sample Volume : 25ul', 'Seal Type : Film', and 'Vessel Type : Plates'. A 'SYBR' radio button is selected.
- Plate Grid:** An 8x12 grid representing a 96-well plate. All wells are highlighted in green and contain the number '1', indicating they are all set for SYBR detection.
- Bottom Panel:** Features a table for cycle details and a 'BIO-RAD' logo.

Cycle	Repeats	Step	Dwell Time	Hold	Setpoint	PCR / Melt De Acquisition
1	1					
1	1	1	3:00	<input type="checkbox"/>	95.0	
2	40					
		1	0:10	<input type="checkbox"/>	95.0	

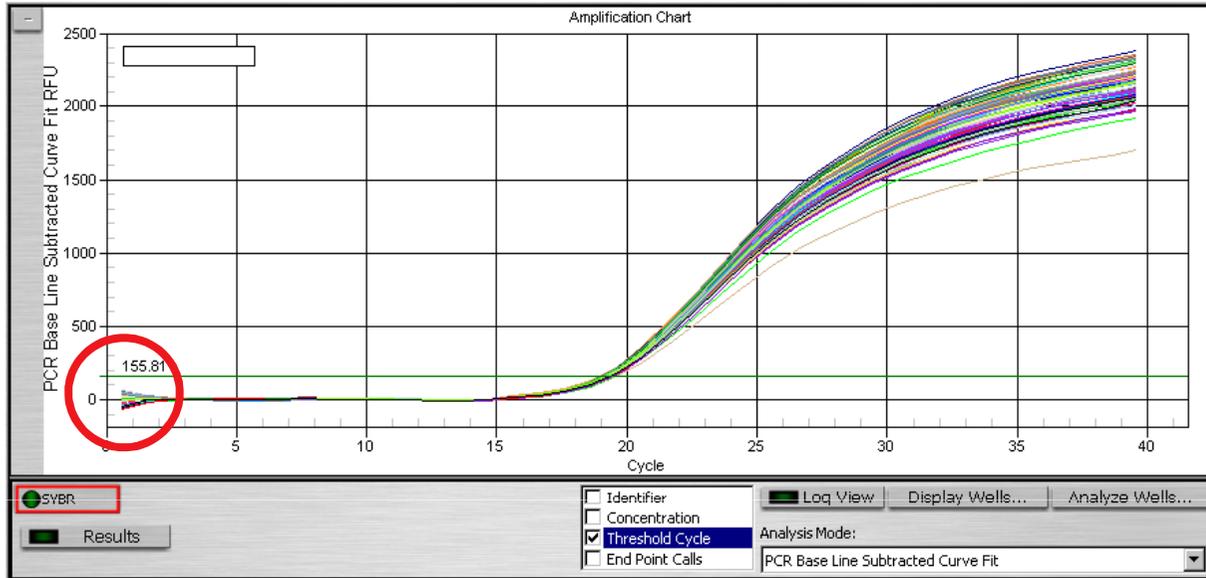
# Ct: Automatic Determination

The screenshot displays the Bio-Rad iQ5 software interface. The main window shows an 'Amplification Chart' with 'PCR Base Line Subtracted Curve Fit RFU' on the y-axis and 'Cycle' on the x-axis. A horizontal threshold line is drawn at 155.81. A context menu is open over the chart, with 'BaseLine Threshold...' selected. A dialog box titled 'Base Line Threshold Parameter-SYBR' is also open, showing 'Auto Calculated' selected for both 'Base Line Cycles' and 'Crossing Threshold'. The 'Crossing Threshold' dialog shows a 'Threshold Position' of 155.81.

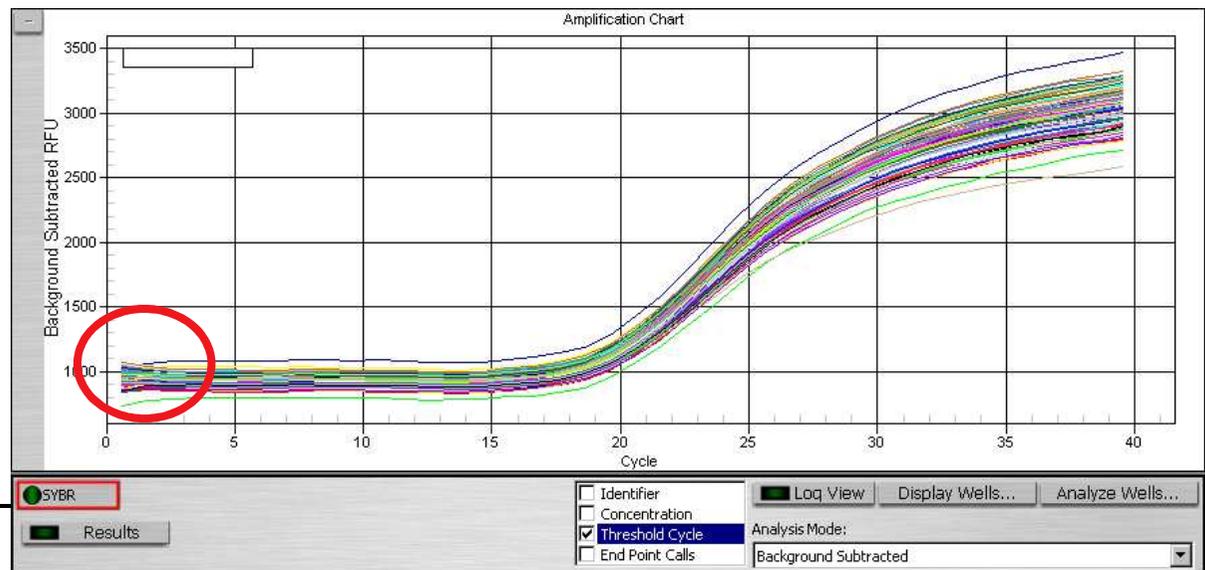
The data table below the chart shows the following information:

	1	2	3	4	5	6	7	8	9	10	11	12
A SampleType	Unkn-1											
A ThresholdCycle	19.58	19.42	19.25	19.20	19.24	19.36	19.09	19.20	19.03	19.29	19.21	19.51
B SampleType	Unkn-1											
B ThresholdCycle	19.24	19.30	19.15	19.07	18.89	19.09	18.98	19.05	19.00	19.56	19.13	19.26
C SampleType	Unkn-1											
C ThresholdCycle	19.18	19.19	19.10	19.03	18.92	19.00	18.95	19.05	19.00	19.05	19.14	19.29
D SampleType	Unkn-1											
D ThresholdCycle	19.23	19.09	19.01	19.09	18.99	19.18	19.04	19.00	19.19	19.10	19.07	19.22

# 3 Analysis Mode for Troubleshooting



- Analysis Mode:
- PCR Base Line Subtracted Curve Fit
  - Background Subtracted
  - PCR Base Line Subtracted
  - PCR Base Line Subtracted Curve Fit

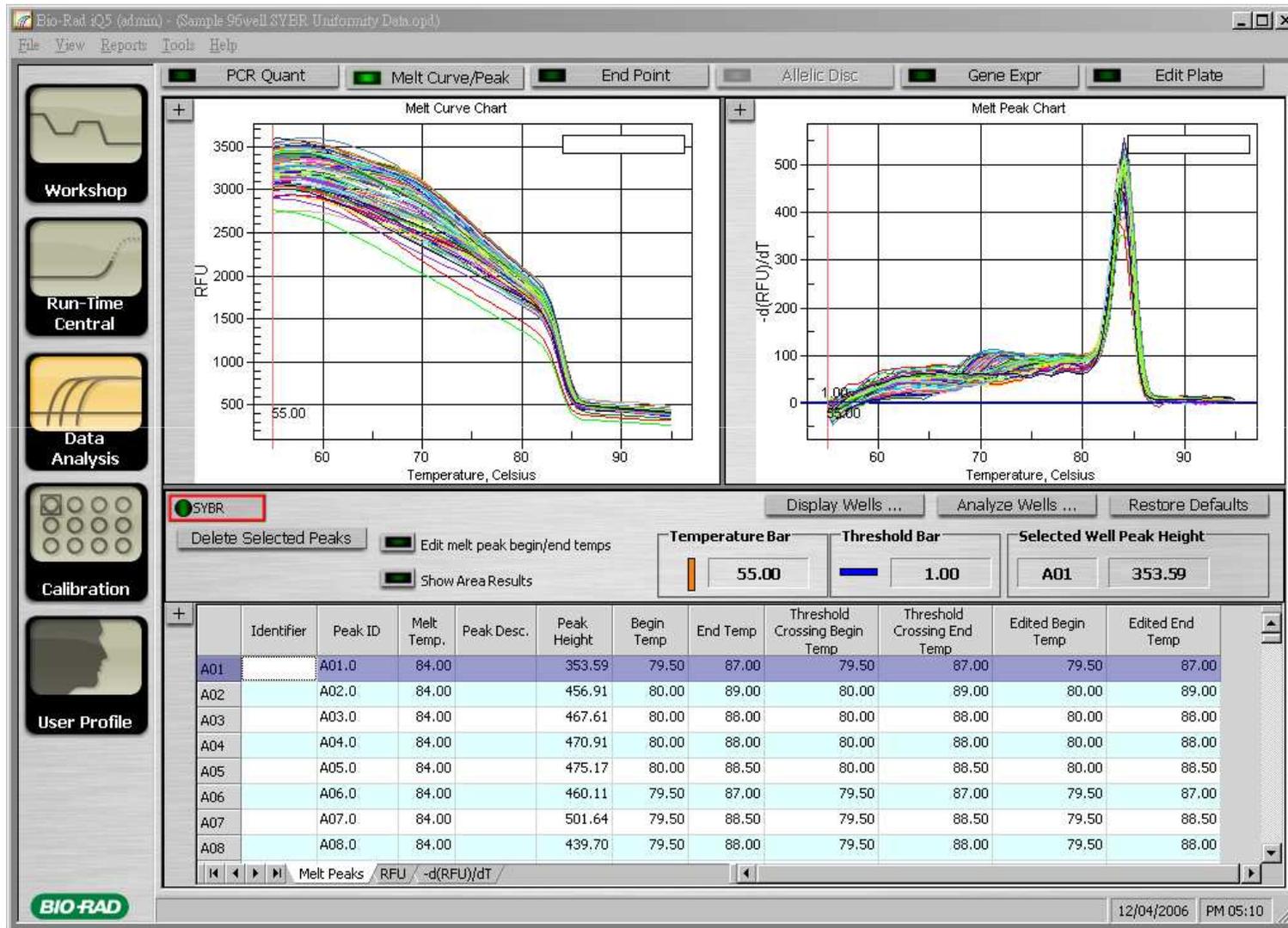


# Detailed Results

The screenshot displays the Bio-Rad iQ5 software interface. The top window shows a single amplification curve with a threshold cycle (Ct) of 155.81. The bottom window shows multiple amplification curves for wells A01 through A07, with a Ct of 155.81 indicated on the y-axis. The 'Results' tab is highlighted in red, and a table below it provides detailed data for each well.

Well	Fluor	Type	Identifier	Replicate #	Threshold Cycle (Ct)	Ct Mean	Ct Std. Dev	Set Point
1	A01	SYBR	Unkn	1	19.58	19.14	0.143	N/A
2	A02	SYBR	Unkn	1	19.42	19.14	0.143	N/A
3	A03	SYBR	Unkn	1	19.25	19.14	0.143	N/A
4	A04	SYBR	Unkn	1	19.20	19.14	0.143	N/A
5	A05	SYBR	Unkn	1	19.24	19.14	0.143	N/A
6	A06	SYBR	Unkn	1	19.36	19.14	0.143	N/A
7	A07	SYBR	Unkn	1	19.09	19.14	0.143	N/A

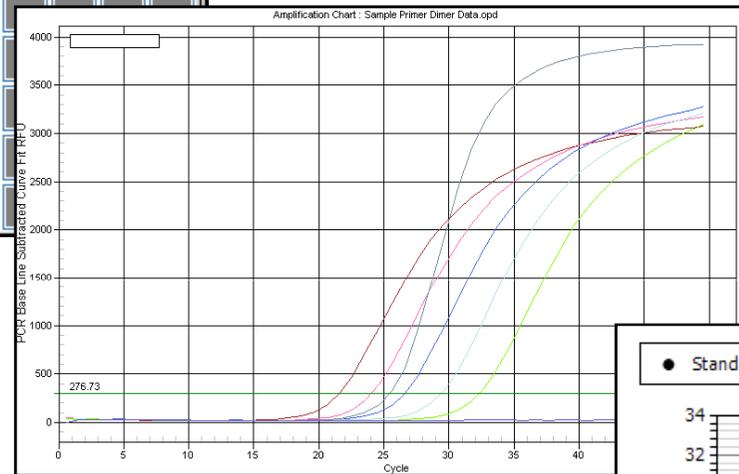
# Melting Curve Analysis



# Absolute Quantization

## 絶対定量

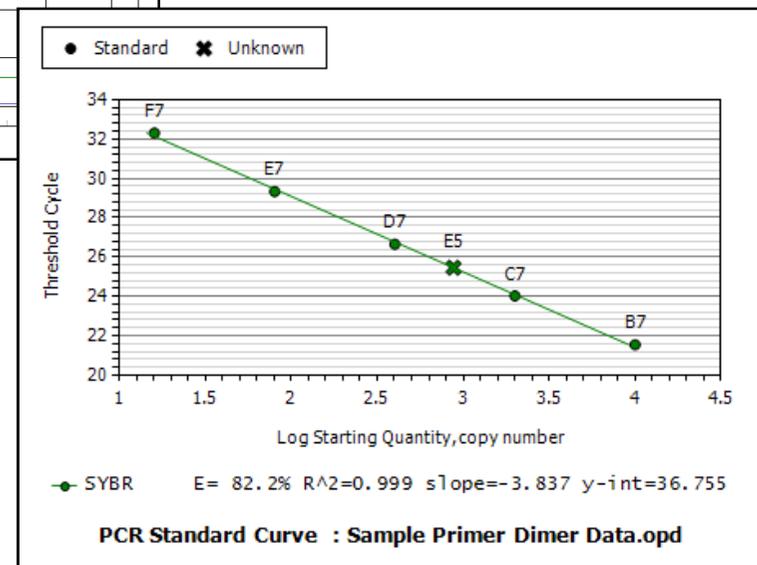
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B							6					
C							7					
D							8					
E				1			9					
F							10					
G												
H							2					



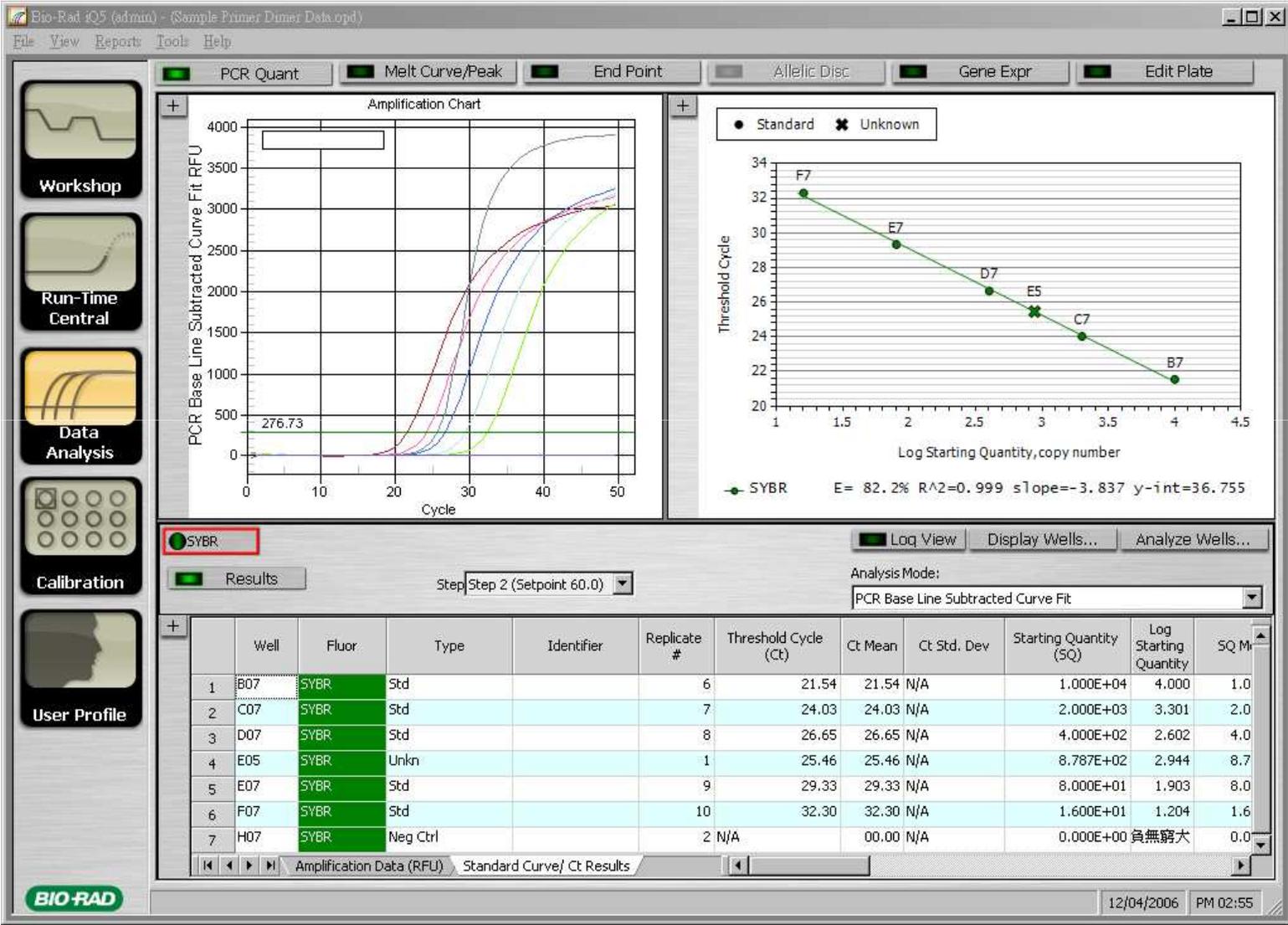
Auto-Calculation

Set up the  
Sample Type and  
Concs:  
Standard or  
Unknown

Determine Ct Value



# AQ Results



# Relative Quantization

## 相對定量 ( $\Delta\Delta\text{Ct}$ method)

	Before	After
Target gene	Ct SB	Ct SA
Reference gene	Ct CB	Ct CA

$\Delta\text{Ct B}$  (change from Ct SB to Ct CB)

$\Delta\text{Ct A}$  (change from Ct SA to Ct CA)

$$\text{Ratio} = ( 2 )^{-\Delta\Delta\text{Ct}}$$

$$\Delta\Delta\text{Ct} = (\Delta\text{Ct A} - \Delta\text{Ct B})$$

# Normalized Expression

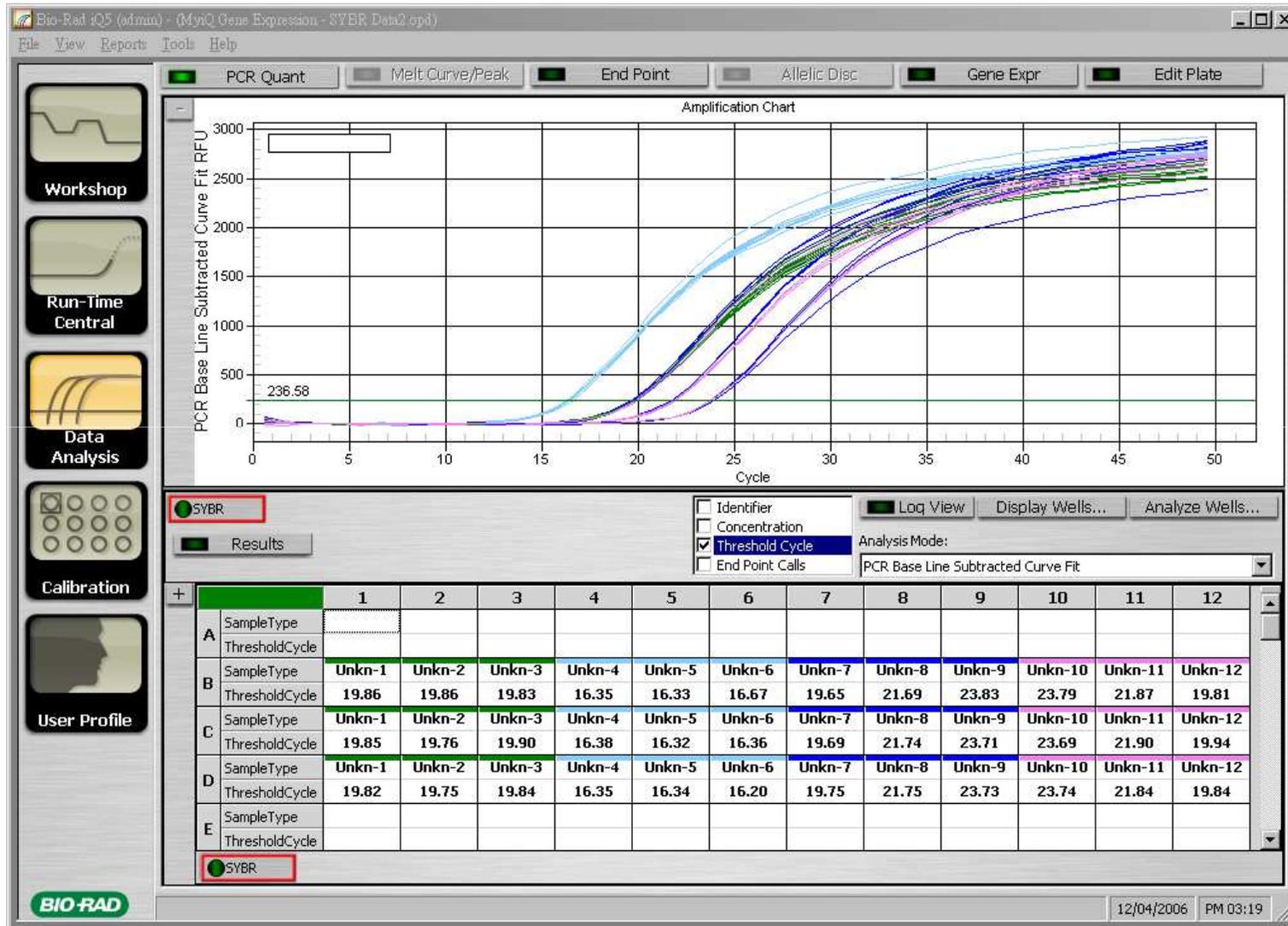
- **$\Delta\Delta C_T$  (Livak)**
  - Assume 100% efficiency
  - Only one Ref Gene
- **Pfaffl Modification**
  - Accounts for efficiency differences
  - Only one Ref Gene
- **Vandesompele**
  - Accounts for **efficiency differences**
  - Allows **multiple reference genes** for normalization

Simple



Complex

# Gene Expression Analysis Ct Determination



# Gene Expression Tab

PCR Quant   Melt Curve/Peak   End Point   Allelic Disc   Gene Expr   Edit Plate

Workshop  
Run-Time Central  
Data Analysis  
Calibration  
User Profile

Graph Data  
 Relative to control  
 Relative to zero

x-Axis Options  
 Condition  
 Gene

y-Axis Options  
 Log 2  
 Linear

Scaling Options  
 Highest  
 Lowest  
 Unscaled

Graph error  
 +/- Std Devs   1

Setting   Data Table  
 Gene List    Condition List    Data Set List

	Name	Full Name	Ref	Color
1	Gapdh	Gapdh	<input checked="" type="checkbox"/>	Blue
2	IL1b	IL1b	<input type="checkbox"/>	Purple
3	Tubulin	Tubulin	<input type="checkbox"/>	Pink
4	Actin	Actin	<input checked="" type="checkbox"/>	Teal

Gene Name:   Condition Name:    Normalized expression (ddCt)   Recalculate  
 Relative quantity (dCt)

Copy condition to all data sets    Enable for Gene Study   Analyze Wells...

	1	2	3	4	5	6	7	8	9	10	11	12
A												
	[actin]	[actin]	[actin]	[gapdh]	[gapdh]	[gapdh]	IL1b	IL1b	IL1b	Tubulin	Tubulin	Tubulin
B	19.86	19.86	19.83	16.35	16.33	16.67	19.65	21.69	23.83	23.79	21.87	19.81
	*O Hr	1 Hr	2 Hr	*O Hr	1 Hr	2 Hr	*O Hr	1 Hr	2 Hr	*O Hr	1 Hr	2 Hr

BIO-RAD   12/04/2006   PM 03:27



# Grouping

Bio-Rad iQ5 (admin) - (MynQ Gene Expression - SYBR Data2.qpd)

File View Reports Tools Help

PCR Quant
  Melt Curve/Peak
  End Point
  Allelic Disc
  Gene Expr
  Edit Plate

Gene Name:  Condition Name:

Normalized expression (ddCt)
  Relative quantity (dCt)
 Recalculate

Copy condition to all data sets
  Enable for Gene Study
 Analyze Wells...

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	[actin]	[actin]	[actin]	[gapdh]	[gapdh]	[gapdh]	IL1b	IL1b	IL1b	Tubulin	Tubulin	Tubulin
	19.86	19.86	19.83	16.35	16.33	16.67	19.65	21.69	23.83	23.79	21.87	19.81
	*O Hr	1 Hr	2 Hr	*O Hr	1 Hr	2 Hr	*O Hr	1 Hr	2 Hr	*O Hr	1 Hr	2 Hr
C	[actin]	[actin]	[actin]	[gapdh]	[gapdh]	[gapdh]	IL1b	IL1b	IL1b	Tubulin	Tubulin	Tubulin
	19.85	19.76	19.90	16.38	16.32	16.36	19.69	21.74	23.71	23.69	21.90	19.94
	*O Hr	1 Hr	2 Hr	*O Hr	1 Hr	2 Hr	*O Hr	1 Hr	2 Hr	*O Hr	1 Hr	2 Hr
D	[actin]	[actin]	[actin]	[gapdh]	[gapdh]	[gapdh]	IL1b	IL1b	IL1b	Tubulin	Tubulin	Tubulin
	19.82	19.75	19.84	16.35	16.34	16.20	19.75	21.75	23.73	23.74	21.84	19.84
	*O Hr	1 Hr	2 Hr	*O Hr	1 Hr	2 Hr	*O Hr	1 Hr	2 Hr	*O Hr	1 Hr	2 Hr
E												
F												

Workshop

Run-Time Central

Data Analysis

Calibration

User Profile

BIO-RAD

12/04/2006 PM 03:28

# Select Ref. Gene and Control

Setting Data Table

Gene List Condition List Data Set List

	Name	Full Name	Ref	Color	Show Graph	Auto Efficiency	Efficiency (%)
1	Gapdh	Gapdh	<input checked="" type="checkbox"/>	Blue	<input checked="" type="checkbox"/>	<input type="checkbox"/>	100.0
2	IL1b	IL1b	<input type="checkbox"/>	Purple	<input checked="" type="checkbox"/>	<input type="checkbox"/>	100.0
3	Tubulin	Tubulin	<input type="checkbox"/>	Pink	<input checked="" type="checkbox"/>	<input type="checkbox"/>	100.0
4	Actin	Actin	<input checked="" type="checkbox"/>	Green	<input checked="" type="checkbox"/>	<input type="checkbox"/>	100.0

Setting Data Table

Gene List Condition List Data Set List

	Name	Full Name	Ctrl	Color	Show Graph
1	0 Hr	0 Hr	<input checked="" type="checkbox"/>	Pink	<input checked="" type="checkbox"/>
2	1 Hr	1 Hr	<input type="checkbox"/>	Brown	<input checked="" type="checkbox"/>
3	2 Hr	2 Hr	<input type="checkbox"/>	Blue	<input checked="" type="checkbox"/>



Bio-Rad iQ5 (admin) - (MvIQ Gene Expression - SYBR Data2.qpd)

File View Reports Tools Help

PCR Quant Melt Curve/Peak End Point Allelic Disc Gene Expr Edit Plate

Workshop Run-Time Central Data Analysis Calibration User Profile

Normalized Fold Expression

IL1b Tubulin

Condition

Graph Data

- Relative to control
- Relative to zero

x-Axis Options

- Condition
- Gene

y-Axis Options

- Log 2
- Linear

Scaling Options

- Highest
- Lowest
- Unscaled

Graph error

+/- Std Devs 1

Setting Data Table

- Gene List
- Condition List
- Data Set List

	Name	Full Name	Ctrl	Color
1	1 Hr	1 Hr	<input type="checkbox"/>	Red
2	2 Hr	2 Hr	<input type="checkbox"/>	Blue
3	0 Hr	0 Hr	<input checked="" type="checkbox"/>	Green

Gene Name: Condition Name: 0 Hr

- Normalized expression (ddCt)
- Relative quantity (dCt)

Recalculate

Analyze Wells...

	1	2	3	4	5	6	7	8	9	10	11	12
B	[actin]	[actin]	[actin]	[gapdh]	[gapdh]	[gapdh]	IL1b	IL1b	IL1b	Tubulin	Tubulin	Tubulin
	19.86	19.86	19.83	16.35	16.33	16.67	19.65	21.69	23.83	23.79	21.87	19.81
	*0 Hr	1 Hr	2 Hr	*0 Hr	1 Hr	2 Hr	*0 Hr	1 Hr	2 Hr	*0 Hr	1 Hr	2 Hr
	Unknown											
C	[actin]	[actin]	[actin]	[gapdh]	[gapdh]	[gapdh]	IL1b	IL1b	IL1b	Tubulin	Tubulin	Tubulin
	19.85	19.76	19.90	16.38	16.32	16.36	19.69	21.74	23.71	23.69	21.90	19.94
	*0 Hr	1 Hr	2 Hr	*0 Hr	1 Hr	2 Hr	*0 Hr	1 Hr	2 Hr	*0 Hr	1 Hr	2 Hr

BIO-RAD

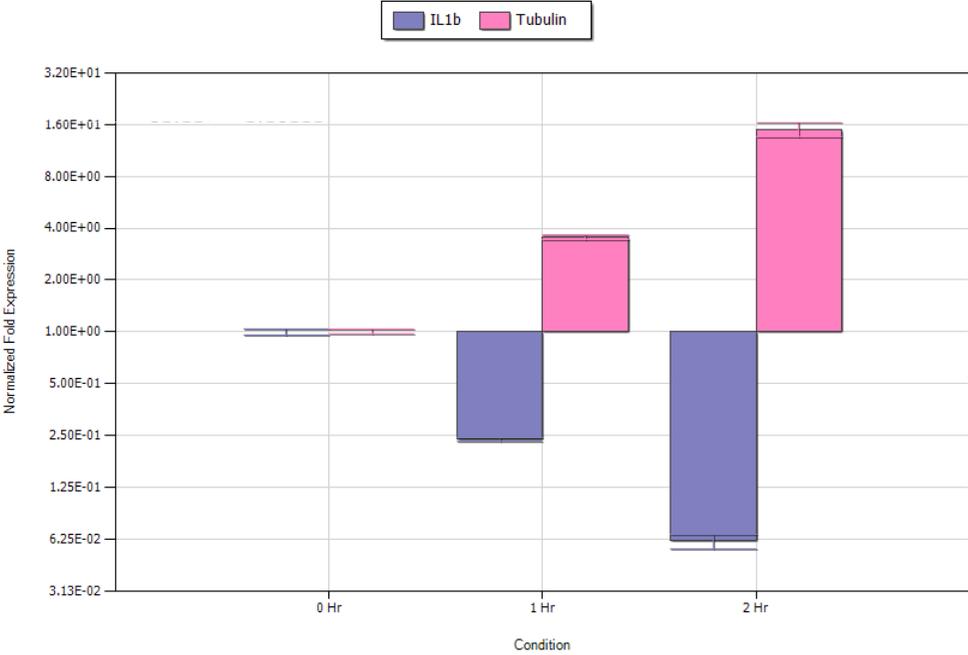
12/04/2006 PM 03:38

# RQ Results

Setting    Data Table

Show Details

	Condition ^	Gene ^	Ctrl	Expression	Expression SD	Corrected Expression SD	Mean Ct	Ct SD
1	0 Hr	Actin	*	N/A	N/A	N/A	19.85	0.02230
2	0 Hr	Gapdh	*	N/A	N/A	N/A	16.36	0.01538
3	0 Hr	IL1b	*	1.00000	0.03635			
4	0 Hr	Tubulin	*	1.00000	0.03285			
5	1 Hr	Actin		N/A	N/A			
6	1 Hr	Gapdh		N/A	N/A			
7	1 Hr	IL1b		0.23757	0.00728			
8	1 Hr	Tubulin		3.56056	0.10845			
9	2 Hr	Actin		N/A	N/A			
10	2 Hr	Gapdh		N/A	N/A			
11	2 Hr	IL1b		0.06109	0.00583			
12	2 Hr	Tubulin		15.03916	1.45200			



Gene Expression : MyiQ Gene Expression - SYBR Data2.opd



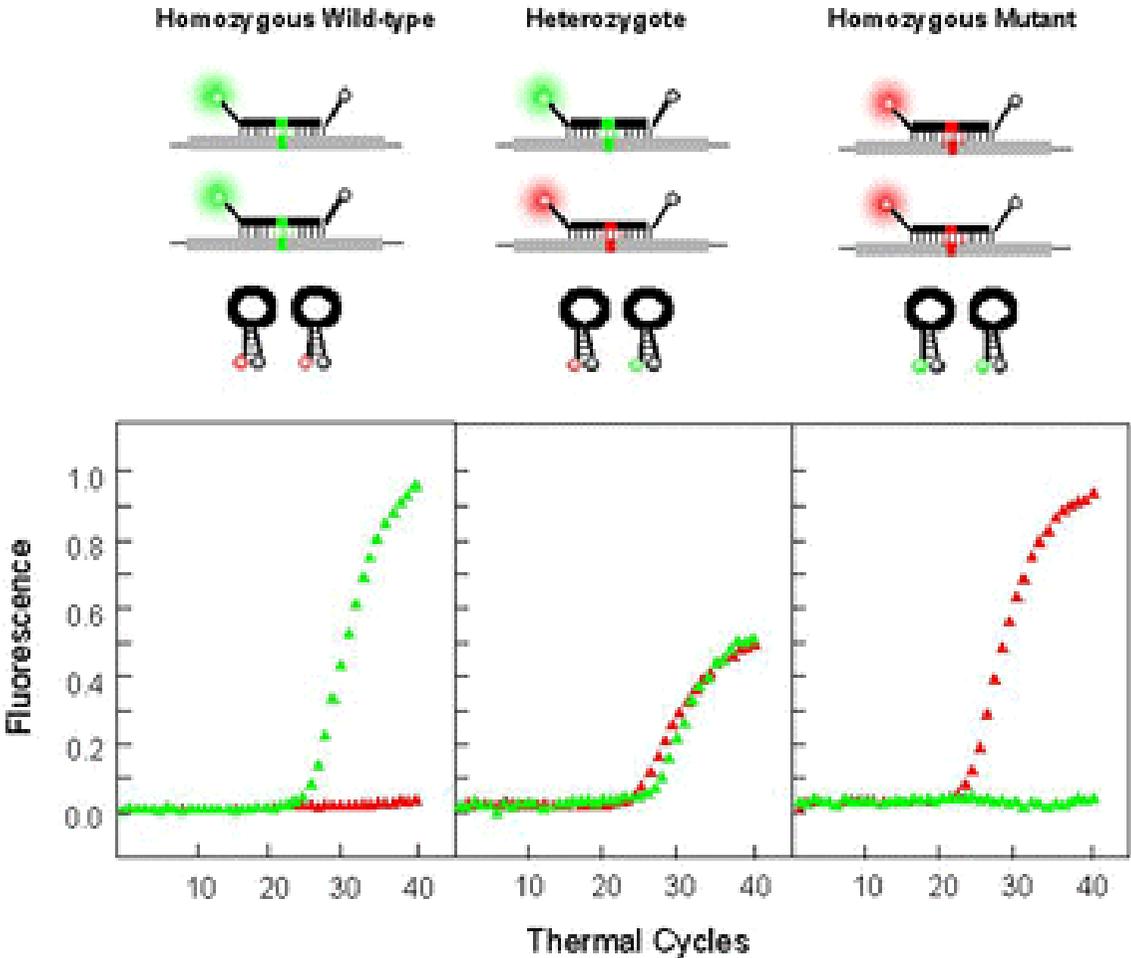


# Multifie Gene Expression - MFGE

- Accomplished through new file type called “Gene Study”
- Created as .gxd file
- .gxd files **maintain Sample ID and C<sub>T</sub> information only**
- Over 50 data files can be imported into a Gene Study
  - Over **5,000** wells of data can be analyzed in a Gene Study using the iQ5 Gene Expression module
  - This is approx. 50 full plates (data files) of single color real-time PCR data or 25 plates of dual-color data, etc...



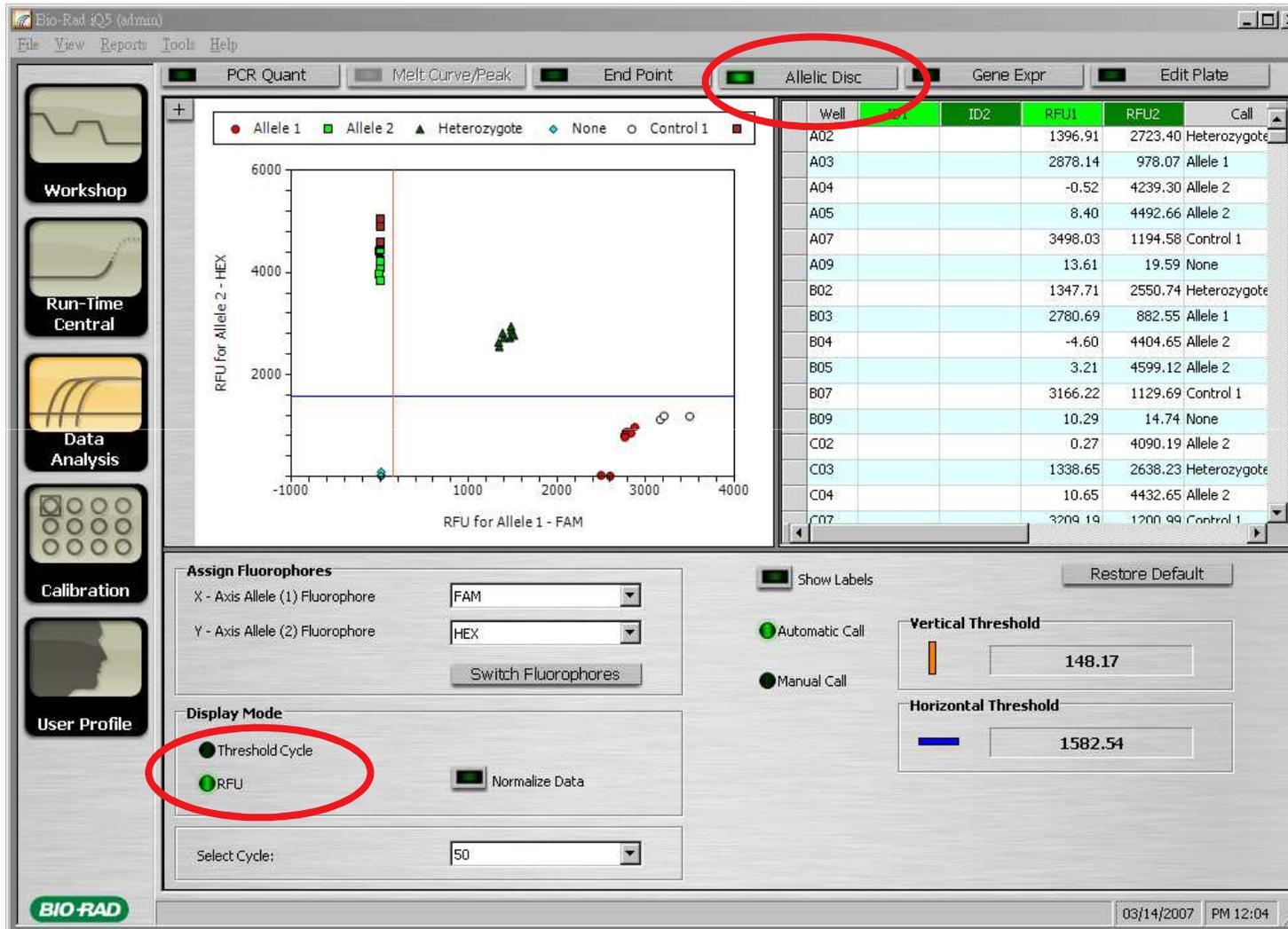
# SNP detection by Probe



Kostrikis et al. Science vol. 279 p. 1228-1229



# Allelic Discrimination



# Detailed Reports

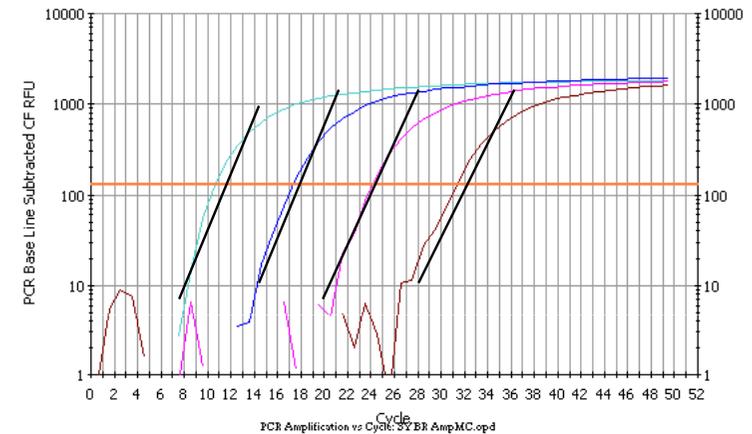
The screenshot shows the Bio-Rad iQ5 software interface. The 'Reports' menu is circled in red. The 'Report Viewer' window displays a 'PCR Quantification Report' for 'PCR Quant Detailed'. The 'Sort Data By' dropdown is set to 'None', and the 'Ascending Order' radio button is selected. The 'Save to File' button is circled in red. The report content includes the Bio-Rad logo, the title 'PCR Quantification Detailed Report', and the subtitle 'PCR Base Line Subtracted Curve Fit Data (Texas) Contains All Available Data'. The 'General Data' section lists the following information:

Field	Value
Data File Name	Sample Dynamic Range Data.opd
Data File Path	C:\Program Files\Bio-Rad\iQ5\SampleFile
Collected Data	Collected Data
Current Date	03/14/2007 PM 12:07:57
Run Date	03/03/2006 PM 03:50:17
User aborted the run	No
Active RMEs	Original
Active Well Factors	Dynamic
Background Readings Valid	Yes
RME Valid	Yes
Well Factors Valid	Yes
Plate Setup File Name	TexrdLin1e10to1e1p&Q25ul.pts
Plate Setup File Path	C:\Program Files\Bio-Rad\iQ5\User1\Nahe
Protocol File Name	2StepStandard.tmo
Protocol File Path	C:\Program Files\Bio-Rad\iQ5\User1\Nahe
Computer name	LSGXP-01004735
Created by app	iQ5.exe (v2.0.104.60221)
Created by user	BioRad\admin
Creation Date	03/03/2006 PM 03:50:17
Created in Security Edition	No
Last Creation GUID	7416edc2-4d9f-4785-995a-e668b490c4e9
Modified by user	BioRad\admin
Last modified date	03/08/2006 AM 10:03:26
OS Build and Service Pack	2600 (Service Pack 2)

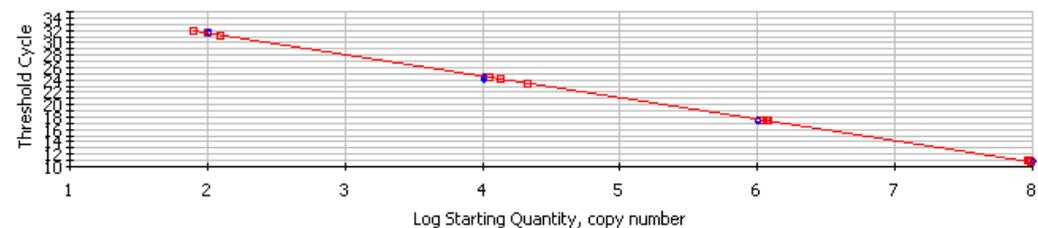
# General QPCR Working Process

## -- Data analysis in iQ5

- Amplification plot
  - Reproducibility? So you need duplication or triplication....
  - Determination Ct Value? Threshold
- Absolute Q., Relative Q. or SNP
  - $\Delta C_t$  or  $\Delta\Delta C_t$
  - Allelic Discrimination
- PCR efficiency from std. Curve
  - <100%
  - >100%
  - Dynamic range
- Reproducibility
  - Duplication or triplication
- Melting curve analysis
  - Primer dimmer
  - Non specific production



Correlation Coefficient: 1.000 Slope: -3.450 Intercept: 38.443  $Y = -3.450 X + 38.443$   
PCR Efficiency: 94.9 %



PCR Standard Curve: SYBR AmpMC.opd





0000524288  
0001013176  
0002097152  
0004191304  
0006308508  
0016777216  
0033651482  
0057108974

- General introduction
- Instrument Operation
- Data analysis
  - 決定 Ct 値
  - Melting Curve
  - Absolute Quantification
  - Relative Quantification



# iQ5 Software Data analysis

---

- General: Ct determination and melting curve
- AQ
- RQ

# Ct: Automatic Determination

The screenshot displays the Bio-Rad iQ5 software interface. At the top, there are menu options: File, View, Reports, Tools, Help. Below the menu is a toolbar with buttons for PCR Quant, Melt Curve/Peak, End Point, Allelic Disc, Gene Expr, and Edit Plate. The main window shows an 'Amplification Chart' with a graph of fluorescence versus cycle number. A vertical line is drawn at cycle 155.81. A context menu is open over the graph, listing options like 'Set Data Analysis Window...', 'Digital Filter...', 'BaseLine Threshold...', 'Single Point', 'All Candidates', 'Adjust Graph...', 'Define Trace Style...', 'Display Data', 'Restore Graph', 'Show All Traces', 'Copy Graph', 'Print Graph...', 'Print Amplification Data', and 'Print Std. Curve Data'. A 'Base Line Threshold Parameter-SYBR' dialog box is also open, showing a table of wells and a 'Crossing Threshold' field set to 155.81.

**Base Line Threshold Parameter-SYBR**

**Base Line Cycles**

Auto Calculated  
 User Defined

	Well	Fluor	Start	End
1	H12	SYBR	2	16
2	H11	SYBR	2	16
3	H10	SYBR	2	15
4	H9	SYBR	2	15
5	H8	SYBR	2	15
6	H7	SYBR	2	15
7	H6	SYBR	2	15

\* Indicates changed value

**Crossing Threshold**

Auto Calculated  
 User Defined

Threshold Position: 155.81

OK Cancel

**Results**

		1	2	3	4	5	6	7	8	9	10	11	12
A	SampleType	Unkn-1											
A	ThresholdCycle	19.58	19.42	19.25	19.20	19.24	19.36	Unkn-1	Unkn-1	Unkn-1	Unkn-1	Unkn-1	Unkn-1
B	SampleType	Unkn-1											
B	ThresholdCycle	19.24	19.30	19.15	19.07	18.89	19.09	Unkn-1	Unkn-1	Unkn-1	Unkn-1	Unkn-1	Unkn-1
C	SampleType	Unkn-1											
C	ThresholdCycle	19.18	19.19	19.10	19.03	18.92	19.00	Unkn-1	Unkn-1	Unkn-1	Unkn-1	Unkn-1	Unkn-1
D	SampleType	Unkn-1											
D	ThresholdCycle	19.23	19.09	19.01	19.09	18.99	19.18	19.04	19.00	19.19	19.10	19.07	19.22

12/04/2006 PM 05:00





Bio-Rad iQ5 (admin) - (MyiQ Gene Expression - SYBR Data2.qpd)

File View Reports Tools Help

PCR Quant Melt Curve/Peak End Point Allelic Disc Gene Expr Edit Plate

Workshop  
Run-Time Central  
Data Analysis  
Calibration  
User Profile

Graph Data  
 Relative to control  
 Relative to zero

x-Axis Options  
 Condition  
 Gene

y-Axis Options  
 Log 2  
 Linear

Scaling Options  
 Highest  
 Lowest  
 Unscaled

Graph error  
 +/- Std Devs 1

Setting Data Table

Gene List  Condition List  Data Set List

	Name	Full Name	Ref	Color
1	Gapdh	Gapdh	<input checked="" type="checkbox"/>	Blue
2	IL1b	IL1b	<input type="checkbox"/>	Purple
3	Tubulin	Tubulin	<input type="checkbox"/>	Pink
4	Actin	Actin	<input checked="" type="checkbox"/>	Teal

Gene Name:  Condition Name:

Copy condition to all data sets  Enable for Gene Study Analyze Wells...  Normalized expression (ddCt)  Relative quantity (dCt) Recalculate

	1	2	3	4	5	6	7	8	9	10	11	12
A												
	[actin]	[actin]	[actin]	[gapdh]	[gapdh]	[gapdh]	IL1b	IL1b	IL1b	Tubulin	Tubulin	Tubulin
B	19.86	19.86	19.83	16.35	16.33	16.67	19.65	21.69	23.83	23.79	21.87	19.81
	*O Hr	1 Hr	2 Hr	*O Hr	1 Hr	2 Hr	*O Hr	1 Hr	2 Hr	*O Hr	1 Hr	2 Hr

BIO-RAD

12/04/2006 PM 03:27