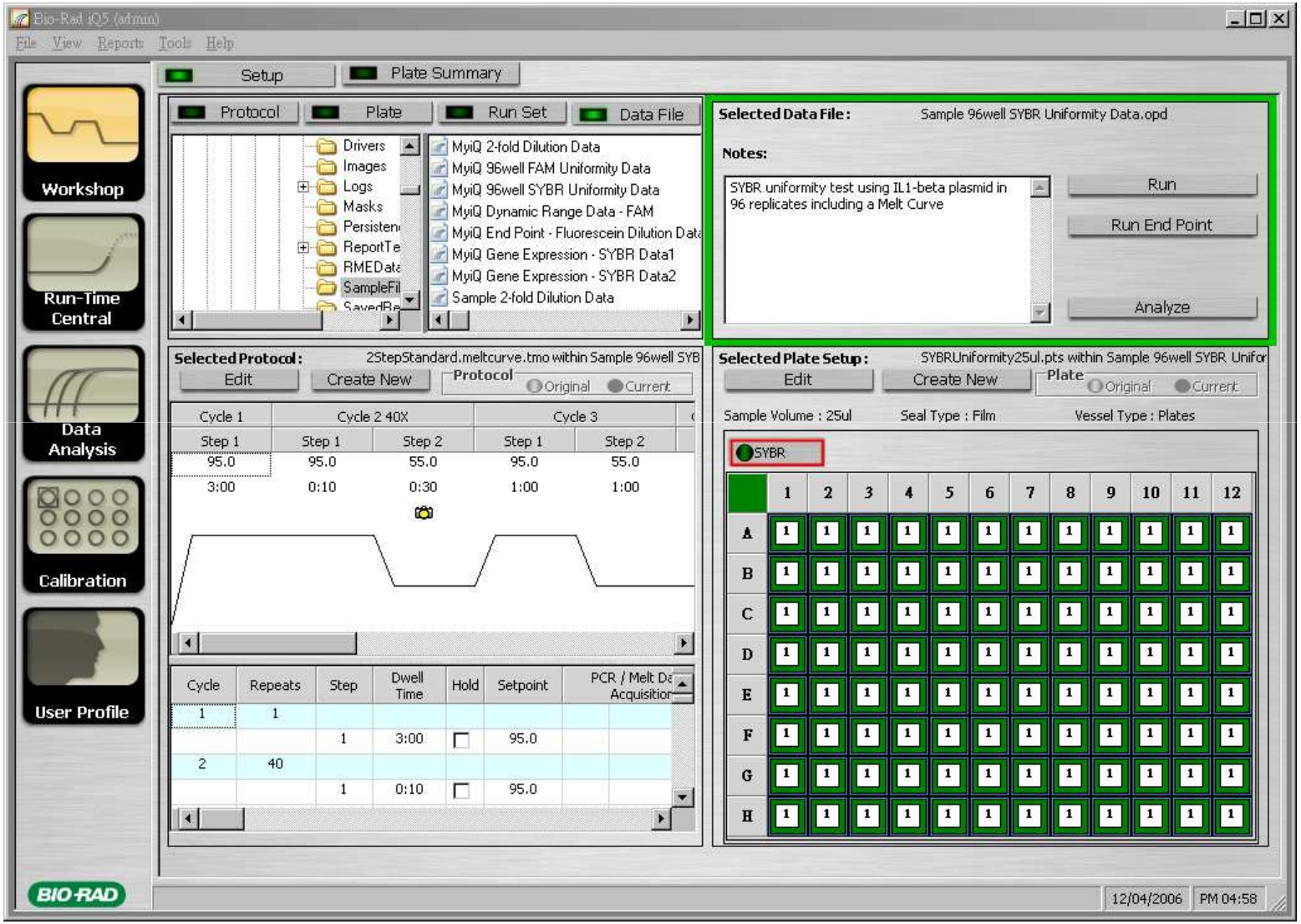
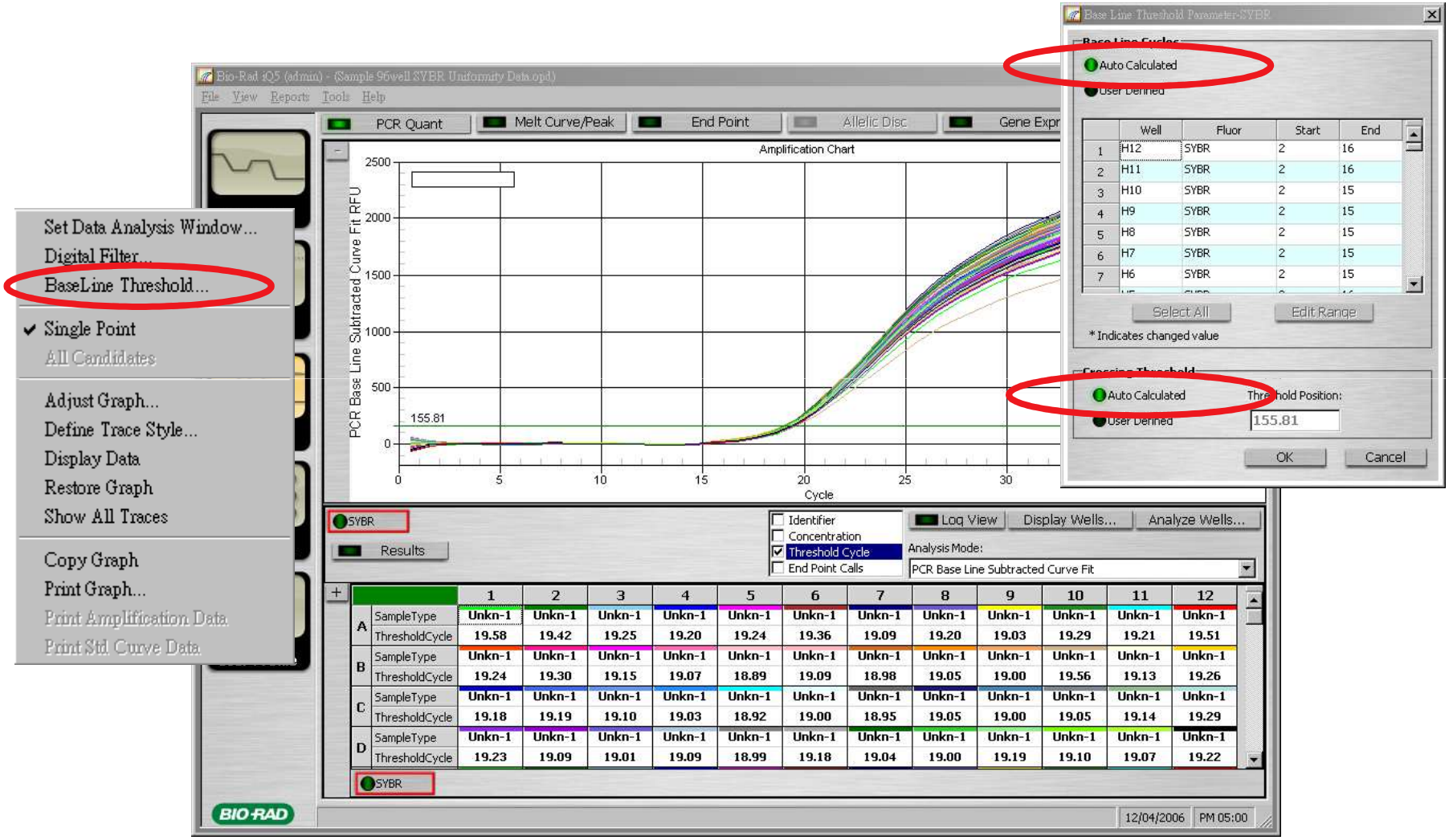
iQ5 Software Training-Data analysis

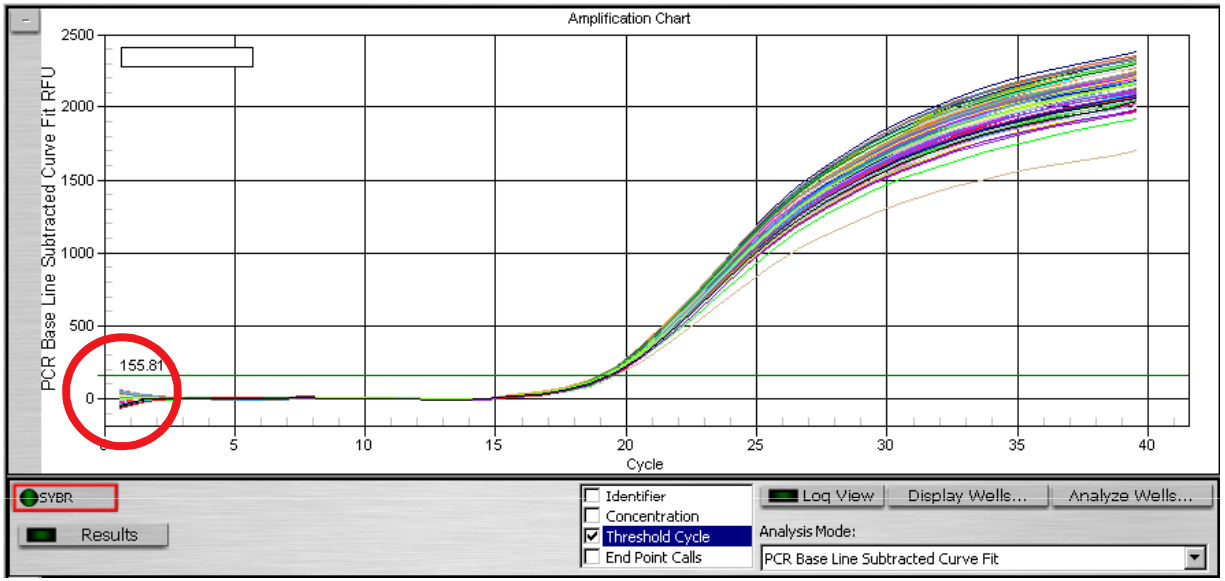
iQ5 Overview

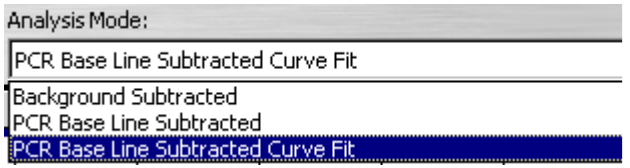


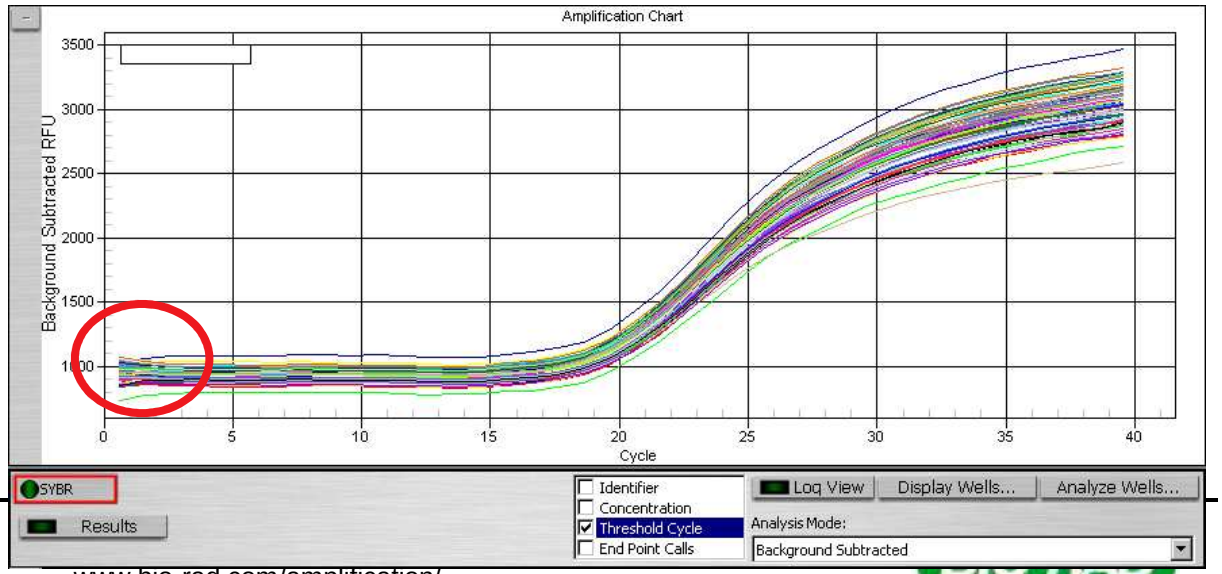
Ct: Automatic Determination



3 Analysis Mode for Troubleshooting



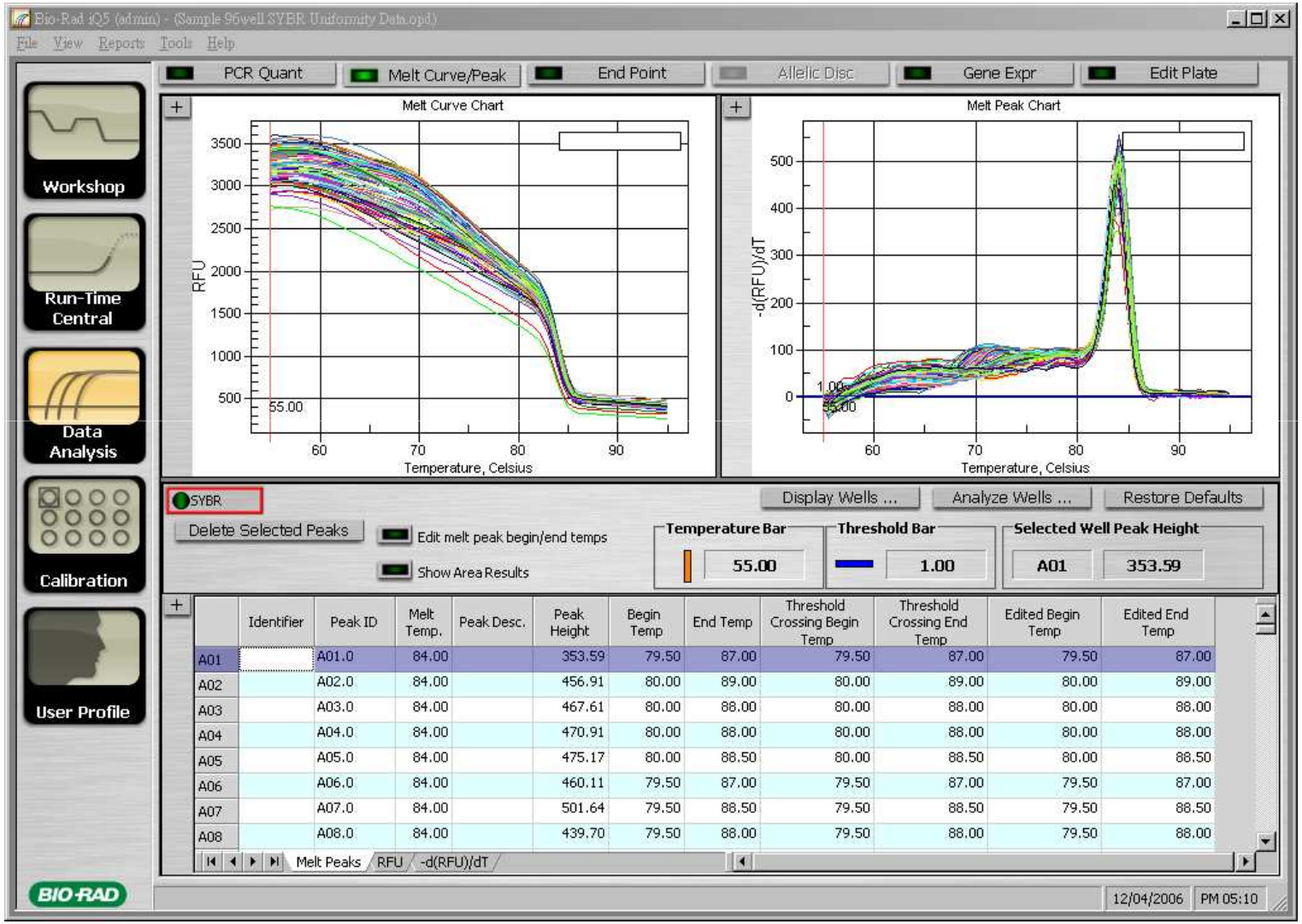




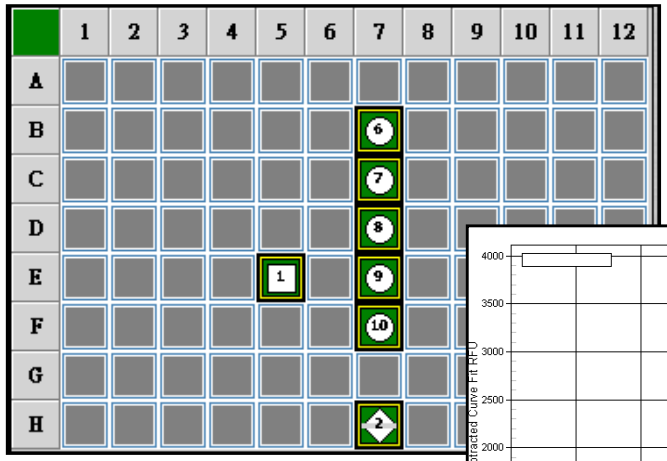
Detailed Results

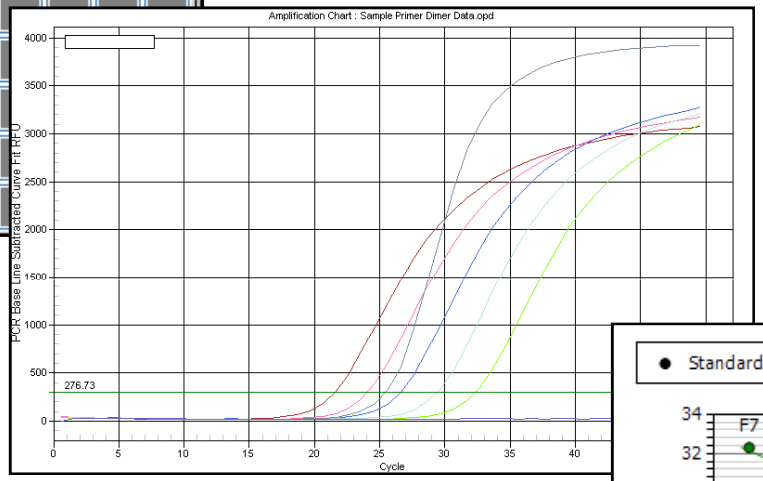
	Well	Fluor	Type	Identifier	Replicate #	Threshold Cycle (Ct)	Ct Mean	Ct Std. Dev	Set Point
1	A01	SYBR	Unkn		1	19.58	19.14	0.143	N/A
2	A02	SYBR	Unkn		1	19.42	19.14	0.143	N/A
3	A03	SYBR	Unkn		1	19.25	19.14	0.143	N/A
4	A04	SYBR	Unkn		1	19.20	19.14	0.143	N/A
5	A05	SYBR	Unkn		1	19.24	19.14	0.143	N/A
6	A06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

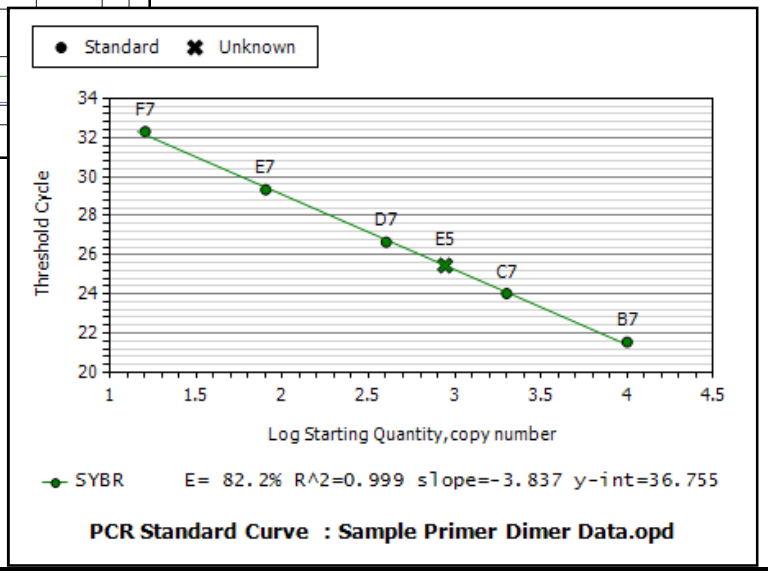




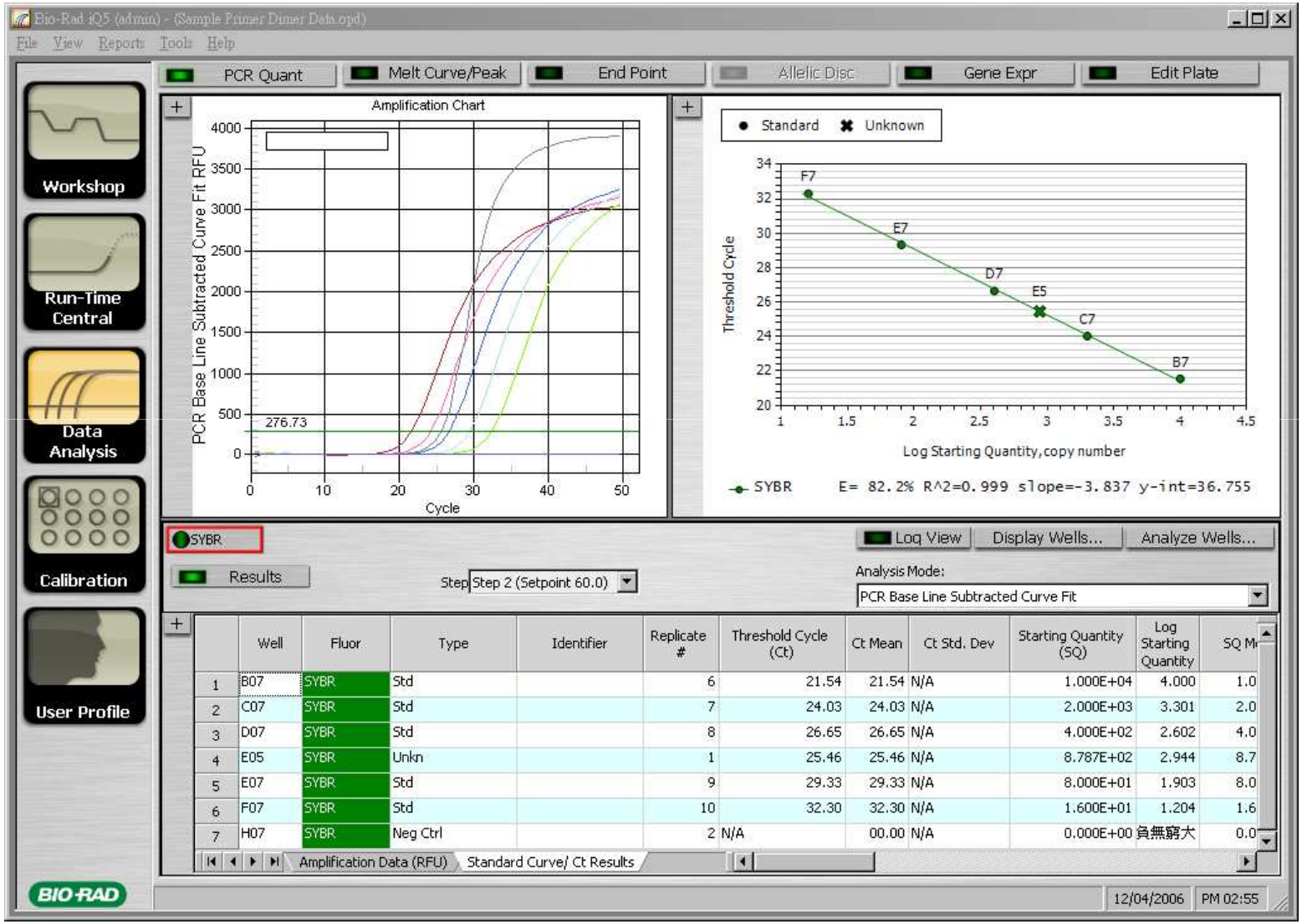
Set up the Sample Type and Concs: Standard or Unknown

Determine Ct Value

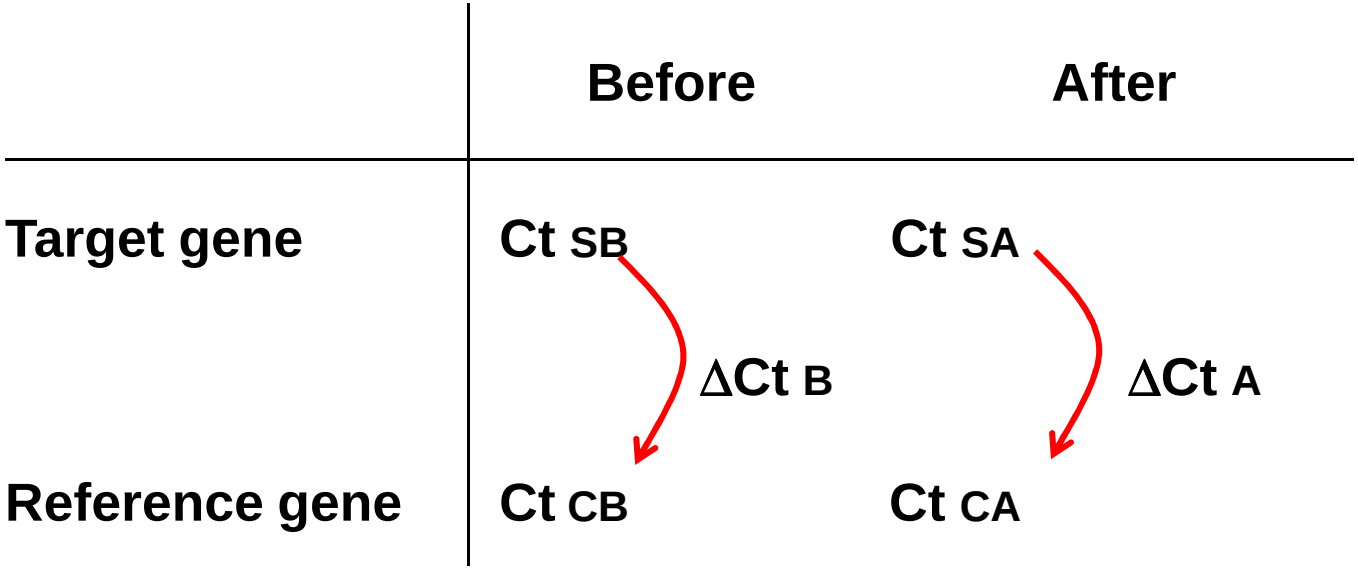
Auto-Calculation



AQ Results


Bio-Rad iQ5 (admin) - (Sample Primer Dimer Data.opd)
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
Amplification Chart
PCR Base Line Subtracted Curve Fit RFU
Cycle
276.73
Standard
Unknown
Threshold Cycle
Log Starting Quantity, copy number
F7
E7
D7
E5
C7
B7
SYBR
E= 82.2% R^2=0.999 slope=-3.837 y-int=36.755
SYBR
Results
Step
Step 2 (Setpoint 60.0)
Log View
Display Wells...
Analyze Wells...
Analysis Mode:
PCR Base Line Subtracted Curve Fit
Well | Fluor | Type | Identifier | Replicate # | Threshold Cycle (Ct) | Ct Mean | Ct Std. Dev | Starting Quantity (SQ) | Log Starting Quantity | SQ M
1 B07 SYBR Std 6 21.54 21.54 N/A 1.000E+04 4.000 1.0
2 C07 SYBR Std 7 24.03 24.03 N/A 2.000E+03 3.301 2.0
3 D07 SYBR Std 8 26.65 26.65 N/A 4.000E+02 2.602 4.0
4 E05 SYBR Unkn 1 25.46 25.46 N/A 8.787E+02 2.944 8.7
5 E07 SYBR Std 9 29.33 29.33 N/A 8.000E+01 1.903 8.0
6 F07 SYBR Std 10 32.30 32.30 N/A 1.600E+01 1.204 1.6
7 H07 SYBR Neg Ctrl 2 N/A 00.00 N/A 0.000E+00 負無窮大 0.0
Amplification Data (RFU)
Standard Curve/ Ct Results
BIO-RAD
12/04/2006
PM 02:55

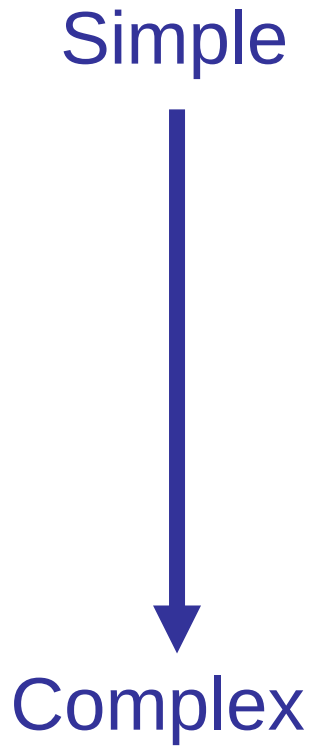
Relative Quantization
相對定量 (ΔΔCt method)



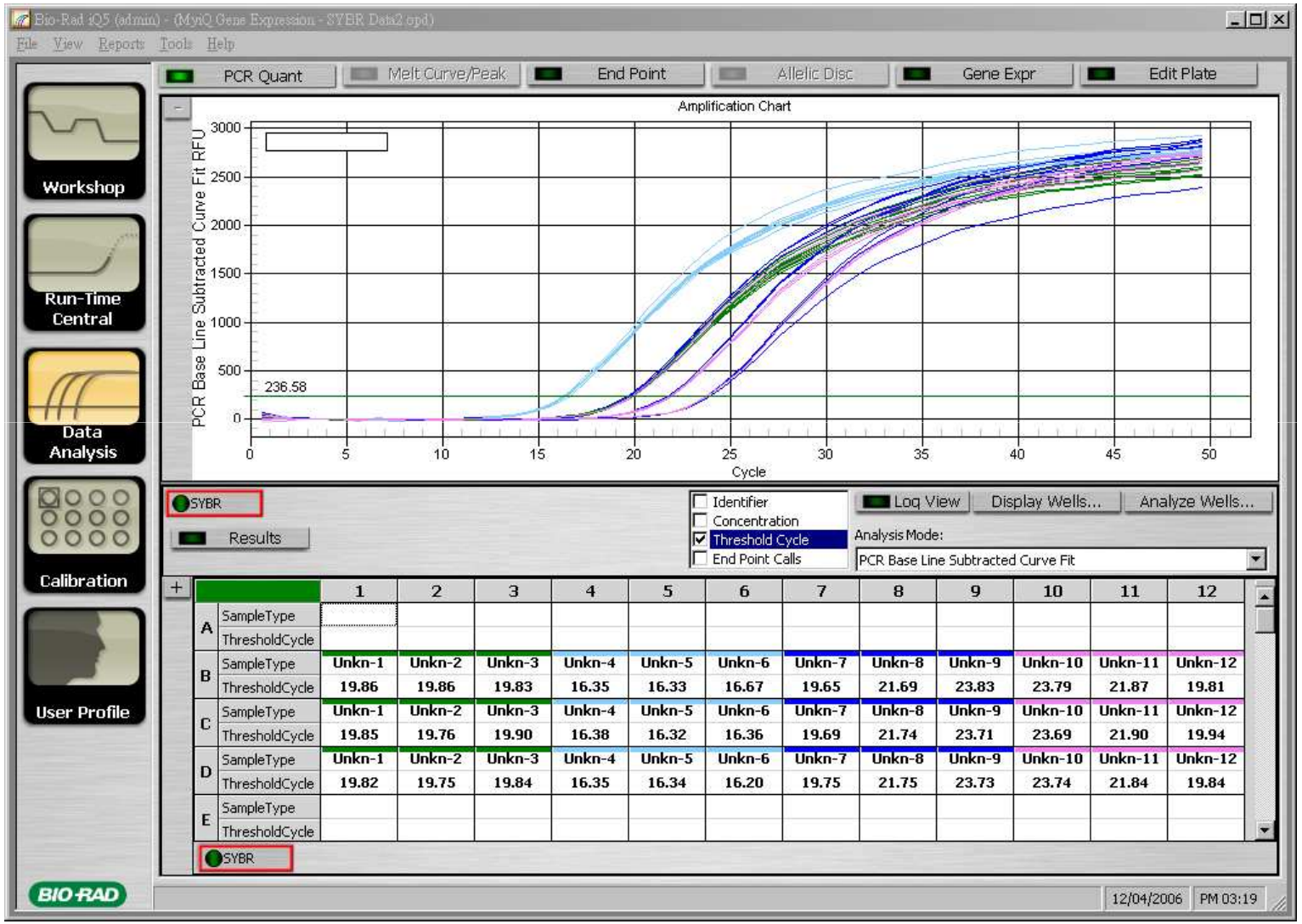
$$\text{Ratio} = (2)^{-\Delta\Delta Ct} \qquad \Delta\Delta Ct = (\Delta Ct_A - \Delta Ct_B)$$

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

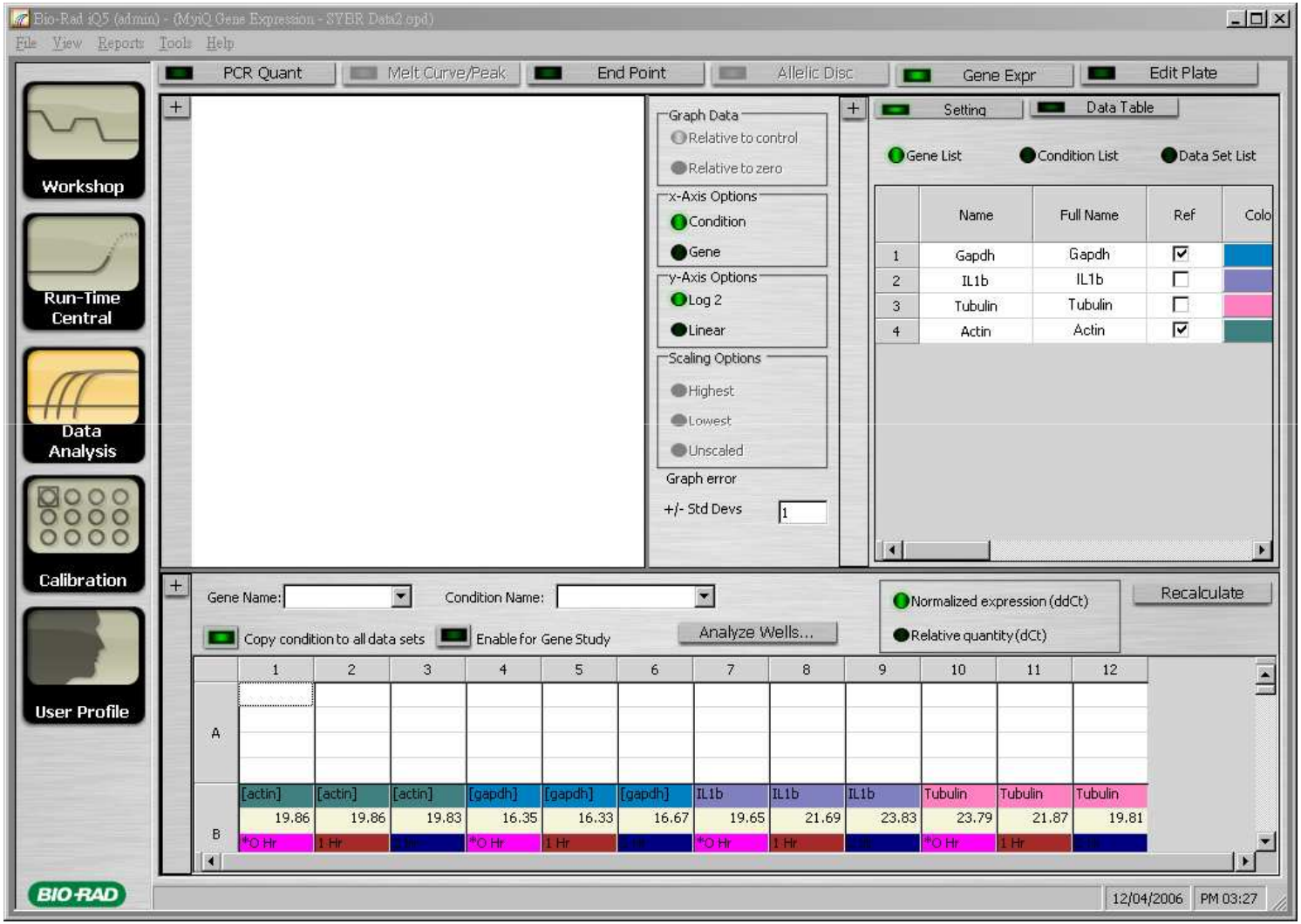


Gene Expression Analysis
Ct Determination

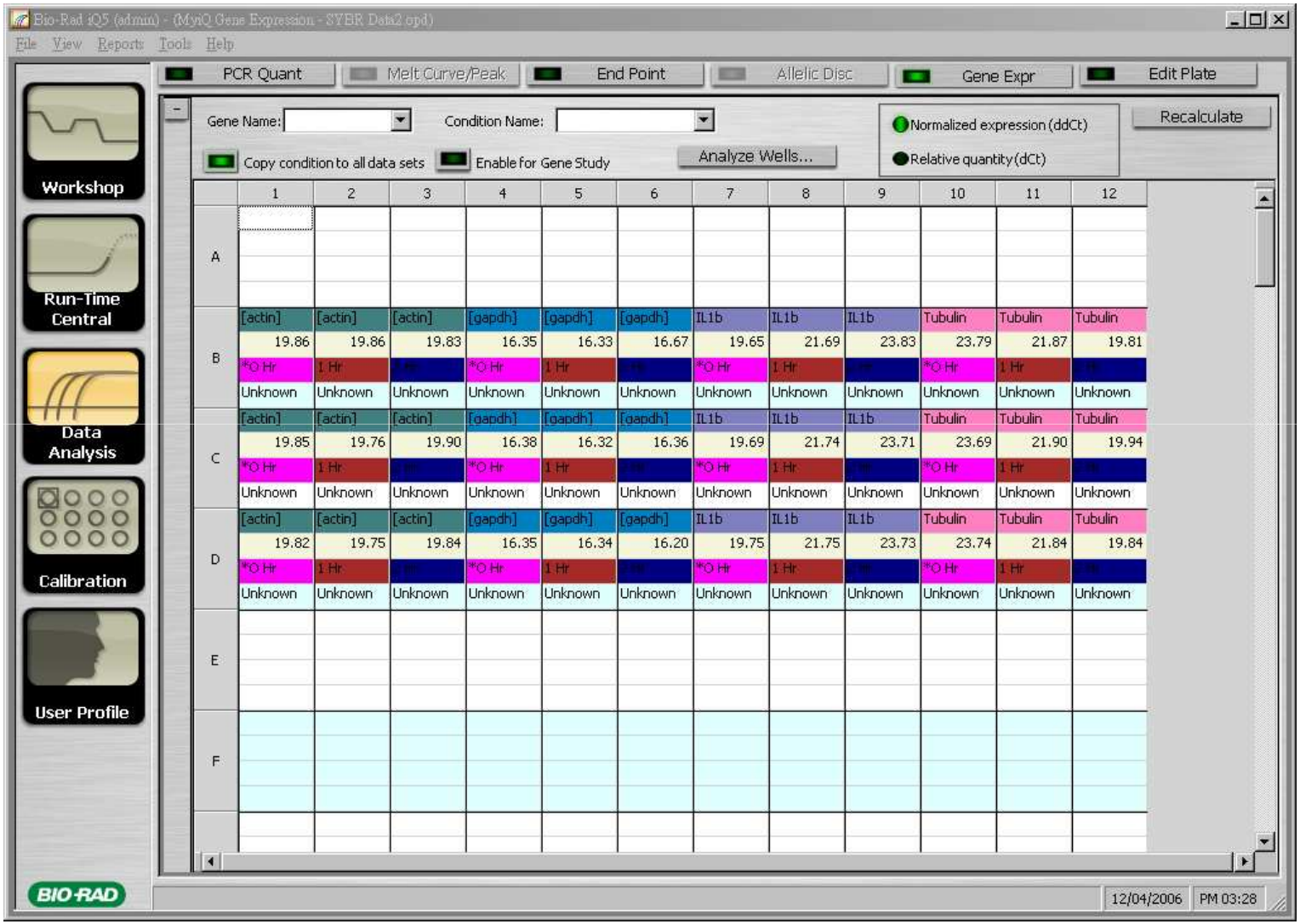


		1	2	3	4	5	6	7	8	9	10	11	12
A	SampleType												
	ThresholdCycle												
B	SampleType	Unkn-1	Unkn-2	Unkn-3	Unkn-4	Unkn-5	Unkn-6	Unkn-7	Unkn-8	Unkn-9	Unkn-10	Unkn-11	Unkn-12
	ThresholdCycle	19.86	19.86	19.83	16.35	16.33	16.67	19.65	21.69	23.83	23.79	21.87	19.81
C	SampleType	Unkn-1	Unkn-2	Unkn-3	Unkn-4	Unkn-5	Unkn-6	Unkn-7	Unkn-8	Unkn-9	Unkn-10	Unkn-11	Unkn-12
	ThresholdCycle	19.85	19.76	19.90	16.38	16.32	16.36	19.69	21.74	23.71	23.69	21.90	19.94
D	SampleType	Unkn-1	Unkn-2	Unkn-3	Unkn-4	Unkn-5	Unkn-6	Unkn-7	Unkn-8	Unkn-9	Unkn-10	Unkn-11	Unkn-12
	ThresholdCycle	19.82	19.75	19.84	16.35	16.34	16.20	19.75	21.75	23.73	23.74	21.84	19.84
E	SampleType												
	ThresholdCycle												

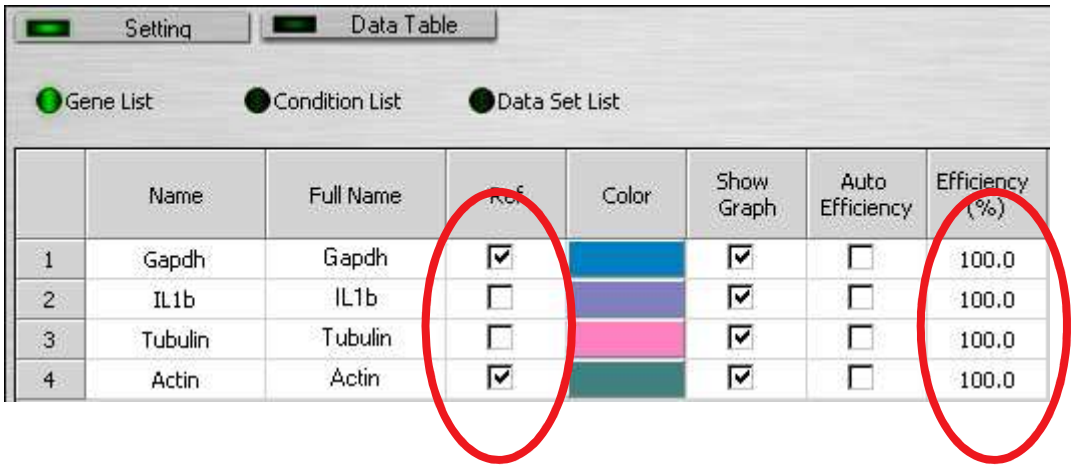
Gene Expression Tab

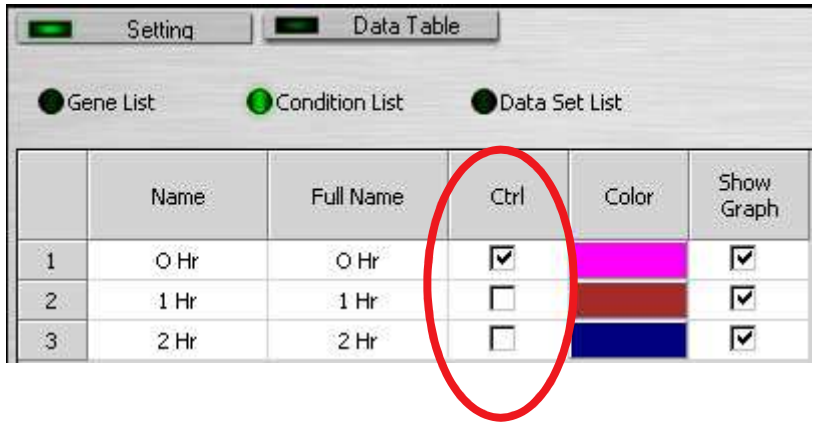


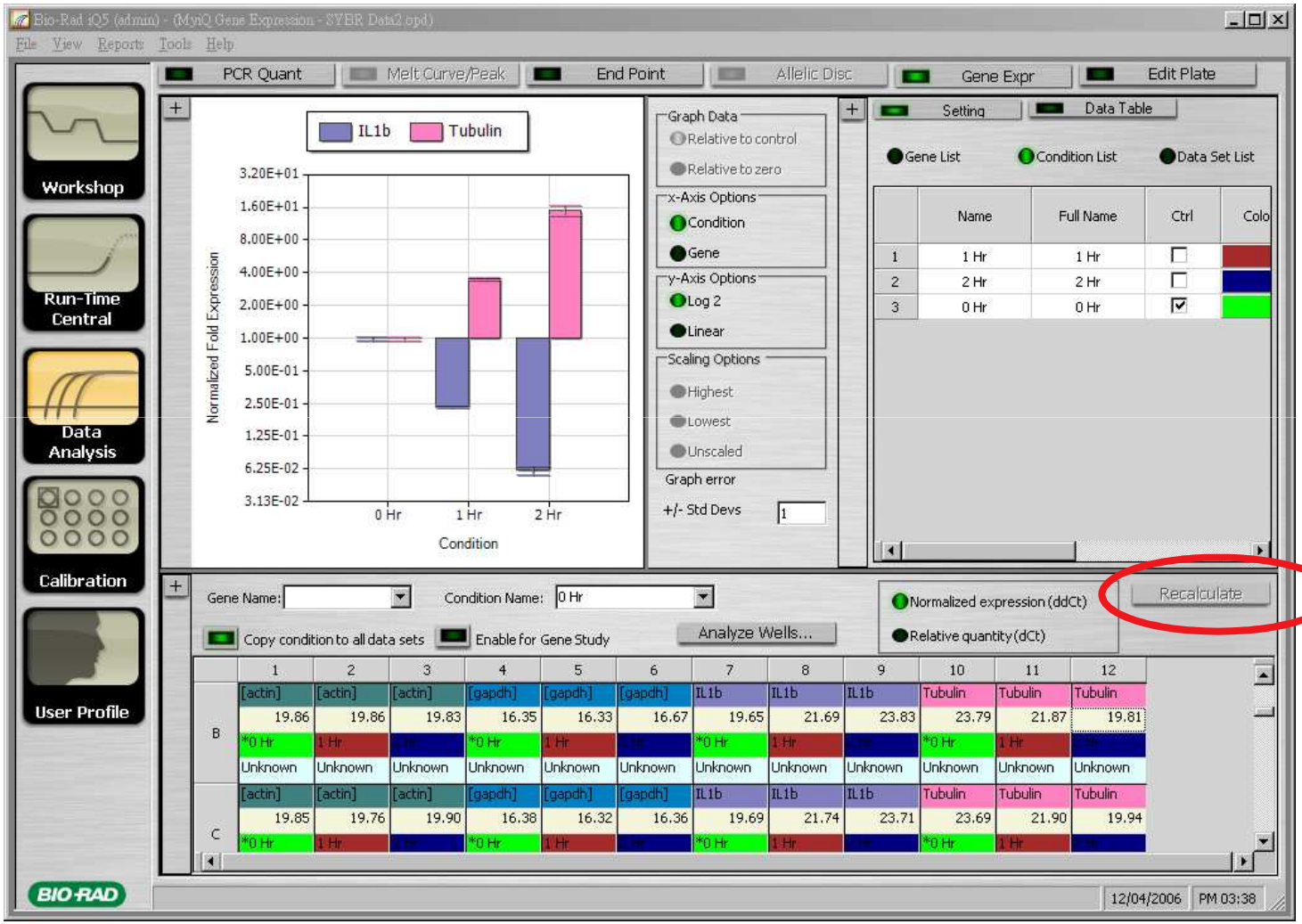
Grouping


Bio-Rad iQ5 (admin) - (MyiQ Gene Expression - SYBR Data2.opd)
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
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
*0 Hr 1 Hr *0 Hr 1 Hr *0 Hr 1 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 *0 Hr 1 Hr *0 Hr 1 Hr
Unknown
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
*0 Hr 1 Hr *0 Hr 1 Hr *0 Hr 1 Hr
Unknown
E
F
BIO-RAD
12/04/2006 PM 03:28

Select Ref. Gene and Control





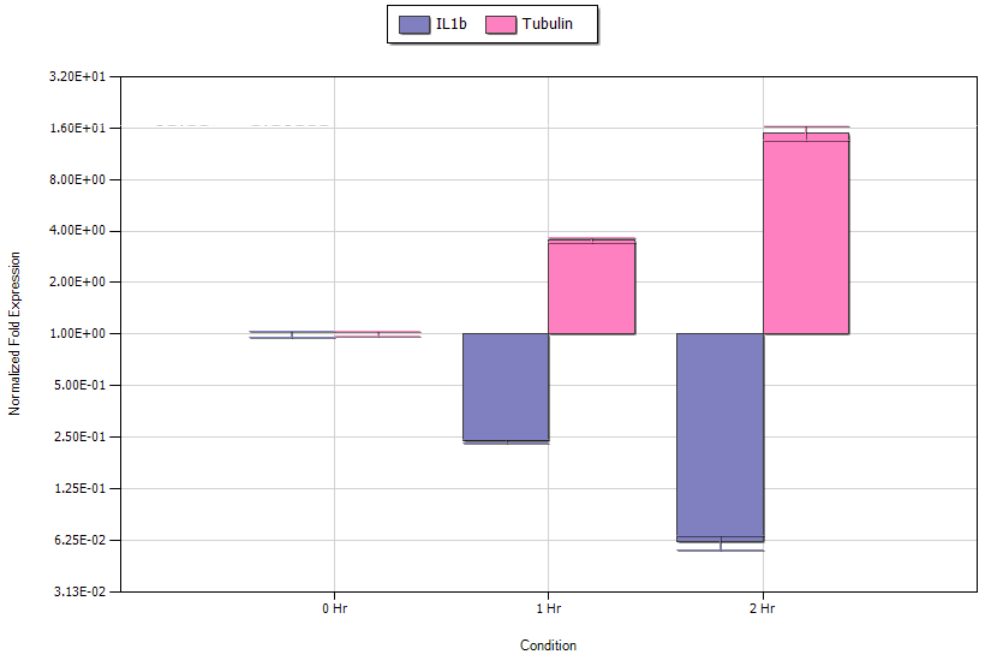

Bio-Rad iQ5 (admin) - (MyiQ Gene Expression - SYBR Data2.opd)
File View Reports Tools Help
PCR Quant
Melt Curve/Peak
End Point
Allelic Disc
Gene Expr
Edit Plate
Workshop
Run-Time Central
Data Analysis
Calibration
User Profile
IL1b
Tubulin
Normalized Fold Expression
3.20E+01
1.60E+01
8.00E+00
4.00E+00
2.00E+00
1.00E+00
5.00E-01
2.50E-01
1.25E-01
6.25E-02
3.13E-02
0 Hr
1 Hr
2 Hr
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
Colo
1 Hr
2 Hr
0 Hr
Gene Name:
Condition Name:
0 Hr
Copy condition to all data sets
Enable for Gene Study
Analyze Wells...
Normalized expression (ddCt)
Relative quantity (dCt)
Recalculate
[actin]
[gapdh]
IL1b
Tubulin
Unknown
12/04/2006
PM 03:38

RQ Resluts

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			

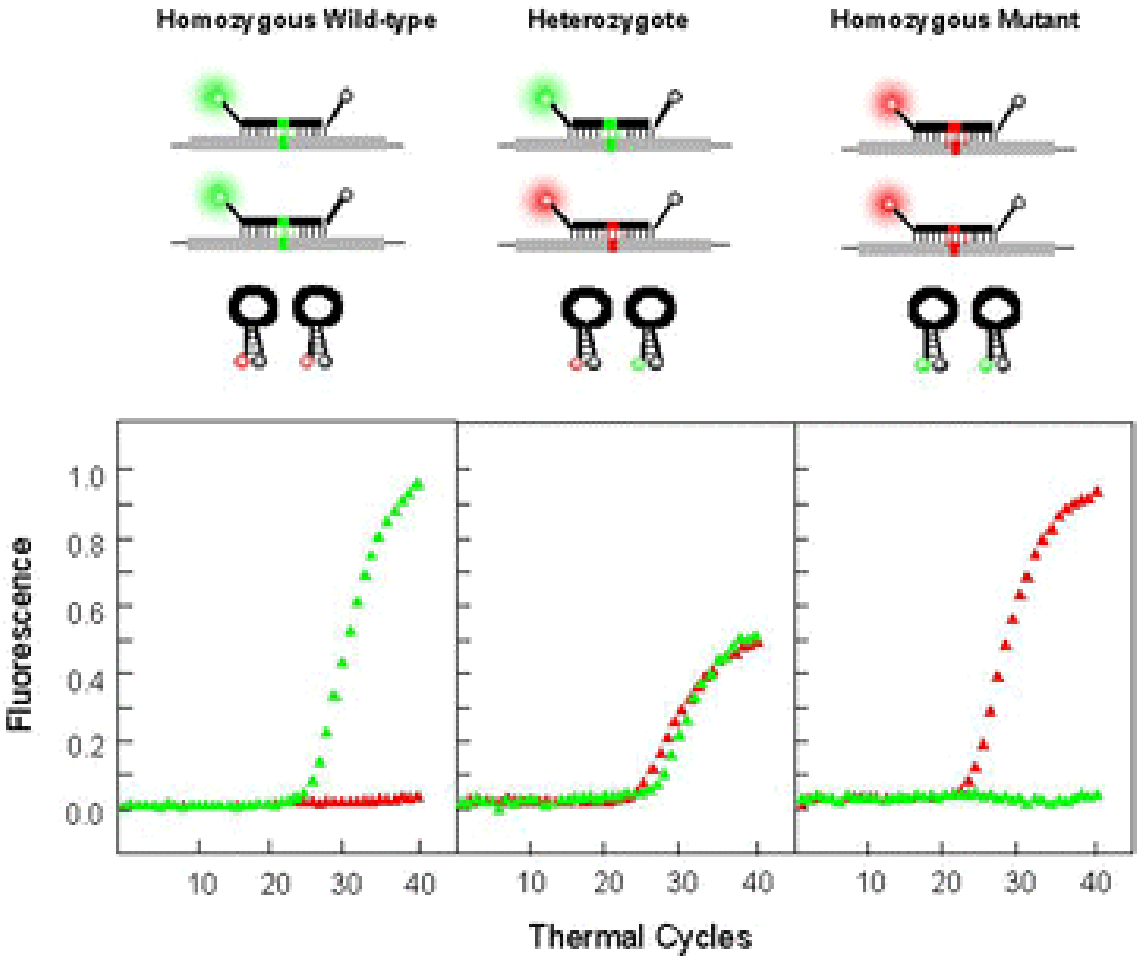


Gene Expression : MyiQ Gene Expression - SYBR Data2.opd

Multifile Gene Expression - MFGE

- Accomplished through new file type called “Gene Study”
- Created as .gxd file
- .gxd files maintain Sample ID and C_T 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…

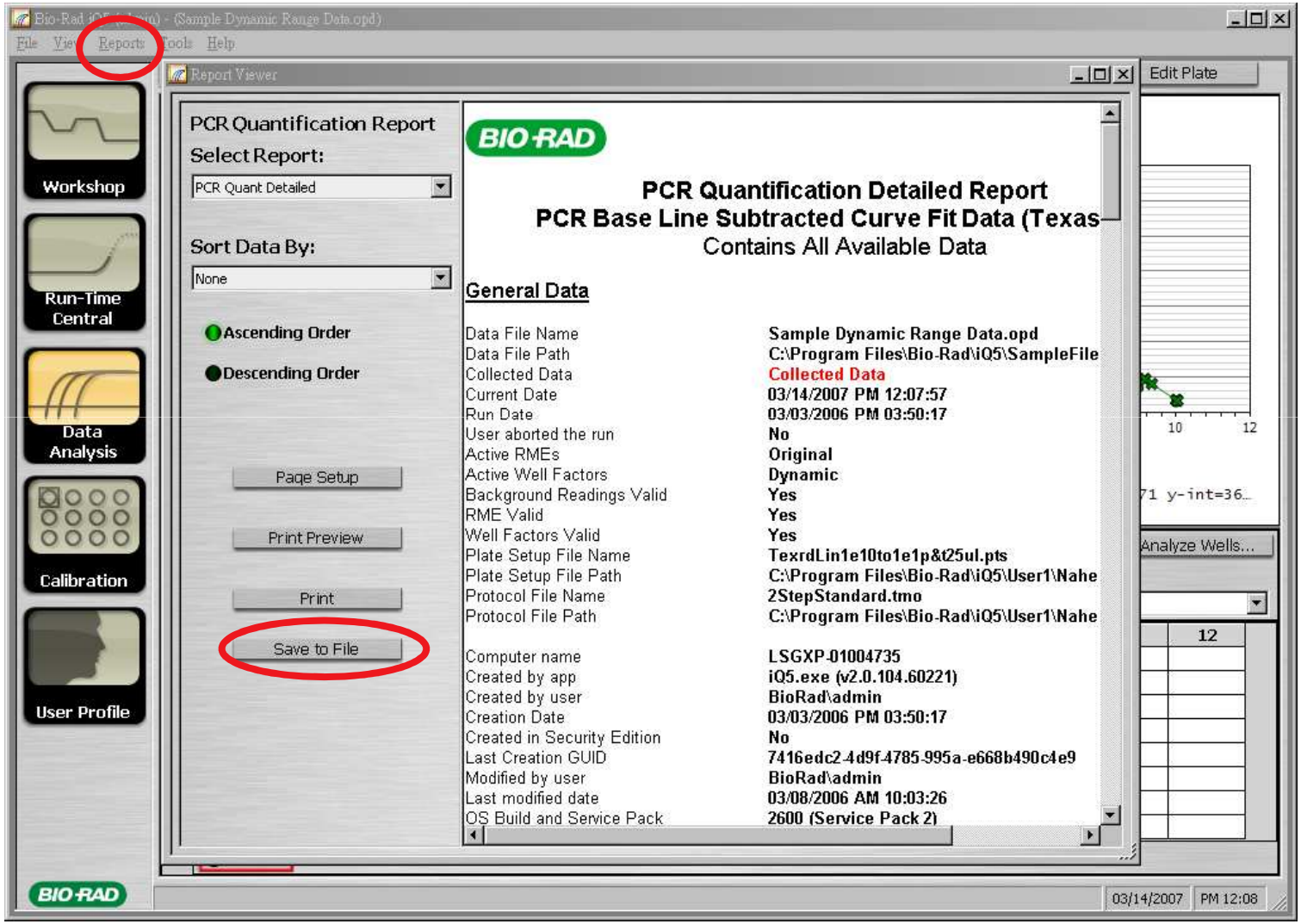
SNP detection by Probe



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

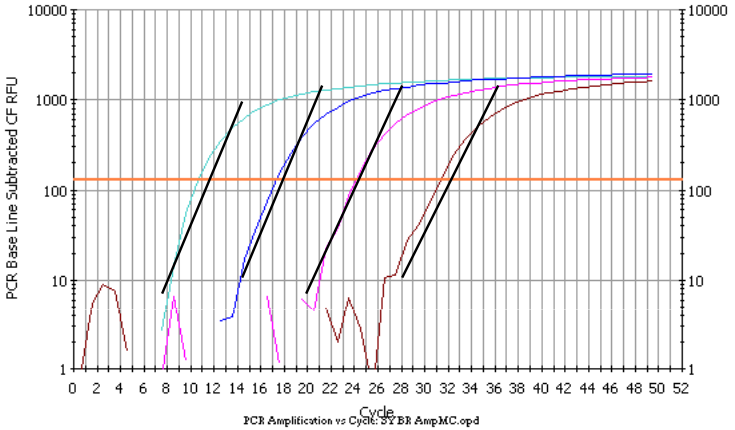
Allelic Discrimination

Detailed Reports



General QPCR Working Process -- Data analysis in iQ5

- Amplification plot
  - Reproducibility? So you need duplication or triplication….
  - Determination Ct Value? Threshold

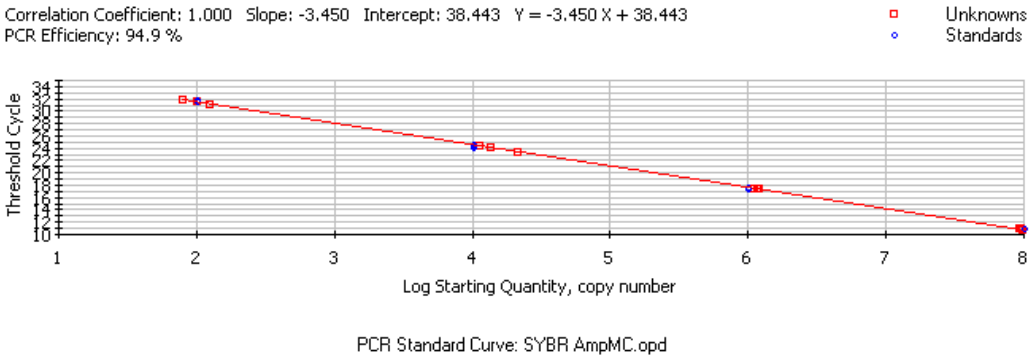


- Absolute Q., Relative Q. or SNP
  - ΔCt or ΔΔCt
  - Allelic Discrimination

- PCR efficiency from std. Curve
  - <100%
  - >100%
  - Dynamic range

- Reproducibility
  - Duplication or triplication

- Melting curve analysis
  - Primer dimmer
  - Non specific production

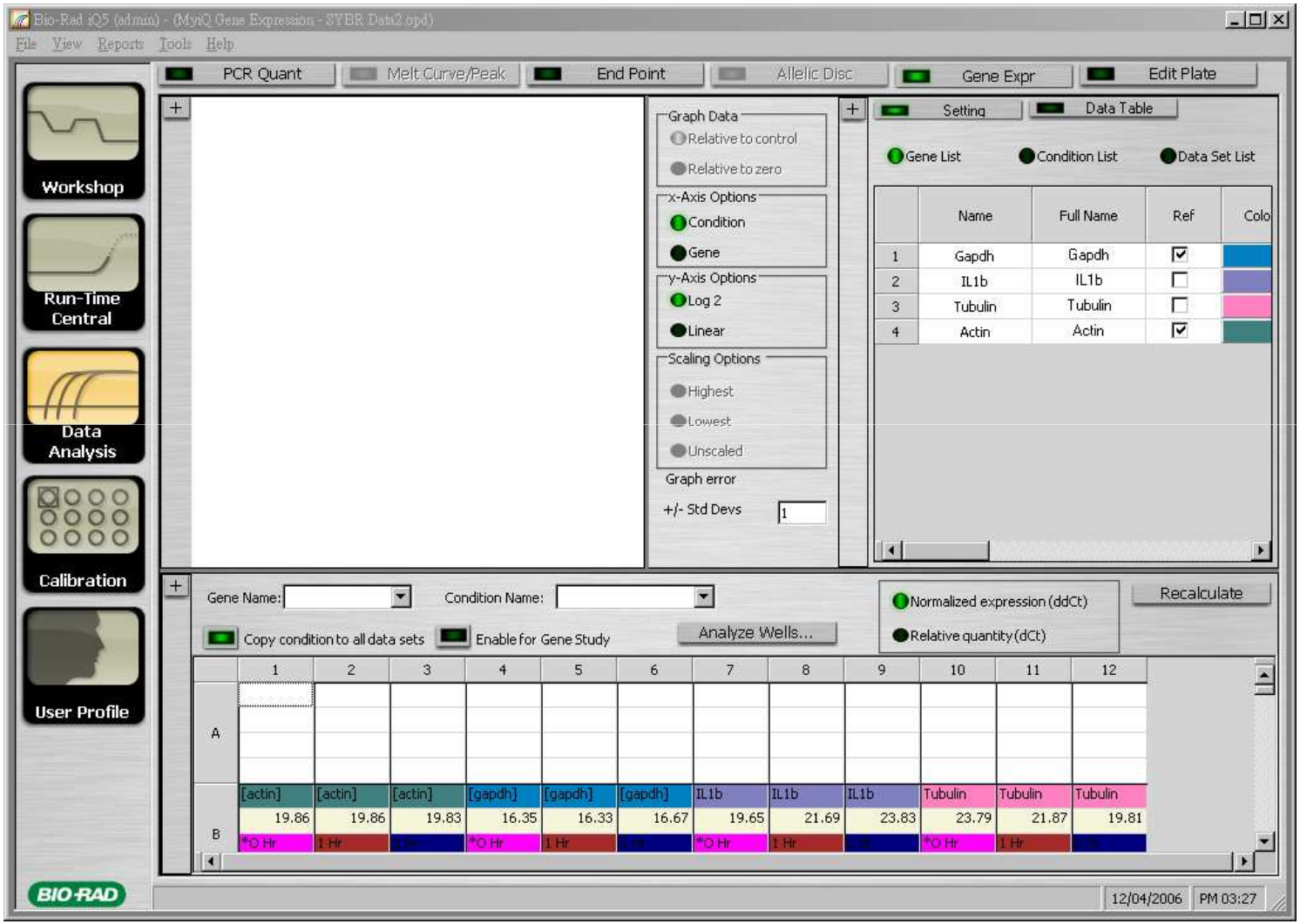


- General introduction
- Instrument Operation
- Data analysis
  - 決定 Ct 値
  - Melting Curve
  - Absolute Quantification
  - Relative Quantification

iQ5 Software Data analysis

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

Ct: Automatic Determination


Bio-Rad iQ5 (admin) - (MyiQ Gene Expression - SYBR Data2.opd)
File View Reports Tools Help
PCR Quant
Melt Curve/Peak
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Allelic Disc
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Edit Plate
Workshop
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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
Colo
1 Gapdh Gapdh
2 IL1b IL1b
3 Tubulin Tubulin
4 Actin Actin
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
19.86 19.86 19.83 16.35 16.33 16.67 19.65 21.69 23.83 23.79 21.87 19.81
B
*0 Hr 1 Hr *0 Hr 1 Hr *0 Hr 1 Hr
BIO-RAD
12/04/2006 PM 03:27