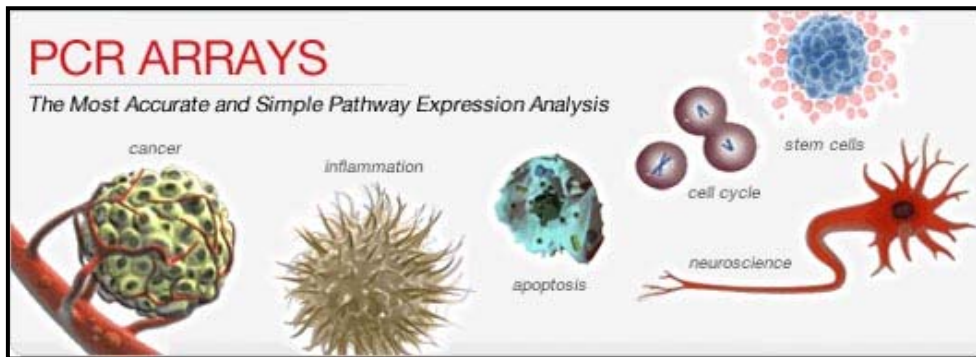


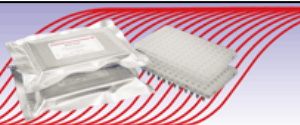
# RT<sup>2</sup>Profiler PCR Array: Web-Based Data Analysis Tutorial



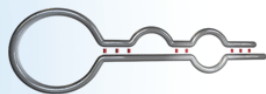
**Samuel J. Rulli, Jr., Ph.D.**  
**qPCR-Applications Scientist**  
**[Samuel.Rulli@QIAGEN.com](mailto:Samuel.Rulli@QIAGEN.com)**

## RT<sup>2</sup> Profiler PCR Arrays

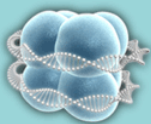
Microarray Profiling Capabilities with Real-Time PCR Performance



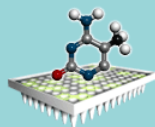
## RT<sup>2</sup> miRNA PCR Arrays



## NEW! High-Throughput Methylation Analysis



Methyl-Profiler™ PCR ARRAY



## shRNA Plasmids & siRNA Arrays

Genome-Wide shRNA for Your Gene & siRNA for Your Pathway



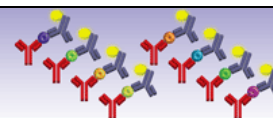
## RNA Interference

High-potency Genome-wide siRNA & shRNA

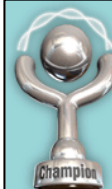


## Multi-Analyte ELISArray Kits

Analyze 12 Cytokines or Chemokines Simultaneously Using ELISA



## NEW! ChampionChIP™ qPCR System



Transcription Factors

Coregulators

ChIP-on-chip Validation

Modified Histones

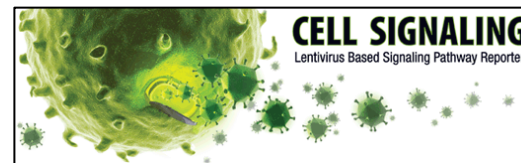
Chromatin Remodeling

Successful ChIP in ONE Day



## CELL SIGNALING

Lentivirus Based Signaling Pathway Reporter



# Topics to be Covered

## Topic I:

Brief Technology and Protocol Overview

**Offered every Month! (Next RT<sup>2</sup> PCR Array Webinar)**

## Topic II (Today):

PCR Array Data Analysis

- Defining Baseline and Threshold
- Web Portal Location / Address
- Uploading Raw C<sub>t</sub> Data
- Analyzing Data & Controls
- Exporting Data

# Anatomy of a PCR Array

## Human Inflammatory Cytokines & Receptors RT<sup>2</sup> Profiler PCR Array

	1	2	3	4	5	6	7	8	9	10	11	12
A	ABCF1	BCL6	C3	C4A	C5	CCL1	CCL11	CCL13	CCL15	CCL16	CCL17	CCL18
B	CCL19	CCL2	CCL20	CCL21	CCL23	CCL24	CCL25	CCL26	CCL3	CCL4	CCL5	CCL7
C	CCL8	CCR1	CCR2	CCR3	CCR4	CCR5	CCR6	CCR7	CCR8	CCR9	CEBPB	CRP
D	CX3CR1	CXCL1	CXCL10	CXCL11	CXCL12	CXCL13	CXCL14	CXCL2	CXCL3	CXCL5	CXCL6	CXCL9
E	ICEBERG	IFNA2	IL10	IL10RA	IL10RB	IL13	IL13RA1	IL17C	IL1A	IL1B	IL1F10	IL1F5
F	IL1F6	IL1F7	IL1F8	IL1F9	IL1R1	IL1RN	IL22	IL5	IL5RA	IL8	IL8RA	IL8RB
G	IL9	IL9R	LTA	LTB	LTB4R	MIF	SCYE1	SPP1	TNF	TNFSF5	TOLLIP	XCR1
H	B2M	HPRT1	RPL13A	GAPDH	ACTB	HGDC	RTC	RTC	RTC	PPC	PPC	PPC
	HOUSEKEEPING GENE CONTROLS					GENOMIC DNA CONTROL	REVERSE TRANSCRIPTION CONTROLS			POSITIVE PCR CONTROLS		

84 Pathway-Specific Genes of Interest

5 Housekeeping Genes

Genomic DNA Contamination Control

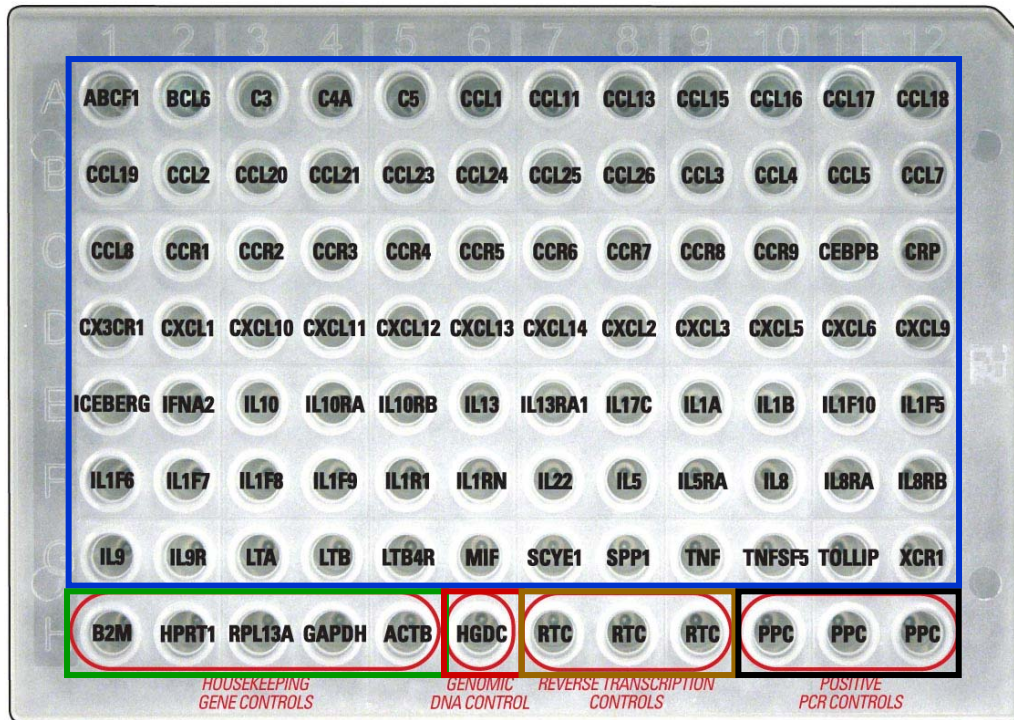
Reverse Transcription Controls (RTC) n=3

Positive PCR Controls (PPC) n=3



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## Human Inflammatory Cytokines & Receptors RT<sup>2</sup> Profiler PCR Array



84 Pathway-Specific Genes of Interest

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# How RT<sup>2</sup> Profiler PCR Arrays Work

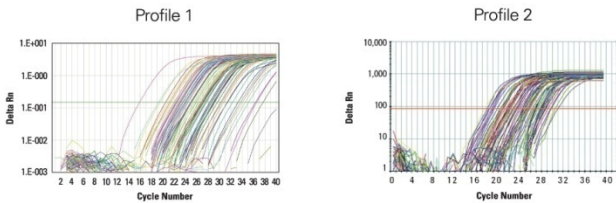
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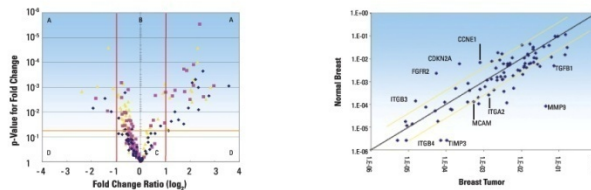
## 2. Add cDNA to RT<sup>2</sup> qPCR Master Mix & Aliquot Mixture Across PCR Array.



## 3. Run in Your Real-Time PCR Instrument.



## 4. Data Analysis.



## cDNA Synthesis (C-03 kit)

- Genomic DNA Removal Step (5 min.)
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## Load Plates

- 1 Sample per PCR Array
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## Run 40 cycle qPCR Program

- Standard cycling conditions for all Real Time PCR Instruments
- 2 hours

## Upload and Analyze Data (FREE)

- 15 minutes from Raw Ct to Fold Change Data

# How RT<sup>2</sup> Profiler PCR Arrays Work

1. Convert Total RNA to cDNA.

control

cDNA 1

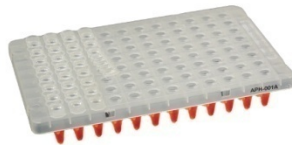
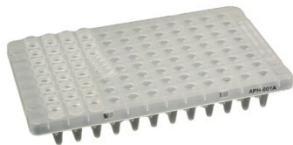


Hot Sauce

cDNA 2

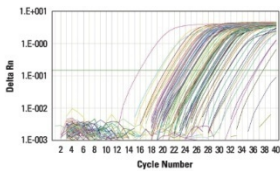


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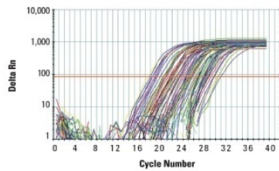


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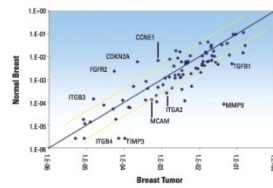
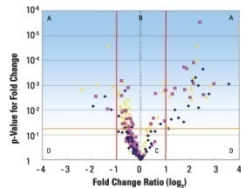
Profile 1



Profile 2



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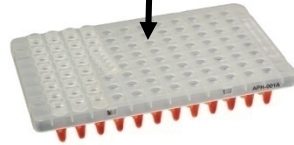
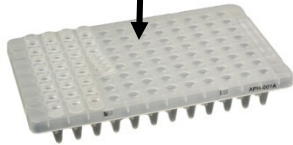
control

Hot Sauce

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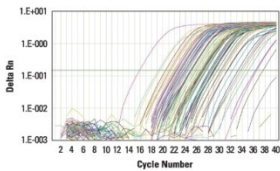
cDNA 2

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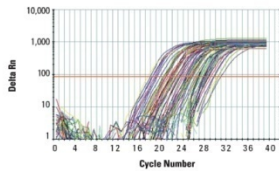


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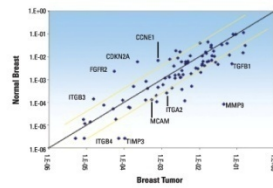
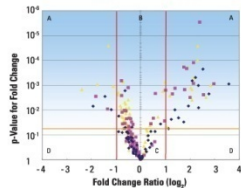
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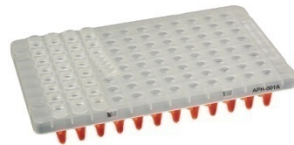
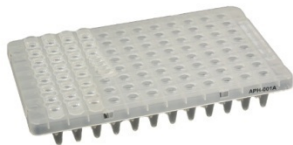


Hot Sauce

cDNA 2

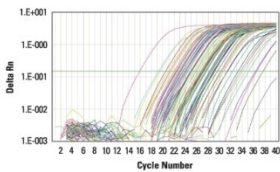


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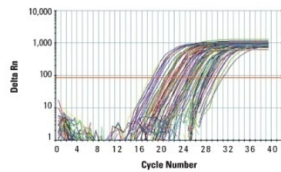


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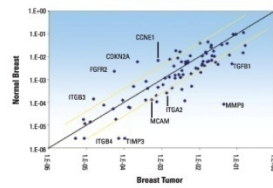
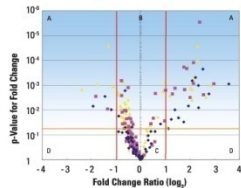
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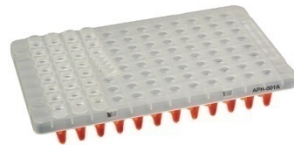
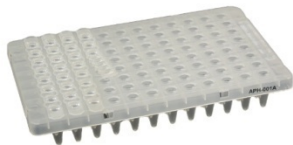


Hot Sauce

cDNA 2

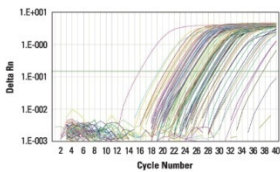


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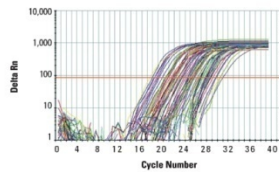


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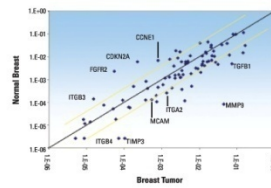
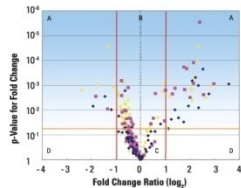
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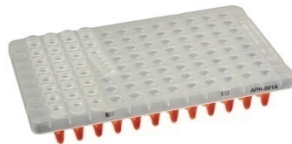
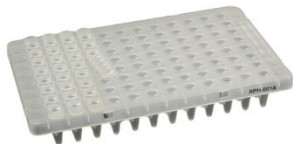
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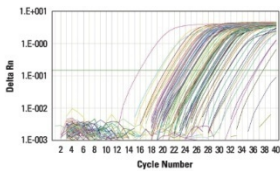


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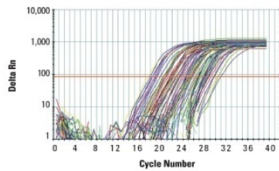


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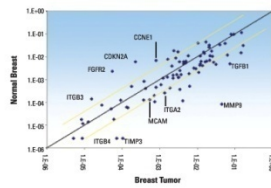
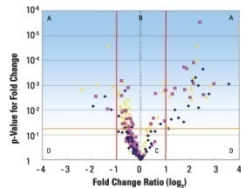
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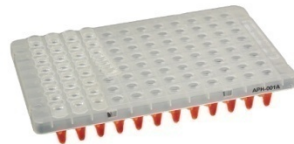
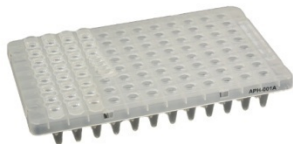
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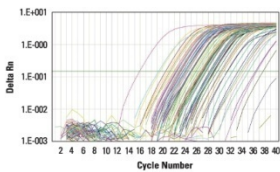


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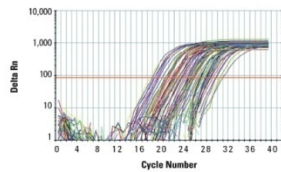


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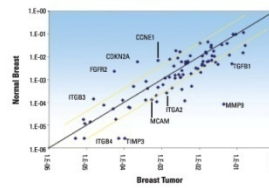
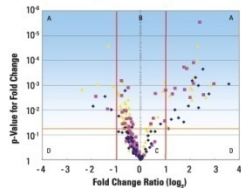
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## Fold Change Results

- Using  $\Delta\Delta C(t)$  calculations
- ABL1 is up/down regulated by x fold

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control

Hot Sauce

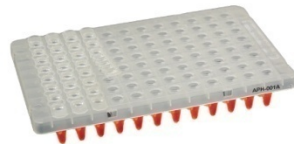
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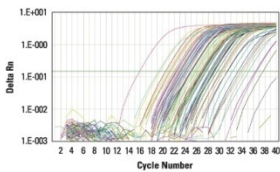


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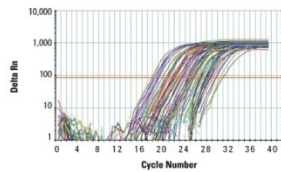


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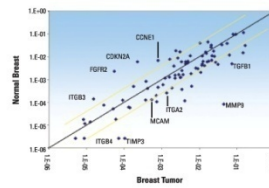
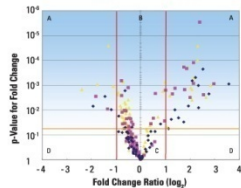
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Thursday January 6<sup>th</sup> @ 1pm EST (Next RT<sup>2</sup> PCR Array Webinar)

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- Defining Baseline and Threshold
- Web Portal Location / Address
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# Defining Baseline and Threshold

**For ABI, Stratagene, Bio-Rad, and Eppendorf Real-Time PCR Instruments\*:**

## Baseline

- Use Automated Baseline
  - (if your instrument has Adaptive Baseline function) OR
- Manually Set Baseline
  - Using Linear View:  
Set to Cycle #2 or #3 up to 1 or 2 cycle values before earliest amplification (with highest cycle being cycle #15)

## Threshold Value

- Use Log View
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Export  $C_t$  values to blank spread sheet (Excel).

Threshold Must Be Same Between Runs (important for PPC and RTC and selecting house keeping genes)

\*For Roche LC480: Use Second Derivative Maximum

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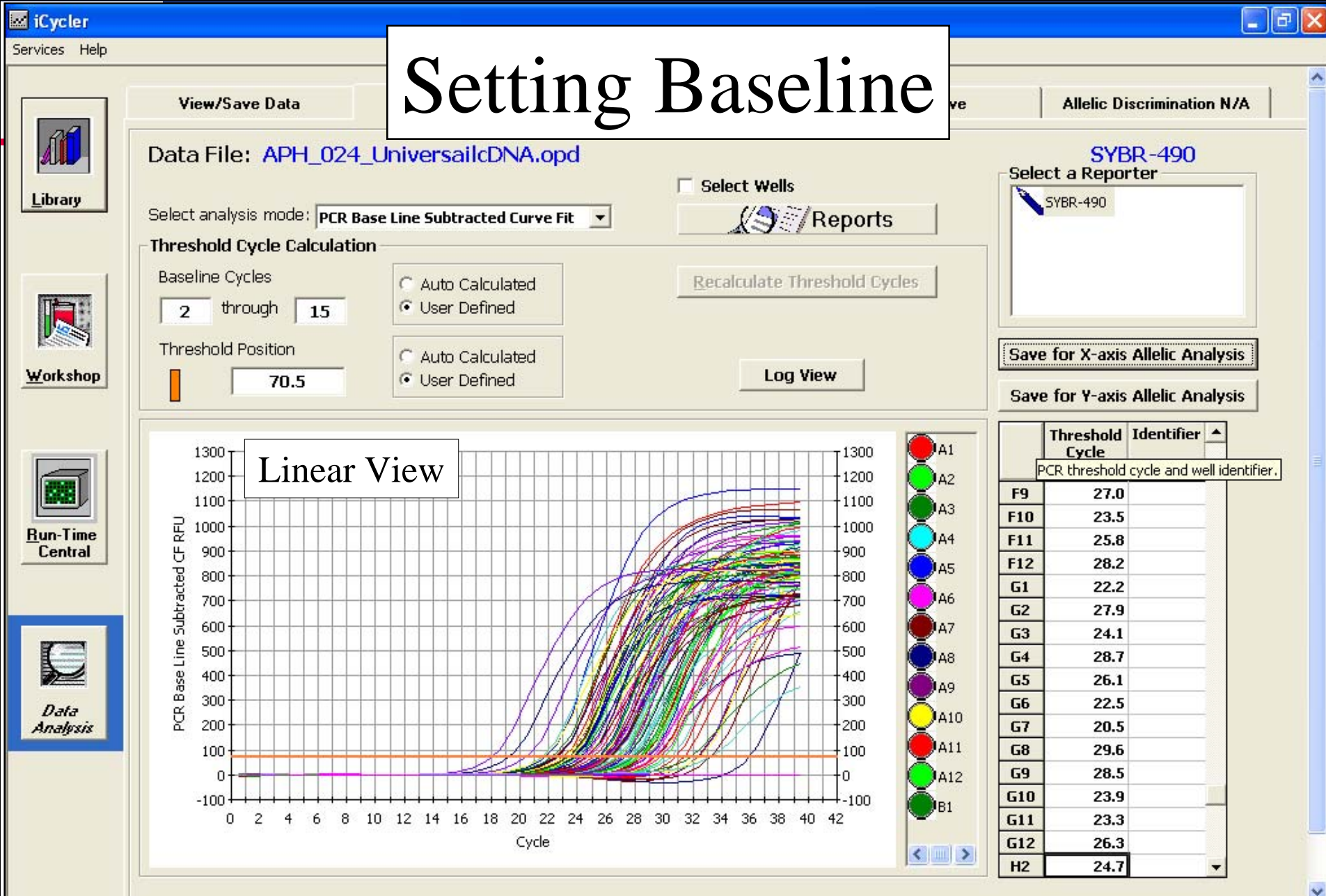
## Baseline

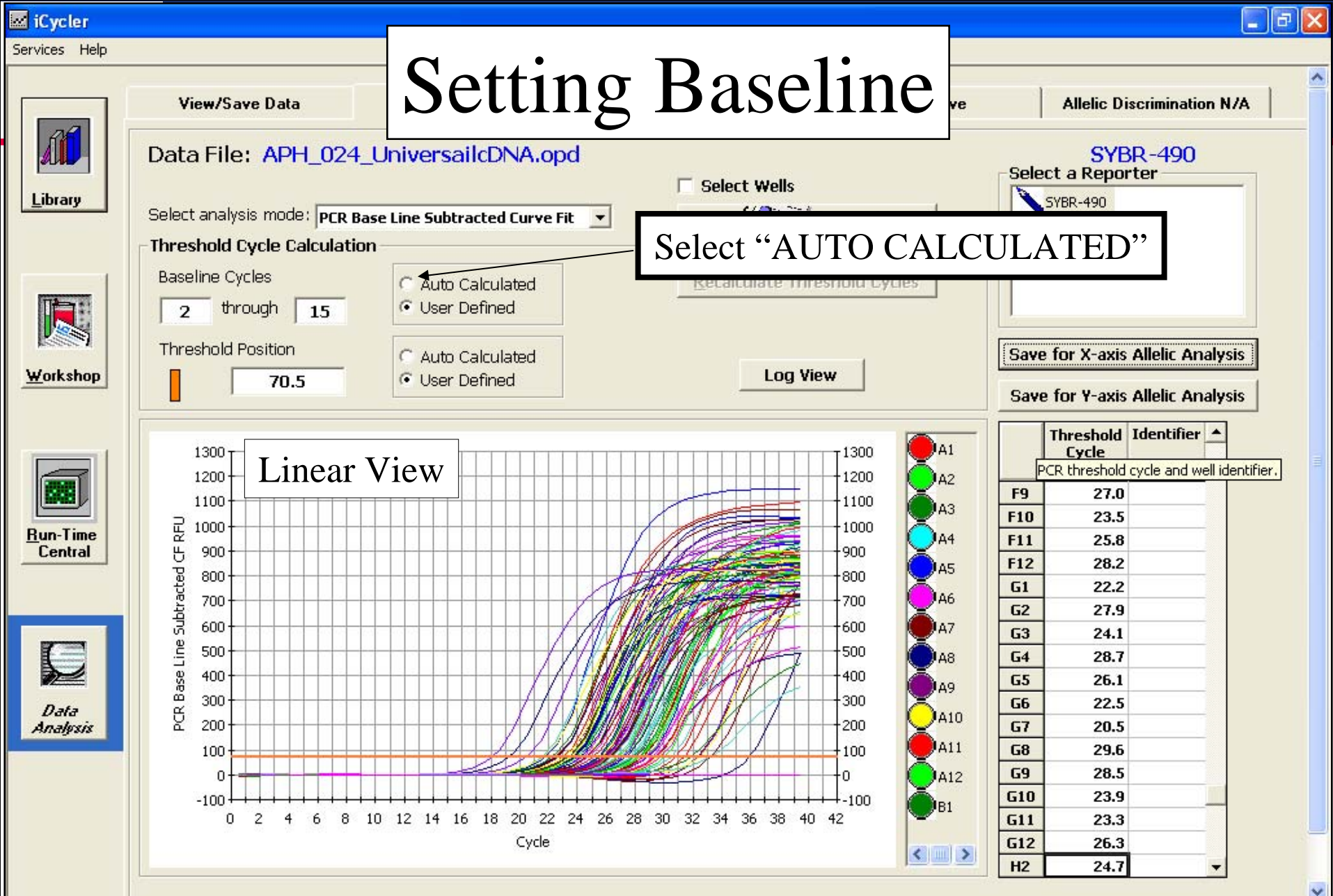
- Use Automated Baseline
    - (if your instrument has Adaptive Baseline function) OR
  - Manually Set Baseline
    - Using Linear View.
- Set to Cycle #2 or #3 up to 4 or 2 cycle values before earliest amplification (with highest cycle being cycle #15)

## Threshold Value

- Use Log View
- Place in
  - 1) Linear phase of amplification curve
  - 2) Above background signal, but within lower half to one third of curve

Export  $C_t$  values to blank spread sheet (Excel).







# Setting Threshold

View/Save Data

Data File: APH\_024\_UniversailcDNA.opd

Select analysis mode: PCR Base Line Subtracted Curve Fit

☐ Select Wells

Reports

Threshold Cycle Calculation

Baseline Cycles

2 through 15

☐ Auto Calculated☒ User Defined

Recalculate Threshold Cycles

Threshold Position

22.6

☐ Auto Calculated☒ User Defined

Normal View

Allelic Discrimination N/A

SYBR-490

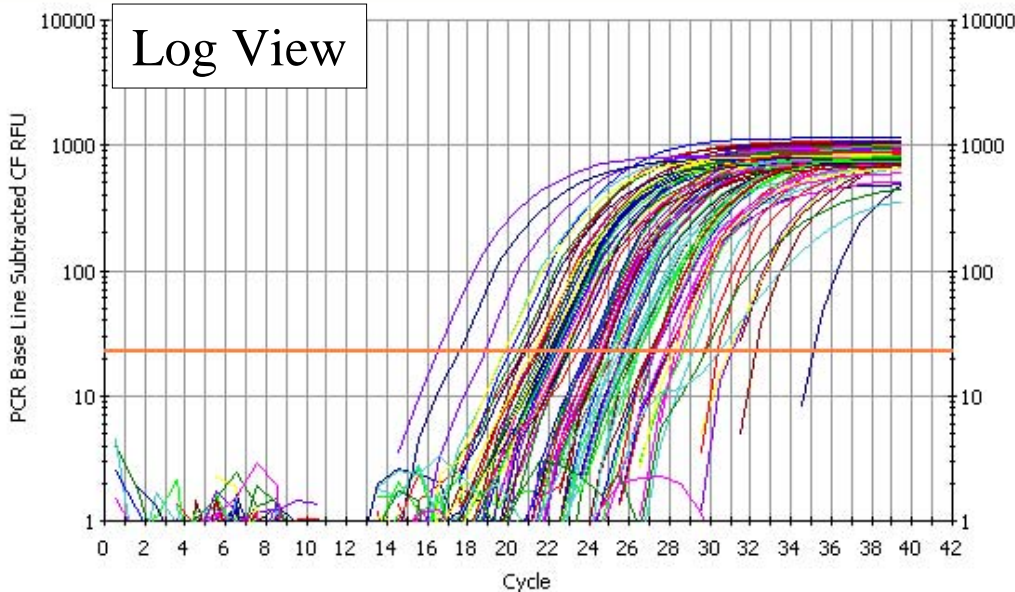
Select a Reporter

SYBR-490

Save for X-axis Allelic Analysis

Save for Y-axis Allelic Analysis

Log View



Save data from current dye layer for allelic analysis (Y-axis).

	Cycle	Ct
A1	19.8	
A2	26.5	
A3	23.8	
A4	25.3	
A5	26.4	
A6	21.7	
A7	31.0	
A8	27.0	
A9	23.5	
A10	21.4	
A11	23.8	
A12	23.9	
B1	26.7	
B2	25.1	
B3	27.1	
B4	27.0	
B5	25.6	



# Setting Threshold

View/Save Data

Data File: APH\_024\_UniversailcDNA.opd

Select analysis mode: PCR Base Line Subtracted Curve Fit

☐ Select Wells

Reports

Threshold Cycle Calculation

Baseline Cycles

2 through 15

☐ Auto Calculated☒ User Defined

Recalculate Threshold Cycles

Threshold Position

22.6

☐ Auto Calculated☒ User Defined

Normal View

Allelic Discrimination N/A

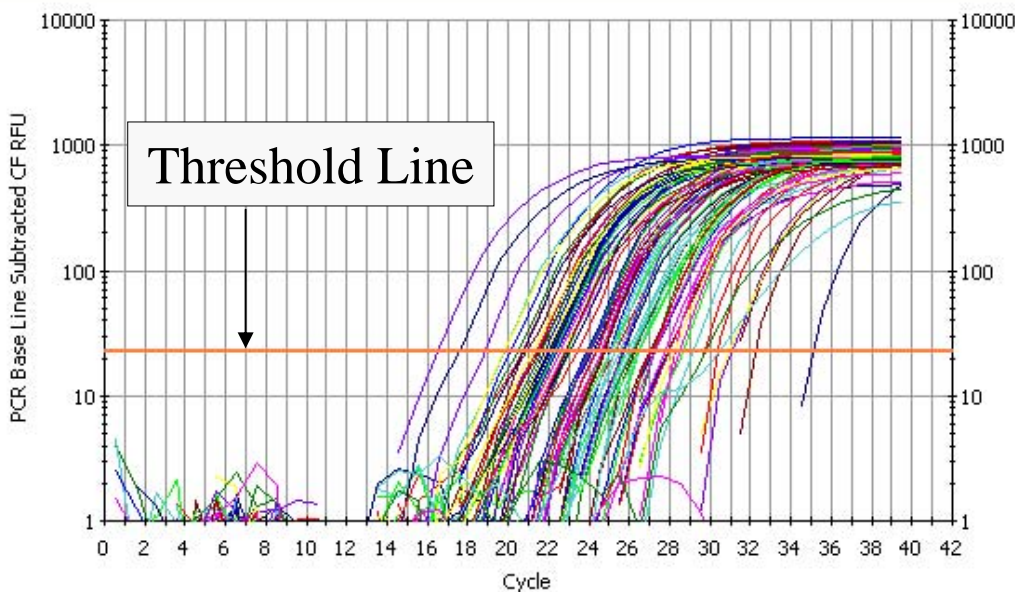
SYBR-490

Select a Reporter

SYBR-490

Save for X-axis Allelic Analysis

Save for Y-axis Allelic Analysis



Save data from current dye layer for allelic analysis (Y-axis).

	Cycle Ct
A1	19.8
A2	26.5
A3	23.8
A4	25.3
A5	26.4
A6	21.7
A7	31.0
A8	27.0
A9	23.5
A10	21.4
A11	23.8
A12	23.9
B1	26.7
B2	25.1
B3	27.1
B4	27.0
B5	25.6

# Setting Threshold

View/Save Data

Data File: APH\_024\_UniversailcDNA.opd

Select analysis mode: PCR Base Line Subtracted Curve Fit

☐ Select Wells

Reports

Threshold Cycle Calculation

Baseline Cycles

2 through 15

☐ Auto Calculated☒ User Defined

Recalculate Threshold Cycles

Threshold Position

22.6

☐ Auto Calculated☒ User Defined

Normal View

Allelic Discrimination N/A

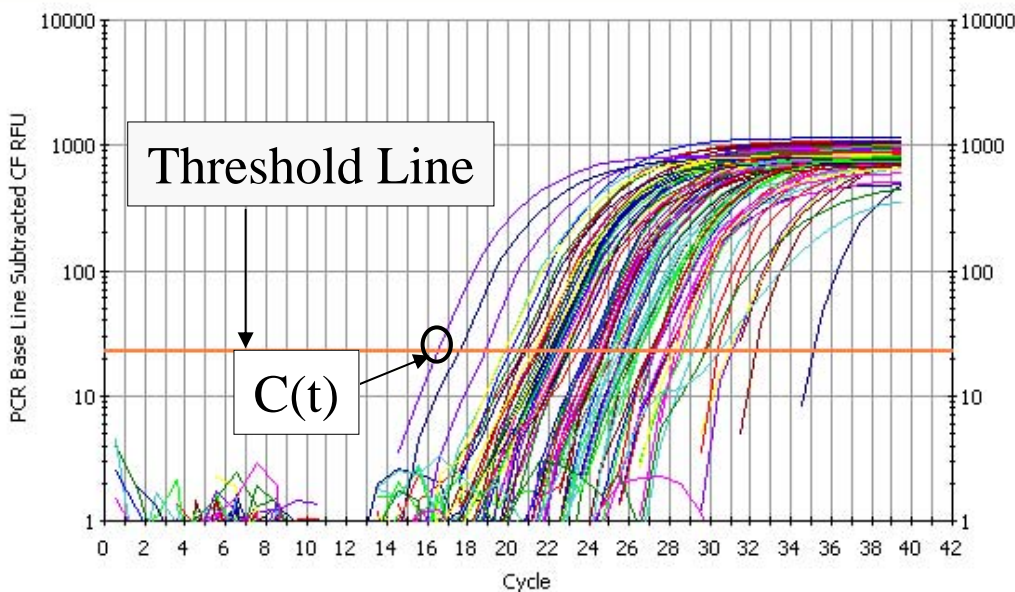
SYBR-490

Select a Reporter

SYBR-490

Save for X-axis Allelic Analysis

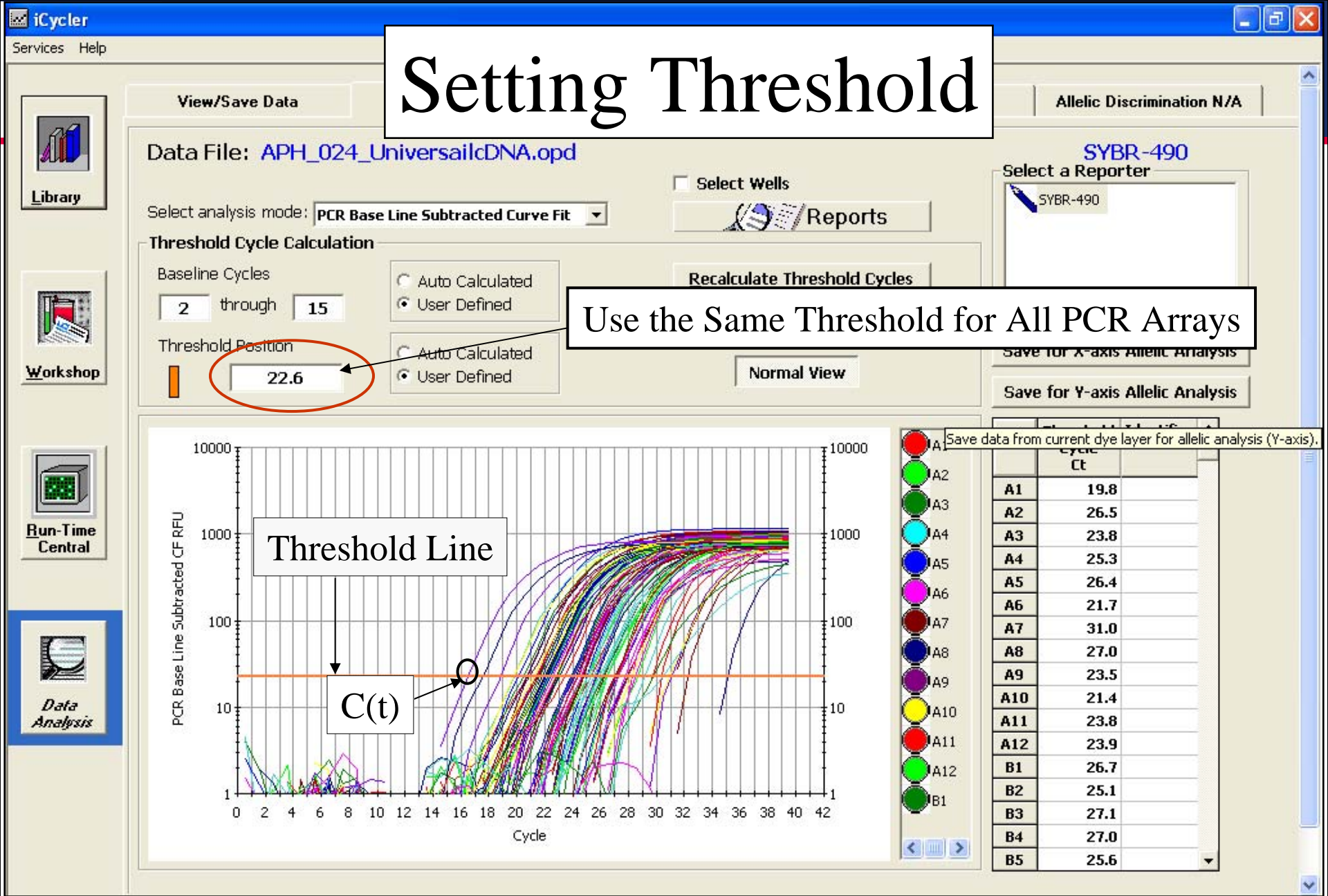
Save for Y-axis Allelic Analysis



Save data from current dye layer for allelic analysis (Y-axis).

	Cycle	Ct
A1	19.8	
A2	26.5	
A3	23.8	
A4	25.3	
A5	26.4	
A6	21.7	
A7	31.0	
A8	27.0	
A9	23.5	
A10	21.4	
A11	23.8	
A12	23.9	
B1	26.7	
B2	25.1	
B3	27.1	
B4	27.0	
B5	25.6	





# Setting Threshold

Use the Same Threshold for All PCR Arrays

## 2 Ways to “CRUNCH” the Data

### Excel Based Templates

- Free!
- Download from <http://www.sabiosciences.com/pcrarraydataanalysis.php>
- Good for 2 Group Comparisons (Control + Experimental)
- 10 PCR Arrays per Group

### Web-Based Data Analysis

- Free!
- Upload Excel spreadsheet at <http://www.sabiosciences.com/pcr/arrayanalysis.php>
- Good for 11 Group Comparisons (Control + 10 Experimental)
- 255 PCR Arrays Total

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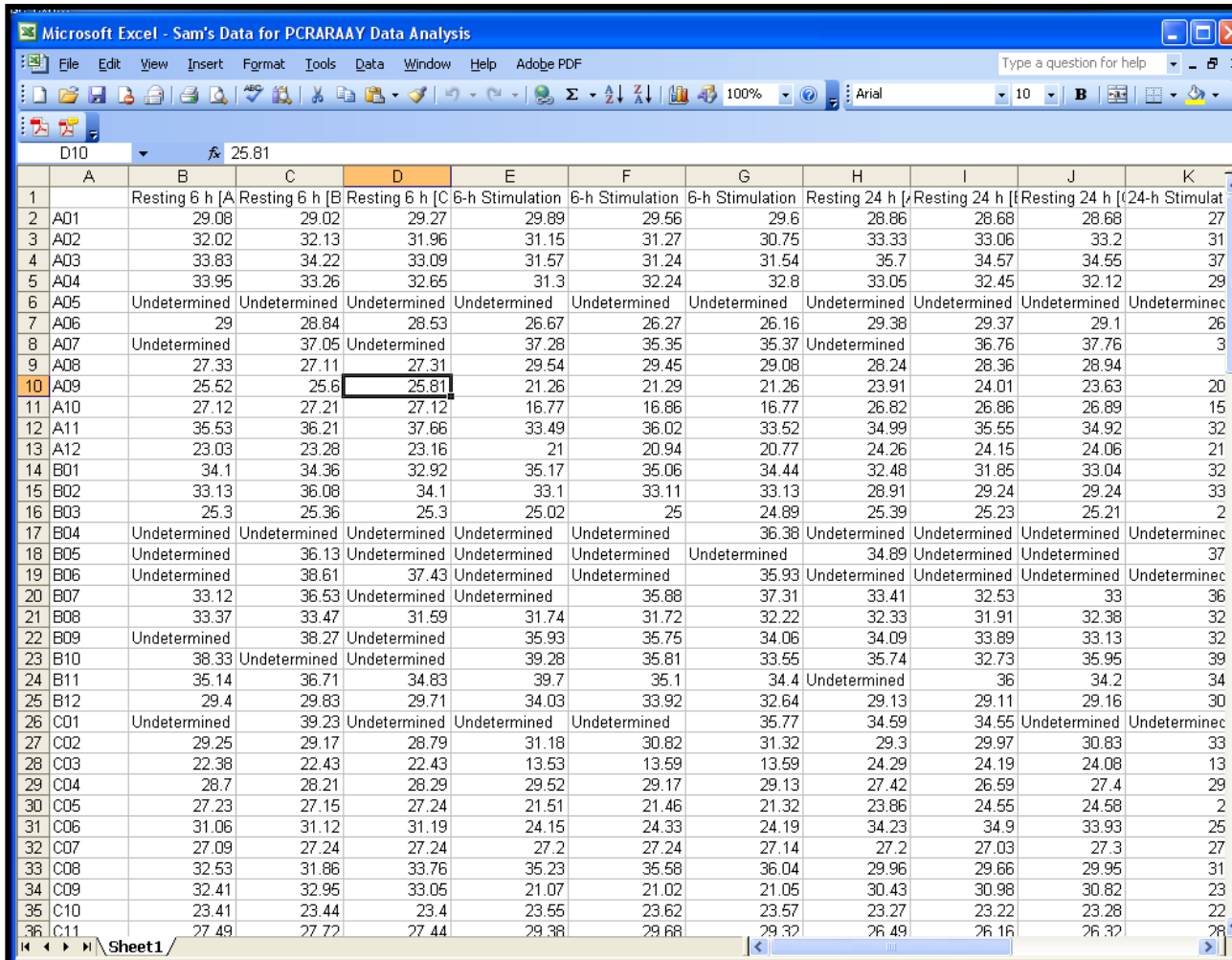
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	A	B	C	D	E	F	G	H	I	J	K
1		Resting 6 h [A	Resting 6 h [B	Resting 6 h [C	6-h Stimulation	6-h Stimulation	6-h Stimulation	Resting 24 h [	Resting 24 h [	Resting 24 h [	24-h Stimulat
2	A01	29.08	29.02	29.27	29.89	29.56	29.6	28.86	28.68	28.68	27
3	A02	32.02	32.13	31.96	31.15	31.27	30.75	33.33	33.06	33.2	31
4	A03	33.83	34.22	33.09	31.57	31.24	31.54	35.7	34.57	34.55	37
5	A04	33.95	33.26	32.65	31.3	32.24	32.8	33.05	32.45	32.12	29
6	A05	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined
7	A06	29	28.84	28.53	26.67	26.27	26.16	29.38	29.37	29.1	26
8	A07	Undetermined	37.05	Undetermined	37.28	35.35	35.37	Undetermined	36.76	37.76	3
9	A08	27.33	27.11	27.31	29.54	29.45	29.08	28.24	28.36	28.94	
10	A09	25.52	25.6	25.81	21.26	21.29	21.26	23.91	24.01	23.63	20
11	A10	27.12	27.21	27.12	16.77	16.86	16.77	26.82	26.86	26.89	15
12	A11	35.53	36.21	37.66	33.49	36.02	33.52	34.99	35.55	34.92	32
13	A12	23.03	23.28	23.16	21	20.94	20.77	24.26	24.15	24.06	21
14	B01	34.1	34.36	32.92	35.17	35.06	34.44	32.48	31.85	33.04	32
15	B02	33.13	36.08	34.1	33.1	33.11	33.13	28.91	29.24	29.24	33
16	B03	25.3	25.36	25.3	25.02	25	24.89	25.39	25.23	25.21	2
17	B04	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	36.38	Undetermined	Undetermined	Undetermined	Undetermined
18	B05	Undetermined	36.13	Undetermined	Undetermined	Undetermined	Undetermined	34.89	Undetermined	Undetermined	37
19	B06	Undetermined	38.61	37.43	Undetermined	Undetermined	35.93	Undetermined	Undetermined	Undetermined	Undetermined
20	B07	33.12	36.53	Undetermined	Undetermined	35.88	37.31	33.41	32.53	33	36
21	B08	33.37	33.47	31.59	31.74	31.72	32.22	32.33	31.91	32.38	32
22	B09	Undetermined	38.27	Undetermined	35.93	35.75	34.06	34.09	33.89	33.13	32
23	B10	38.33	Undetermined	Undetermined	39.28	35.81	33.55	35.74	32.73	35.95	39
24	B11	35.14	36.71	34.83	39.7	35.1	34.4	Undetermined	36	34.2	34
25	B12	29.4	29.83	29.71	34.03	33.92	32.64	29.13	29.11	29.16	30
26	C01	Undetermined	39.23	Undetermined	Undetermined	Undetermined	35.77	34.59	34.55	Undetermined	Undetermined
27	C02	29.25	29.17	28.79	31.18	30.82	31.32	29.3	29.97	30.83	33
28	C03	22.38	22.43	22.43	13.53	13.59	13.59	24.29	24.19	24.08	13
29	C04	28.7	28.21	28.29	29.52	29.17	29.13	27.42	26.59	27.4	29
30	C05	27.23	27.15	27.24	21.51	21.46	21.32	23.86	24.55	24.58	2
31	C06	31.06	31.12	31.19	24.15	24.33	24.19	34.23	34.1	33.93	25
32	C07	27.09	27.24	27.24	27.2	27.24	27.14	27.2	27.03	27.3	27
33	C08	32.53	31.86	33.76	35.23	35.58	36.04	29.96	29.66	29.95	31
34	C09	32.41	32.95	33.05	21.07	21.02	21.05	30.43	30.98	30.82	23
35	C10	23.41	23.44	23.4	23.55	23.62	23.57	23.27	23.22	23.28	22
36	C11	27.49	27.72	27.44	29.38	29.68	29.32	26.49	26.16	26.32	28

Cataloged Array

Row 1  
Sample Name

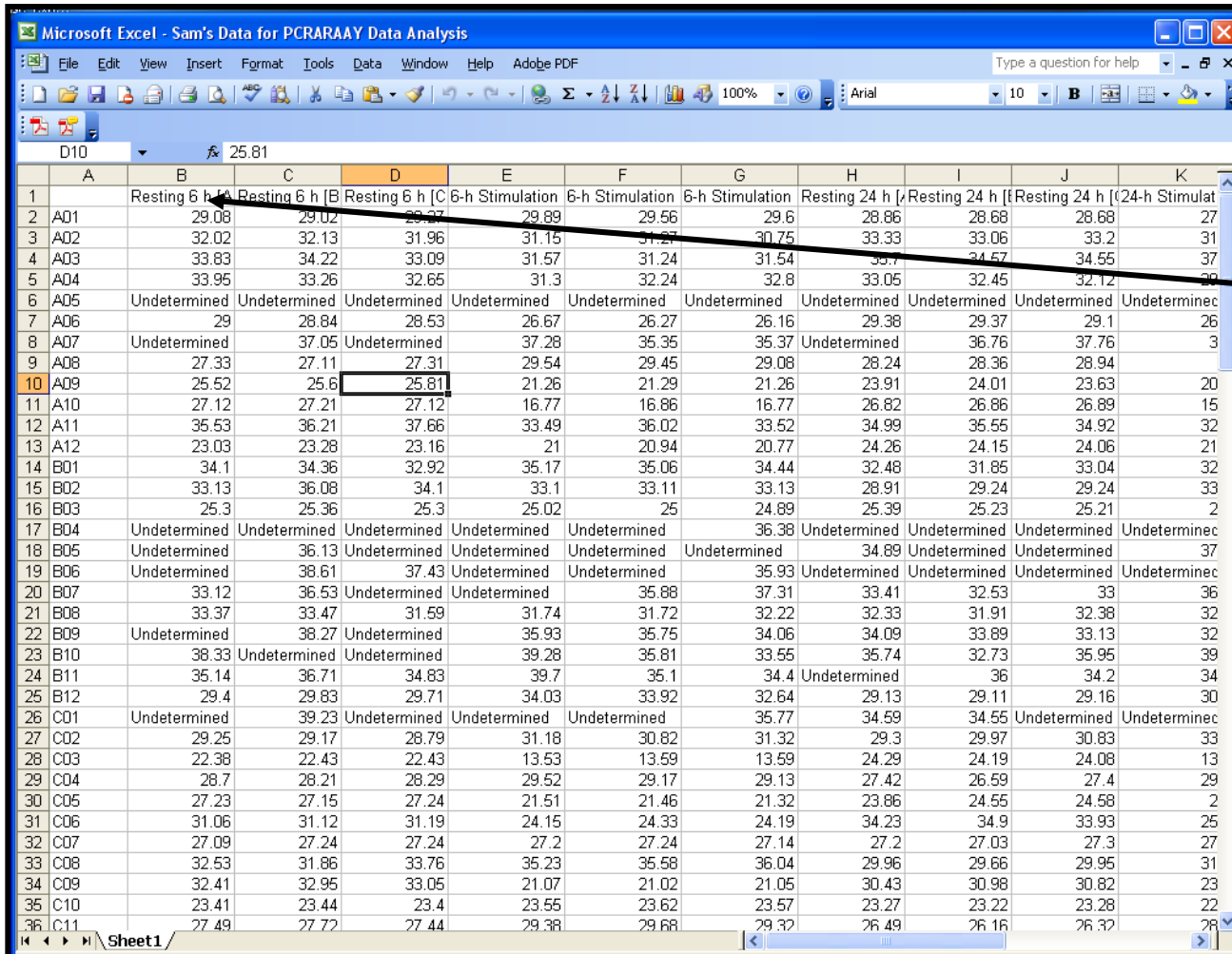
Column A:  
Well Location

Column B-??:

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	A	B	C	D	E	F	G	H	I	J	K
1		Resting 6 h	Resting 6 h	Resting 6 h	6-h Stimulation	6-h Stimulation	6-h Stimulation	Resting 24 h	Resting 24 h	Resting 24 h	24-h Stimulation
2	A01	29.08	29.02	29.07	29.89	29.56	29.6	28.86	28.68	28.68	27
3	A02	32.02	32.13	31.96	31.15	31.27	30.75	33.33	33.06	33.2	31
4	A03	33.83	34.22	33.09	31.57	31.24	31.54	33.7	34.57	34.55	37
5	A04	33.95	33.26	32.65	31.3	32.24	32.8	33.05	32.45	32.12	30
6	A05	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined
7	A06	29	28.84	28.53	26.67	26.27	26.16	29.38	29.37	29.1	26
8	A07	Undetermined	37.05	Undetermined	37.28	35.35	35.37	Undetermined	36.76	37.76	3
9	A08	27.33	27.11	27.31	29.54	29.45	29.08	28.24	28.36	28.94	
10	A09	25.52	25.6	25.81	21.26	21.29	21.26	23.91	24.01	23.63	20
11	A10	27.12	27.21	27.12	16.77	16.86	16.77	26.82	26.86	26.89	15
12	A11	35.53	36.21	37.66	33.49	36.02	33.52	34.99	35.55	34.92	32
13	A12	23.03	23.28	23.16	21	20.94	20.77	24.26	24.15	24.06	21
14	B01	34.1	34.36	32.92	35.17	35.06	34.44	32.48	31.85	33.04	32
15	B02	33.13	36.08	34.1	33.1	33.11	33.13	28.91	29.24	29.24	33
16	B03	25.3	25.36	25.3	25.02	25	24.89	25.39	25.23	25.21	2
17	B04	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	36.38	Undetermined	Undetermined	Undetermined	Undetermined
18	B05	Undetermined	36.13	Undetermined	Undetermined	Undetermined	Undetermined	34.89	Undetermined	Undetermined	37
19	B06	Undetermined	38.61	37.43	Undetermined	Undetermined	35.93	Undetermined	Undetermined	Undetermined	Undetermined
20	B07	33.12	36.53	Undetermined	Undetermined	35.88	37.31	33.41	32.53	33	36
21	B08	33.37	33.47	31.59	31.74	31.72	32.22	32.33	31.91	32.38	32
22	B09	Undetermined	38.27	Undetermined	35.93	35.75	34.06	34.09	33.89	33.13	32
23	B10	38.33	Undetermined	Undetermined	39.28	35.81	33.55	35.74	32.73	35.95	39
24	B11	35.14	36.71	34.83	39.7	35.1	34.4	Undetermined	36	34.2	34
25	B12	29.4	29.83	29.71	34.03	33.92	32.64	29.13	29.11	29.16	30
26	C01	Undetermined	39.23	Undetermined	Undetermined	Undetermined	35.77	34.59	34.55	Undetermined	Undetermined
27	C02	29.25	29.17	28.79	31.18	30.82	31.32	29.3	29.97	30.83	33
28	C03	22.38	22.43	22.43	13.53	13.59	13.59	24.29	24.19	24.08	13
29	C04	28.7	28.21	28.29	29.52	29.17	29.13	27.42	26.59	27.4	29
30	C05	27.23	27.15	27.24	21.51	21.46	21.32	23.86	24.55	24.58	2
31	C06	31.06	31.12	31.19	24.15	24.33	24.19	34.23	34.1	33.93	25
32	C07	27.09	27.24	27.24	27.2	27.24	27.14	27.2	27.03	27.3	27
33	C08	32.53	31.86	33.76	35.23	35.58	36.04	29.96	29.66	29.95	31
34	C09	32.41	32.95	33.05	21.07	21.02	21.05	30.43	30.98	30.82	23
35	C10	23.41	23.44	23.4	23.55	23.62	23.57	23.27	23.22	23.28	22
36	C11	27.49	27.72	27.44	29.38	29.68	29.32	26.49	26.16	26.32	28

Cataloged Array

Row 1  
Sample Name

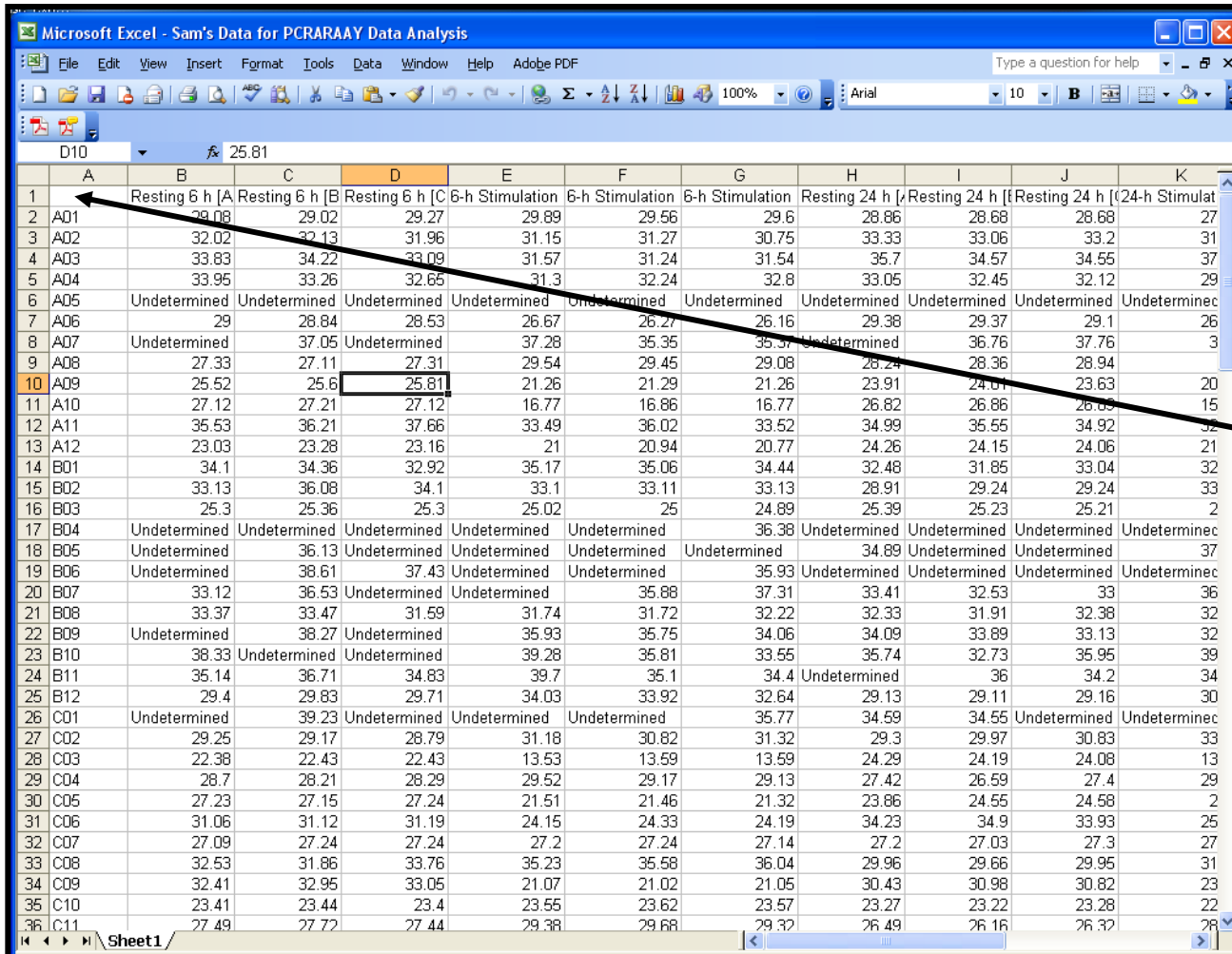
Column A:  
Well Location

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	A	B	C	D	E	F	G	H	I	J	K
1		Resting 6 h [A	Resting 6 h [B	Resting 6 h [C	6-h Stimulation	6-h Stimulation	6-h Stimulation	Resting 24 h [	Resting 24 h [	Resting 24 h [	24-h Stimulat
2	A01	29.08	29.02	29.27	29.89	29.56	29.6	28.86	28.68	28.68	27
3	A02	32.02	32.13	31.96	31.15	31.27	30.75	33.33	33.06	33.2	31
4	A03	33.83	34.22	33.09	31.57	31.24	31.54	35.7	34.57	34.55	37
5	A04	33.95	33.26	32.65	31.3	32.24	32.8	33.05	32.45	32.12	29
6	A05	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined
7	A06	29	28.84	28.53	26.67	26.27	26.16	29.38	29.37	29.1	26
8	A07	Undetermined	37.05	Undetermined	37.28	35.35	35.37	Undetermined	36.76	37.76	3
9	A08	27.33	27.11	27.31	29.54	29.45	29.08	28.24	28.36	28.94	3
10	A09	25.52	25.6	25.81	21.26	21.29	21.26	23.91	24.01	23.63	20
11	A10	27.12	27.21	27.12	16.77	16.86	16.77	26.82	26.86	26.86	15
12	A11	35.53	36.21	37.66	33.49	36.02	33.52	34.99	35.55	34.92	32
13	A12	23.03	23.28	23.16	21	20.94	20.77	24.26	24.15	24.06	21
14	B01	34.1	34.36	32.92	35.17	35.06	34.44	32.48	31.85	33.04	32
15	B02	33.13	36.08	34.1	33.1	33.11	33.13	28.91	29.24	29.24	33
16	B03	25.3	25.36	25.3	25.02	25	24.89	25.39	25.23	25.21	2
17	B04	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	36.38	Undetermined	Undetermined	Undetermined	Undetermined
18	B05	Undetermined	36.13	Undetermined	Undetermined	Undetermined	Undetermined	34.89	Undetermined	Undetermined	37
19	B06	Undetermined	38.61	37.43	Undetermined	Undetermined	35.93	Undetermined	Undetermined	Undetermined	Undetermined
20	B07	33.12	36.53	Undetermined	Undetermined	35.88	37.31	33.41	32.53	33	36
21	B08	33.37	33.47	31.59	31.74	31.72	32.22	32.33	31.91	32.38	32
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24	B11	35.14	36.71	34.83	39.7	35.1	34.4	Undetermined	36	34.2	34
25	B12	29.4	29.83	29.71	34.03	33.92	32.64	29.13	29.11	29.16	30
26	C01	Undetermined	39.23	Undetermined	Undetermined	Undetermined	35.77	34.59	34.55	Undetermined	Undetermined
27	C02	29.25	29.17	28.79	31.18	30.82	31.32	29.3	29.97	30.83	33
28	C03	22.38	22.43	22.43	13.53	13.59	13.59	24.29	24.19	24.08	13
29	C04	28.7	28.21	28.29	29.52	29.17	29.13	27.42	26.59	27.4	29
30	C05	27.23	27.15	27.24	21.51	21.46	21.32	23.86	24.55	24.58	2
31	C06	31.06	31.12	31.19	24.15	24.33	24.19	34.23	34.1	33.93	25
32	C07	27.09	27.24	27.24	27.2	27.24	27.14	27.2	27.03	27.3	27
33	C08	32.53	31.86	33.76	35.23	35.58	36.04	29.96	29.66	29.95	31
34	C09	32.41	32.95	33.05	21.07	21.02	21.05	30.43	30.98	30.82	23
35	C10	23.41	23.44	23.4	23.55	23.62	23.57	23.27	23.22	23.28	22
36	C11	27.49	27.72	27.44	29.38	29.68	29.37	26.49	26.16	26.37	28

Cataloged Array

Row 1  
Sample Name

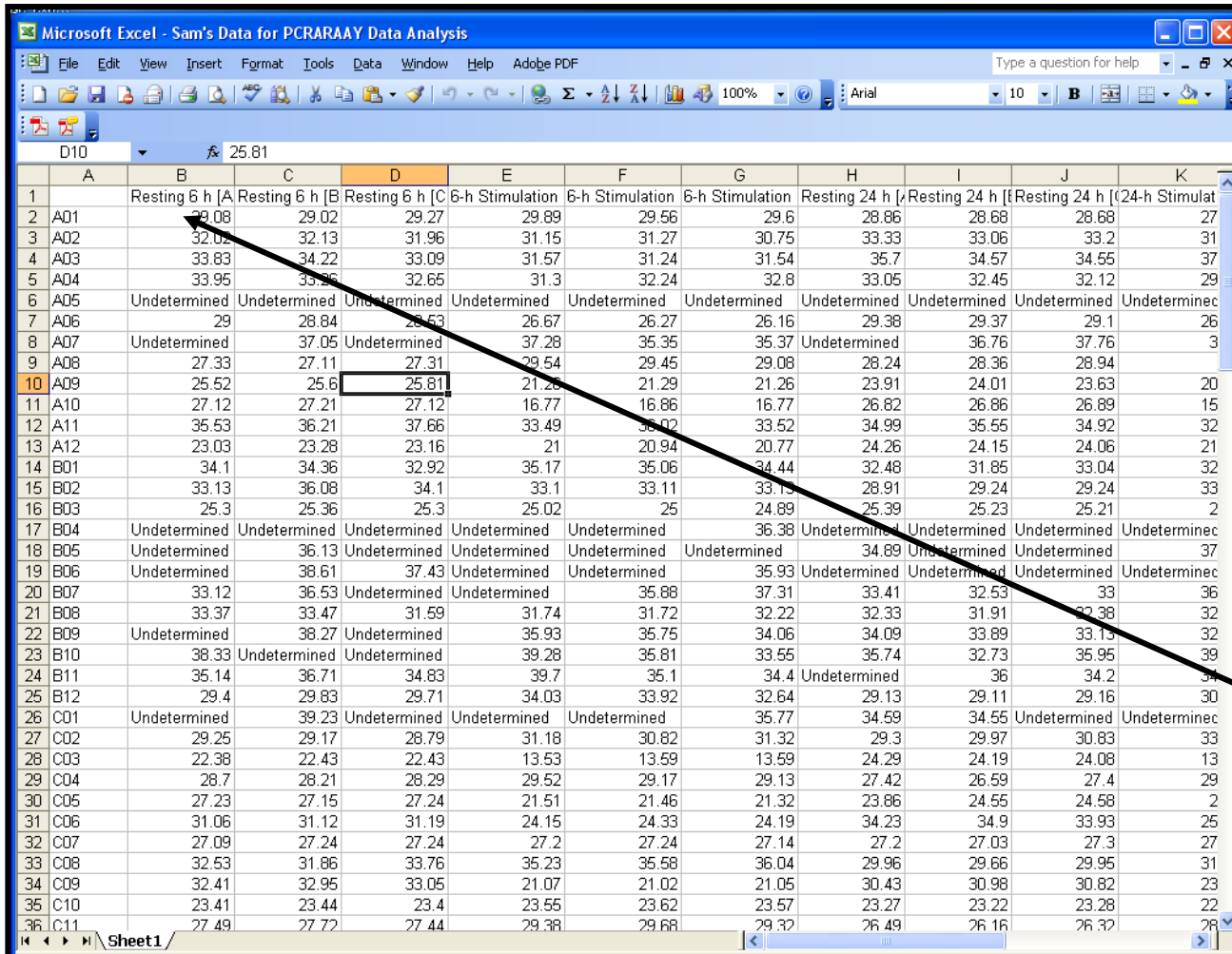
Column A:  
Well Location

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2	A01	29.08	29.02	29.27	29.89	29.56	29.6	28.86	28.68	28.68	27
3	A02	32.02	32.13	31.96	31.15	31.27	30.75	33.33	33.06	33.2	31
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7	A06	29	28.84	28.53	26.67	26.27	26.16	29.38	29.37	29.1	26
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10	A09	25.52	25.6	25.81	21.28	21.29	21.26	23.91	24.01	23.63	20
11	A10	27.12	27.21	27.12	16.77	16.86	16.77	26.82	26.86	26.89	15
12	A11	35.53	36.21	37.66	33.49	35.02	33.52	34.99	35.55	34.92	32
13	A12	23.03	23.28	23.16	21	20.94	20.77	24.26	24.15	24.06	21
14	B01	34.1	34.36	32.92	35.17	35.06	34.44	32.48	31.85	33.04	32
15	B02	33.13	36.08	34.1	33.1	33.11	33.15	28.91	29.24	29.24	33
16	B03	25.3	25.36	25.3	25.02	25	24.89	25.39	25.23	25.21	2
17	B04	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	36.38	Undetermined	Undetermined	Undetermined	Undetermined
18	B05	Undetermined	36.13	Undetermined	Undetermined	Undetermined	Undetermined	34.89	Undetermined	Undetermined	37
19	B06	Undetermined	38.61	37.43	Undetermined	Undetermined	35.93	Undetermined	Undetermined	Undetermined	Undetermined
20	B07	33.12	36.53	Undetermined	Undetermined	35.88	37.31	33.41	32.53	33	36
21	B08	33.37	33.47	31.59	31.74	31.72	32.22	32.33	31.91	32.38	32
22	B09	Undetermined	38.27	Undetermined	35.93	35.75	34.06	34.09	33.89	33.13	32
23	B10	38.33	Undetermined	Undetermined	39.28	35.81	33.55	35.74	32.73	35.95	39
24	B11	35.14	36.71	34.83	39.7	35.1	34.4	Undetermined	36	34.2	34
25	B12	29.4	29.83	29.71	34.03	33.92	32.64	29.13	29.11	29.16	30
26	C01	Undetermined	39.23	Undetermined	Undetermined	Undetermined	35.77	34.59	34.55	Undetermined	Undetermined
27	C02	29.25	29.17	28.79	31.18	30.82	31.32	29.3	29.97	30.83	33
28	C03	22.38	22.43	22.43	13.53	13.59	13.59	24.29	24.19	24.08	13
29	C04	28.7	28.21	28.29	29.52	29.17	29.13	27.42	26.59	27.4	29
30	C05	27.23	27.15	27.24	21.51	21.46	21.32	23.86	24.55	24.58	2
31	C06	31.06	31.12	31.19	24.15	24.33	24.19	34.23	34.1	33.93	25
32	C07	27.09	27.24	27.24	27.2	27.24	27.14	27.2	27.03	27.3	27
33	C08	32.53	31.86	33.76	35.23	35.58	36.04	29.96	29.66	29.95	31
34	C09	32.41	32.95	33.05	21.07	21.02	21.05	30.43	30.98	30.82	23
35	C10	23.41	23.44	23.4	23.55	23.62	23.57	23.27	23.22	23.28	22
36	C11	27.49	27.72	27.44	29.38	29.68	29.32	26.49	26.16	26.32	28

Cataloged Array

Row 1  
Sample Name

Column A:  
Well Location

Column B-??:

Raw C(t) Values

# Organizing Raw C(t) values

Download Excel Template from SABiosciences' Web Portal...or make your own.

http://www.sabiosciences.com/pcr/customarraytemplate.xls - Microsoft Internet Explorer

File Edit View Insert Format Tools Data Go To Favorites Help

Address http://www.sabiosciences.com/pcr/customarraytemplate.xls

Links SABiosciences WebEx Enterprise Site SABiosciences- PCR Arrays, shRNA, Cell-based Assays.

	A	B	C	D	E	F	G	H	I
		Gene Symbol	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
1									
2	1	ADAMTS1	24.2	24.19	24.33	26.67	27.01	24.45	25.54
3	2	ADAMTS13	31.71	31.99	31.61	26.04	27.59	24.53	33.56
4	3	ADAMTS8	27.41	27.59	27.58	23.91	24.37	24.54	30.86
5	4	CD44	25.93	26.04	25.98	23.91	24.84	26.09	28.55
6	5	CDH1	24.9	24.68	24.56	26.3	26.3	25.37	26.1
7	6	CNTN1	31.04	31.48	31.45	25.35	26.75	25.4	32.01
8	7	COL11A1	23.77	23.84	23.85	24.37	26.19	25.56	26.27
9	8	COL12A1	27.76	28.02	27.8	25.21	25.7	30.43	30.02
10	9	COL14A1	27.36	27.53	27.4	25.49	27.96	24.12	27.93
11	10	COL15A1	28.75	29.06	28.85	27.65	26.03	24.25	33.59
12	11	COL16A1	27.96	28	27.86	24.26	25.21	24.42	31.81
13	12	COL1A1	25.7	25.67	25.86	25.39	27.16	24.77	31.85
14	13	COL4A2	26.45	26.59	26.53	29.72	28.49	24.6	31.36
15	14	COL5A1	25.28	25.36	25.37	24.15	25.37	24.71	29.46
16	15	COL6A1	27.97	28.43	28.16	25.44	24.28	24.77	30.48
17	16	COL6A2	26.9	27.42	27.3	25.68	24.9	24.52	28.29
18	17	COL7A1	33.1	33.29	32.83	25.48	28.2	26.37	34.2
19	18	COL8A1	24.03	24.13	24.02	25.14	25.5	25.67	26.28
20	19	VCAN	26.73	27.1	26.83	24.79	27.86	24.03	30.49
21	20	CTGF	Undetermined	25.16	25.14	24.26	24.9	25.4	25.46
22	21	CTNNA1	26.48	26.85	26.57	25.18	25.69	24.42	29.8
23	22	CTNNA1	31.12	30.99	31.01	24.17	29.8	24.93	32.91
24	23	CTNND1	24.05	24.19	24.12	23.91	28.33	23.35	27.02
25	24	CTNND2	26.52	26.68	26.82	26.07	25.48	24.25	29.16
26	25	ECM1	32.71	34.81	33.67	24.35	26.2	26.1	34.26
27	26	FN1	24.47	24.66	24.67	23.28	25.95	24.3	24.44
28	27	HAS1	27.93	28.02	28.01	24.3	25.74	24.09	32.81
29	28	ICAM1	25.76	25.72	25.8	Undetermined	26.53	24.16	27.88
30	29	ITGA1	34.55	33.54	33.76	24.18	25.98	24.01	Undetermined
31	30	ITGA2	33.27	33.79	34.48	23.7	25.88	25.33	Undetermined
32	31	ITGA3	28.69	29.15	28.92	25.48	25.81	28.88	28.05
33	32	ITGA4	23.67	23.71	23.69	24.39	26.35	24.33	27.58
34	33	ITGA5	24.22	24.38	24.17	25.75	Undetermined	24.85	26.96

Sheet1 / Sheet2 / Sheet3 /

Unknown Zone

## Custom Array

Row 1  
Sample Name

Column A:  
Well Location

Column B:  
Gene Name

Column C-??:  
Raw C(t) Values



# Organizing Raw C(t) values

Download Excel Template from SABiosciences' Web Portal...or make your own.

http://www.sabiosciences.com/pcr/customarraytemplate.xls - Microsoft Internet Explorer

File Edit View Insert Format Tools Data Go To Favorites Help

Address http://www.sabiosciences.com/pcr/customarraytemplate.xls

Links SABiosciences WebEx Enterprise Site SABiosciences- PCR Arrays, shRNA, Cell-based Assays.

	A	B	C	D	E	F	G	H	I
		Gene Symbol	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
1									
2	1	ADAMTS1	24.2	24.19	24.33	26.67	27.01	24.45	25.54
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4	3	ADAMTS8	27.41	27.59	27.58	23.91	24.37	24.54	30.86
5	4	CD44	25.93	26.04	25.98	23.91	24.84	26.09	28.55
6	5	CDH1	24.9	24.68	24.56	26.3	26.3	25.37	26.1
7	6	CNTN1	31.04	31.48	31.45	25.35	26.75	25.4	32.01
8	7	COL11A1	23.77	23.84	23.85	24.37	26.19	25.56	26.27
9	8	COL12A1	27.76	28.02	27.8	25.21	25.7	30.43	30.02
10	9	COL14A1	27.36	27.53	27.4	25.49	27.96	24.12	27.93
11	10	COL15A1	28.75	29.06	28.85	27.65	26.03	24.25	33.59
12	11	COL16A1	27.96	28	27.86	24.26	25.21	24.42	31.81
13	12	COL1A1	25.7	25.67	25.86	25.39	27.16	24.77	31.85
14	13	COL4A2	26.45	26.59	26.53	29.72	28.49	24.6	31.36
15	14	COL5A1	25.28	25.36	25.37	24.15	25.37	24.71	29.46
16	15	COL6A1	27.97	28.43	28.16	25.44	24.28	24.77	30.48
17	16	COL6A2	26.9	27.42	27.3	25.68	24.9	24.52	28.29
18	17	COL7A1	33.1	33.29	32.83	25.48	28.2	26.37	34.2
19	18	COL8A1	24.03	24.13	24.02	25.14	25.5	25.67	26.28
20	19	VCAN	26.73	27.1	26.83	24.79	27.86	24.03	30.49
21	20	CTGF	Undetermined	25.16	25.14	24.26	24.9	25.4	25.46
22	21	CTNNA1	26.48	26.85	26.57	25.18	25.69	24.42	29.8
23	22	CTNNA1	31.12	30.99	31.01	24.17	29.8	24.93	32.91
24	23	CTNND1	24.05	24.19	24.12	23.91	28.33	23.35	27.02
25	24	CTNND2	26.52	26.68	26.82	26.07	25.48	24.25	29.16
26	25	ECM1	32.71	34.81	33.67	24.35	26.2	26.1	34.26
27	26	FN1	24.47	24.66	24.67	23.28	25.95	24.3	24.44
28	27	HAS1	27.93	28.02	28.01	24.3	25.74	24.09	32.81
29	28	ICAM1	25.76	25.72	25.8	Undetermined	26.53	24.16	27.88
30	29	ITGA1	34.55	33.54	33.76	24.18	25.98	24.01	Undetermined
31	30	ITGA2	33.27	33.79	34.48	23.7	25.88	25.33	Undetermined
32	31	ITGA3	33.27	33.79	34.48	23.7	25.88	25.33	Undetermined
33	32	ITGA4	33.27	33.79	34.48	23.7	25.88	25.33	Undetermined
34	33	ITGA5	33.27	33.79	34.48	23.7	25.88	25.33	Undetermined

Custom Array format can be adapted for Individual PCR Assays

## Custom Array

Row 1  
Sample Name

Column A:  
Well Location

Column B:  
Gene Name

Column C-??:  
Raw C(t) Values

# Organizing Raw C(t) values

Download Excel Template from SABiosciences' Web Portal...or make your own.  
LEAVE BLANK

	A	B	C	D	E
1		Gene Symbol	Sample 1	Sample 2	Sample 3
2	1	ADAMTS1	24.2	24.19	24.33
3	2	ADAMTS13	31.71	31.99	31.61
4	3	ADAMTS8	27.41	27.59	27.58
5	4	CD44	25.93	26.04	25.98
6	5	CDH1	24.5	24.68	24.56
7	6	CNTN1	31.04	31.48	31.45
8	7	COL11A1	23.77	23.84	23.85

Well #

UPLOAD AS CUSTOM ARRAY: **CPCRDATA**

## Custom Array

Row 1  
Sample Name

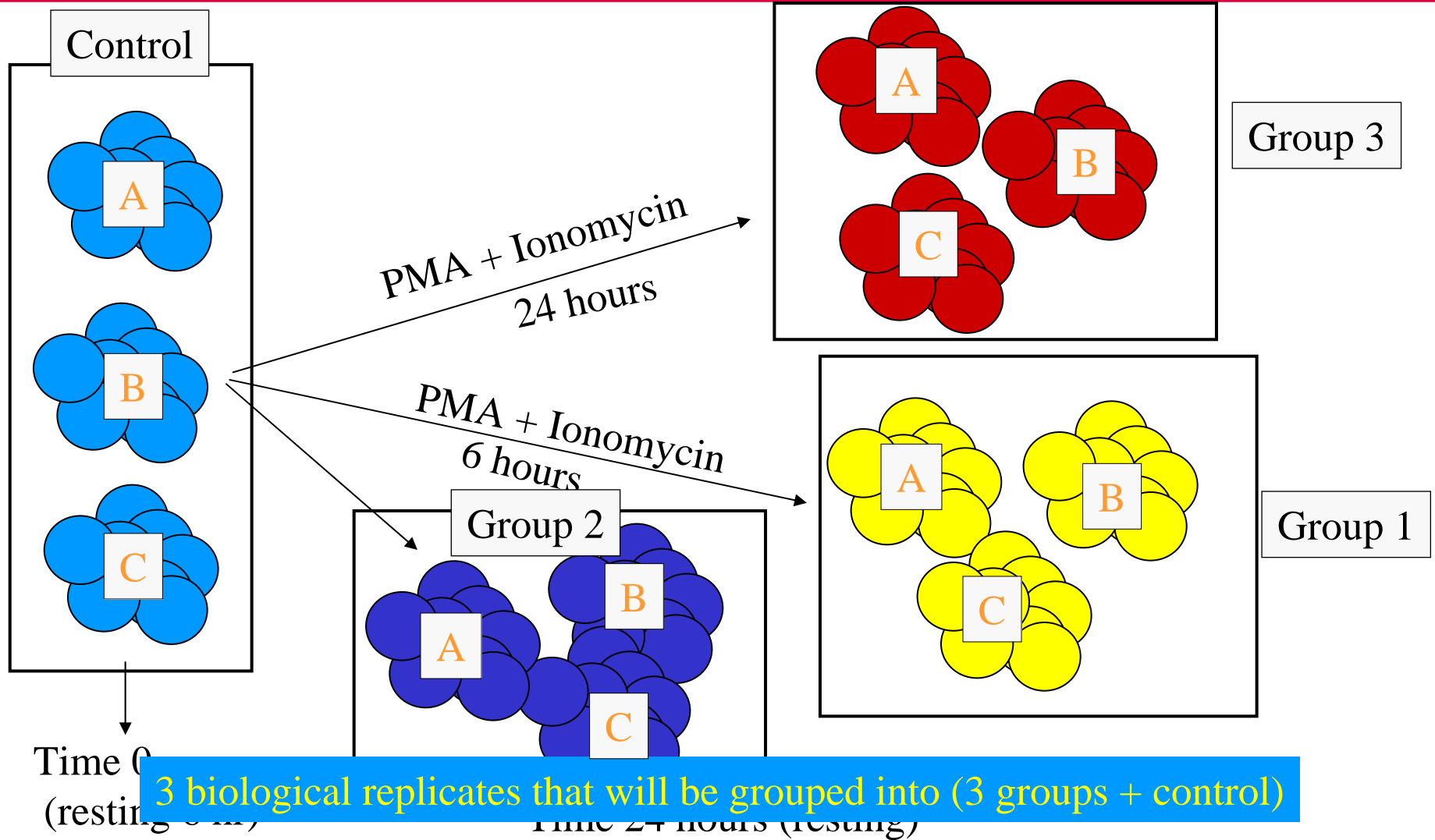
Column A:  
Well Location

Column B:  
Gene Name

Column C-??:  
Raw C(t) Values

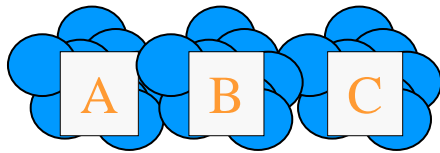
Custom Array format can be adapted for Individual PCR Assays

# Our Experiment

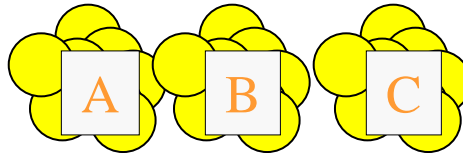


# Our Experiment-Data Analysis Overview

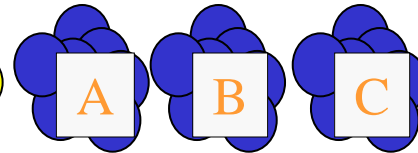
Control



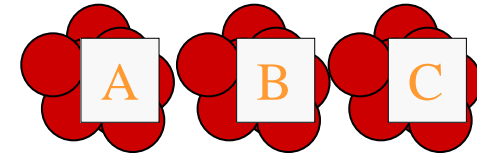
Group 1



Group 2

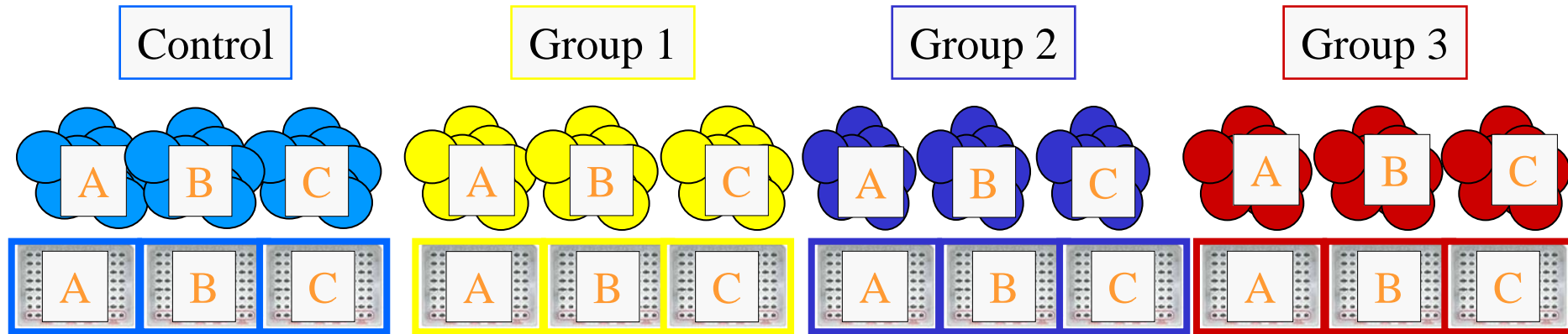


Group 3



3 biological replicates that will be grouped into (3 groups + control)

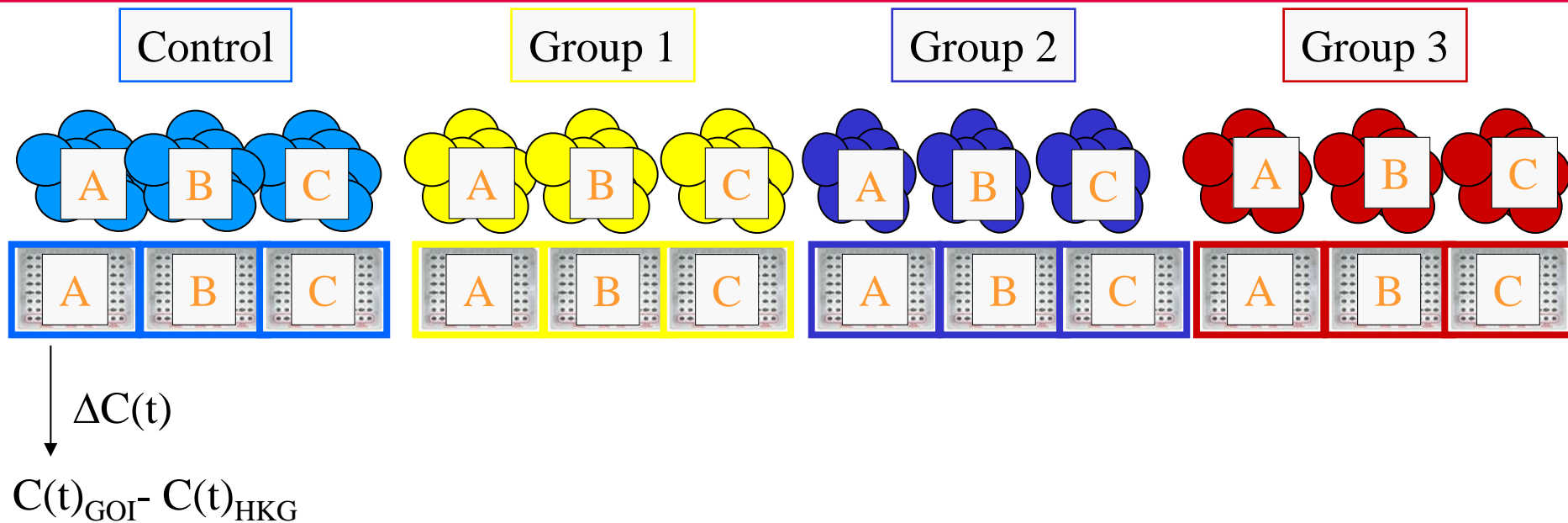
# Our Experiment-Data Analysis Overview



1 PCR Array for Each Sample (12 PCR Arrays Total)

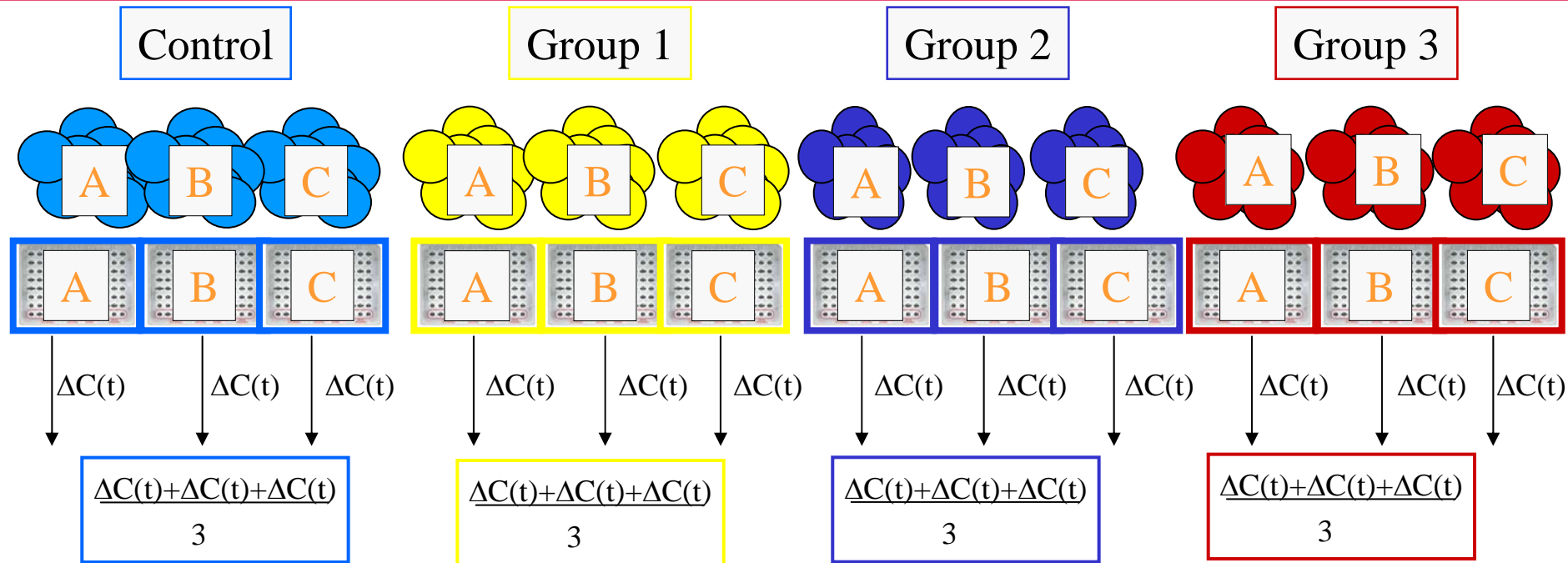


# Our Experiment-Data Analysis Overview



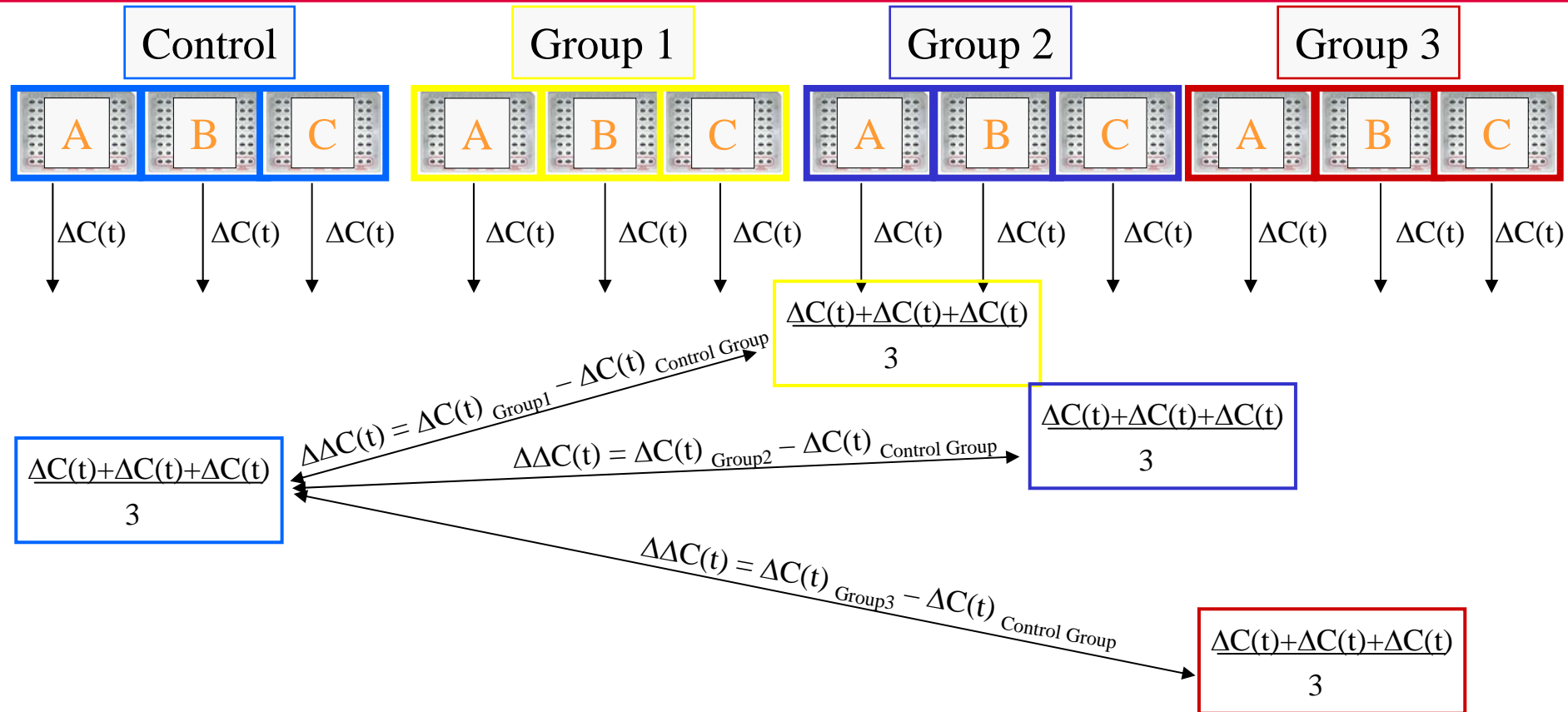
1. Calculate  $\Delta C(t)$  for on each array for each GOI (Gene Of Interest)

# Our Experiment-Data Analysis Overview



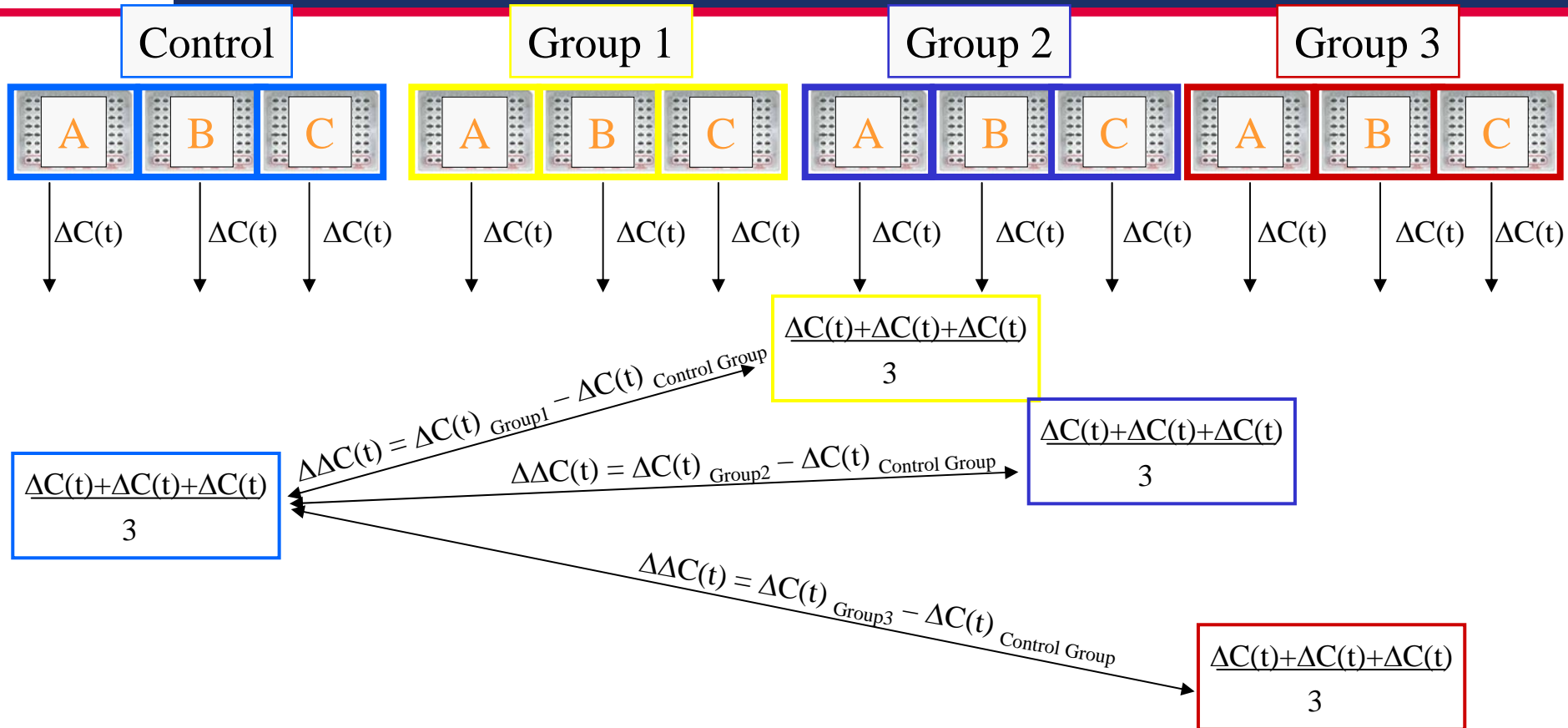
1. Calculate  $\Delta C(t)$  for on each array for each GOI (Gene Of Interest)
2. **Calculate Average  $\Delta C(t)$  for each gene within a Group**

# Our Experiment-Data Analysis Overview



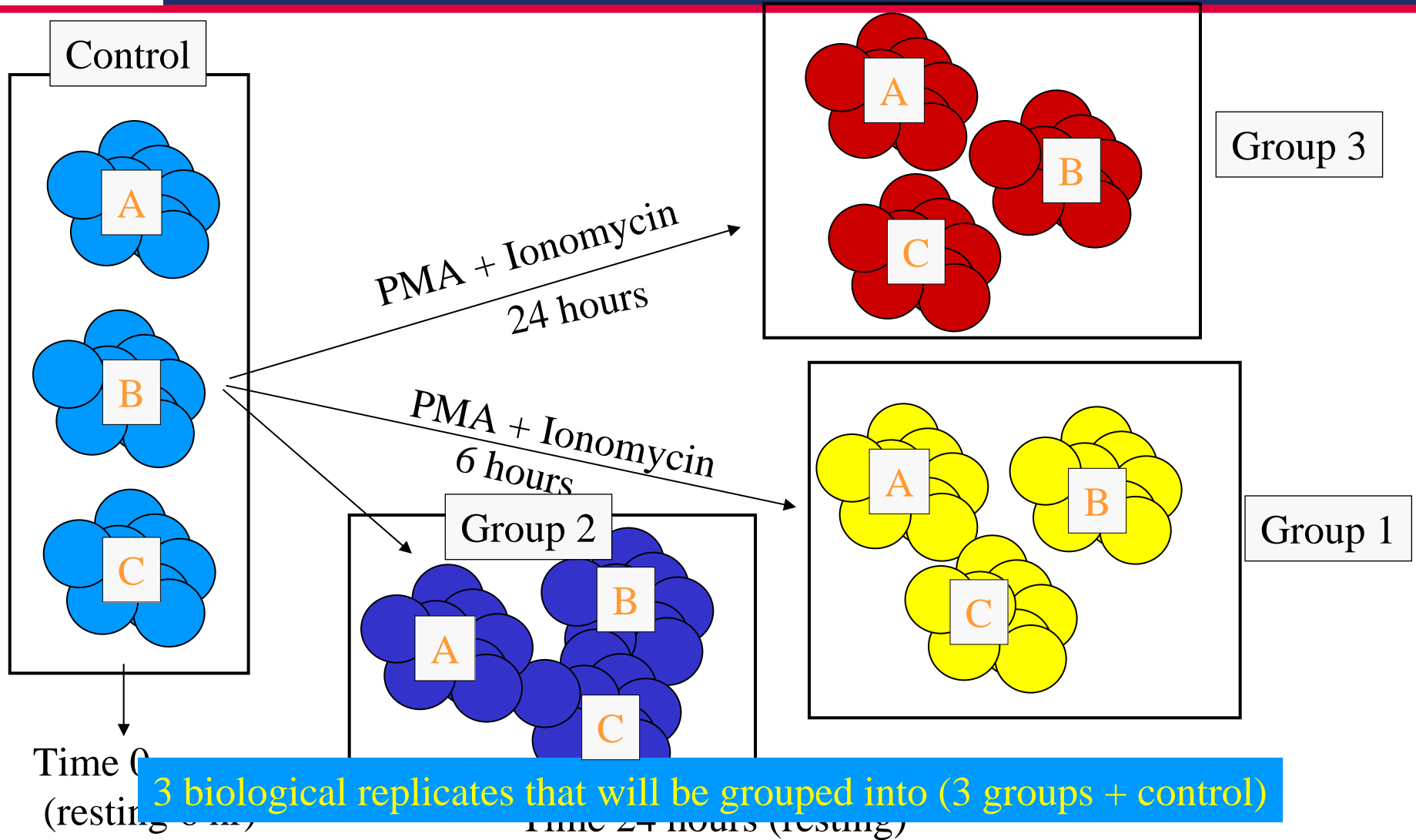
1. Calculate  $\Delta C(t)$  for on each array for each GOI (Gene Of Interest)
2. Calculate Average  $\Delta C(t)$  for each gene within a Group
3. Calculate  $\Delta\Delta C(t)$  for each gene between Groups

# Our Experiment-Data Analysis Overview



1. Calculate  $\Delta C(t)$  for on each array for each GOI (Gene Of Interest)
2. Calculate Average  $\Delta C(t)$  for each gene within a Group
3. Calculate  $\Delta\Delta C(t)$  for each gene between Groups
4. Calculate Fold Change:  $2^{(-\Delta\Delta C(t))}$

# Our Experiment





## TECHNICAL ARTICLE

### **RT<sup>2</sup> Profiler™ PCR Array Application Examples** *Pathway-Focused Gene Expression Profiling in Toxicology, Oncology, and Immunology Research*

Emi Arikawa, Savita Prabhakar, Hewen Zhang, Min You, Yexun (Bill) Wang, George Quellhorst, Xiao Zeng, Jeffrey Hung and Jingping Yang

SABiosciences 6951 Executive Way, Frederick, MD 21703 USA  
Phone: +1 (301) 682-9200 Fax: +1 (301) 682-7300 Web: [www.SABiosciences.com](http://www.SABiosciences.com)  
Email: [support@SABiosciences.com](mailto:support@SABiosciences.com)

**Abstract:** The RT<sup>2</sup> Profiler PCR Array System is the most reliable and accurate tool for analyzing the expression of a focused panel of genes using SYBR® Green-based real-time PCR. This paper describes the latest technique in pathway-focused gene expression profiling: the RT<sup>2</sup> Profiler PCR Array System from SABiosciences. The PCR Array System is comprised of a 96- or 384-well plate containing qPCR primer assays (84 pathway- or disease-focused genes, 5 housekeeping genes, and 3 replicate controls), instrument-specific SYBR® Green master mix, and a first strand cDNA synthesis kit. The PCR Arrays can be used for research on signal transduction, cancer, immunology, stem cells, toxicology, biomarker discovery and validation, and analysis of phenotypes.

In this paper, we report on three application-specific studies in the fields of toxicology, cancer, and immunology. In the first study, the PCR Arrays were used to profile gene expression changes due to compound-induced cytotoxicity in liver cells. We identified idiosyncratic patterns of expression changes with three drugs that induced liver toxicity, suggesting different mechanisms of action for liver toxicity. In the second study, the expression of cancer-related extracellular matrix and cellular adhesion genes were compared between breast tumors and normal tissue. We discovered a common set of genes with significant gene expression changes associated with two independent breast tumor samples. In the third study, cytokine gene expression between stimulated and unstimulated cells was shown



Control  
(resting)

[http://www.sabiosciences.com/manuals/PCRArrayWhitePaper\\_App.pdf](http://www.sabiosciences.com/manuals/PCRArrayWhitePaper_App.pdf)

# Topics to be Covered

## Topic I:

Brief Technology and Protocol Overview


Monday November 15<sup>th</sup> @ 1pm EST (Next RT<sup>2</sup> PCR Array Webinar)

## Topic II (Today):

PCR Array Data Analysis

- Defining Baseline and Threshold
- Web Portal Location / Address
- Uploading Raw C<sub>t</sub> Data
- Analyzing Data & Controls
- Exporting Data

# Web Portal Location


  
A QIAGEN Company

Upload Readout
Readout
Analysis Result
Scatter Plot
Volcano Plot
Clustergram
Multigroup Plot
Export Data

**RT<sup>2</sup> Profiler PCR Array Data Analysis**

Instruction - How to use this tool? [Take a Test Run](#) or [Play Movie Guide](#)

**PCR Data File Upload**

Catalog #:  Or 
  
File:

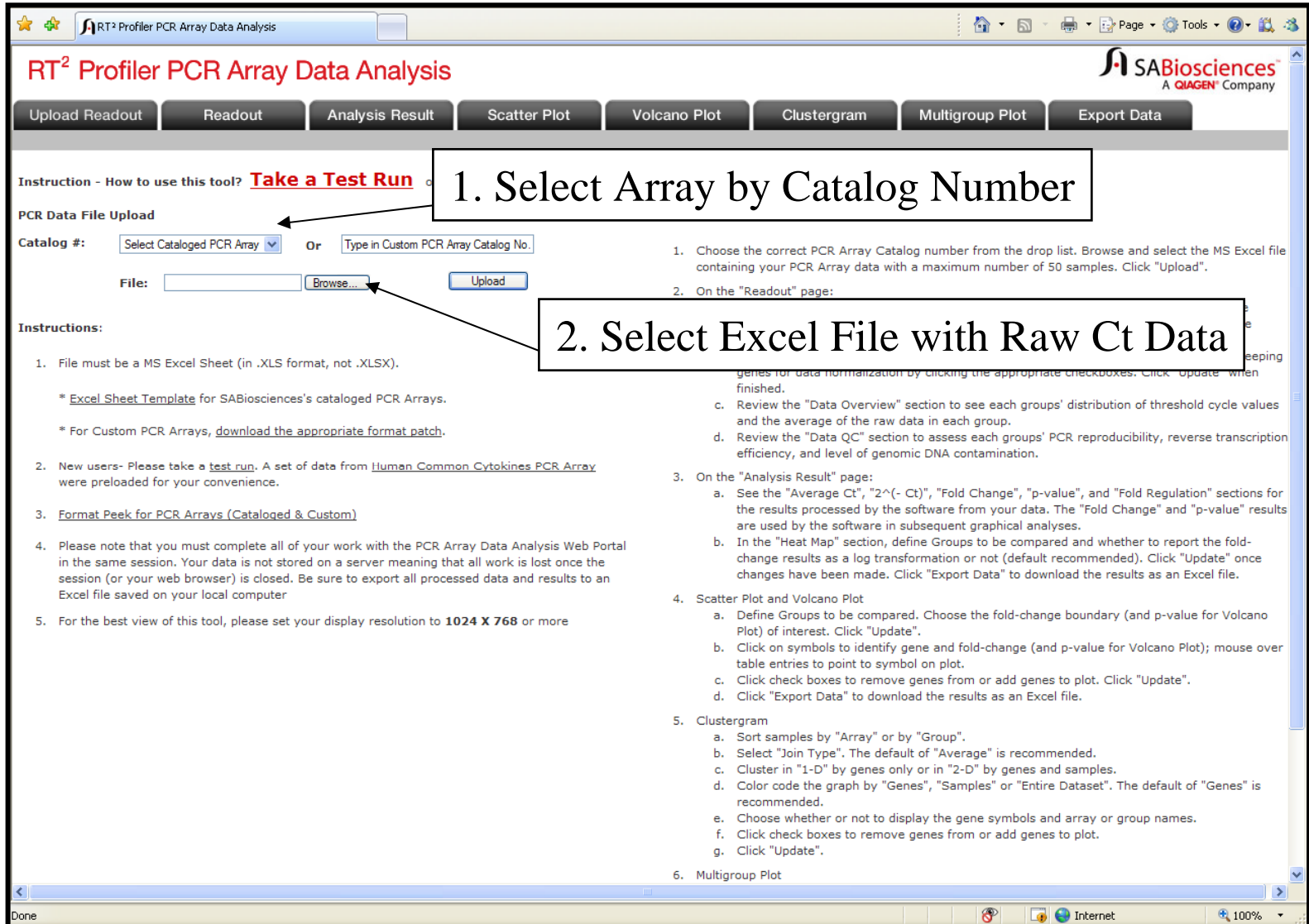
**Instructions:**

- File must be a MS Excel Sheet (in .XLS format, not .XLSX).
  - \* [Excel Sheet Template](#) for SABiosciences's cataloged PCR Arrays.
  - \* For Custom PCR Arrays, [download the appropriate format patch](#).
- New users- Please take a [test run](#). A set of data from [Human Common Cytokines PCR Array](#) were preloaded for your convenience.
- [Format Peek for PCR Arrays \(Cataloged & Custom\)](#)
- Please note that you must complete all of your work with the PCR Array Data Analysis Web Portal in the same session. Your data is not stored on a server meaning that all work is lost once the session (or your web browser) is closed. Be sure to export all processed data and results to an Excel file saved on your local computer
- For the best view of this tool, please set your display resolution to **1024 X 768** or more

- Choose the correct PCR Array Catalog number from the drop list. Browse and select the MS Excel file containing your PCR Array data with a maximum number of 50 samples. Click "Upload".
- On the "Readout" page:
  - In the "Basic Setup" section, assign samples to different groups. At least two groups are needed and up to a maximum of 11 are allowed, where one of those groups must be the control group. Click "Update" when finished.
  - The "View Housekeeping Genes" section allows you to remove or add preferred housekeeping genes for data normalization by clicking the appropriate checkboxes. Click "Update" when finished.
  - Review the "Data Overview" section to see each groups' distribution of threshold cycle values and the average of the raw data in each group.
  - Review the "Data QC" section to assess each groups' PCR reproducibility, reverse transcription efficiency, and level of genomic DNA contamination.
- On the "Analysis Result" page:
  - See the "Average Ct", "2<sup>-Δ(-ΔCt)</sup>", "Fold Change", "p-value", and "Fold Regulation" sections for the results processed by the software from your data. The "Fold Change" and "p-value" results are used by the software in subsequent graphical analyses.
  - In the "Heat Map" section, define Groups to be compared and whether to report the fold-change results as a log transformation or not (default recommended). Click "Update" once changes have been made. Click "Export Data" to download the results as an Excel file.
- Scatter Plot and Volcano Plot
  - Define Groups to be compared. Choose the fold-change boundary (and p-value for Volcano Plot) of interest. Click "Update".
  - Click on symbols to identify gene and fold-change (and p-value for Volcano Plot); mouse over table entries to point to symbol on plot.
  - Click check boxes to remove genes from or add genes to plot. Click "Update".
  - Click "Export Data" to download the results as an Excel file.
- Clustergram
  - Sort samples by "Array" or by "Group".
  - Select "Join Type". The default of "Average" is recommended.
  - Cluster in "1-D" by genes only or in "2-D" by genes and samples.
  - Color code the graph by "Genes", "Samples" or "Entire Dataset". The default of "Genes" is recommended.
  - Choose whether or not to display the gene symbols and array or group names.
  - Click check boxes to remove genes from or add genes to plot.
  - Click "Update"

<http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php>

# Uploading Raw Ct Data



The screenshot shows the RT² Profiler PCR Array Data Analysis web portal. The top navigation bar includes buttons for Upload Readout, Readout, Analysis Result, Scatter Plot, Volcano Plot, Clustergram, Multigroup Plot, and Export Data. The main content area is titled "RT² Profiler PCR Array Data Analysis" and features a "Take a Test Run" link. Below this, the "PCR Data File Upload" section contains a "Catalog #" dropdown menu and a "File:" input field with a "Browse..." button. Two callout boxes provide instructions: Box 1 points to the "Catalog #" dropdown and says "1. Select Array by Catalog Number". Box 2 points to the "Browse..." button and says "2. Select Excel File with Raw Ct Data". The "Instructions:" section lists five steps for using the tool, including file format requirements, test run instructions, and analysis options. The bottom of the page shows a status bar with "Done" and a progress indicator.

**RT<sup>2</sup> Profiler PCR Array Data Analysis**

Upload Readout | Readout | Analysis Result | Scatter Plot | Volcano Plot | Clustergram | Multigroup Plot | Export Data

Instruction - How to use this tool? [Take a Test Run](#)

**PCR Data File Upload**

Catalog #:  Or

File:

**Instructions:**

- File must be a MS Excel Sheet (in .XLS format, not .XLSX).
  - \* [Excel Sheet Template](#) for SABiosciences's cataloged PCR Arrays.
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- For the best view of this tool, please set your display resolution to **1024 X 768** or more

**1. Select Array by Catalog Number**

- Choose the correct PCR Array Catalog number from the drop list. Browse and select the MS Excel file containing your PCR Array data with a maximum number of 50 samples. Click "Upload".
- On the "Readout" page:

**2. Select Excel File with Raw Ct Data**

- genes for data normalization by clicking the appropriate checkboxes. Click "Update" when finished.
- Review the "Data Overview" section to see each groups' distribution of threshold cycle values and the average of the raw data in each group.
- Review the "Data QC" section to assess each groups' PCR reproducibility, reverse transcription efficiency, and level of genomic DNA contamination.

**3. On the "Analysis Result" page:**

- See the "Average Ct", "2<sup>-Δ(-Ct)</sup>", "Fold Change", "p-value", and "Fold Regulation" sections for the results processed by the software from your data. The "Fold Change" and "p-value" results are used by the software in subsequent graphical analyses.
- In the "Heat Map" section, define Groups to be compared and whether to report the fold-change results as a log transformation or not (default recommended). Click "Update" once changes have been made. Click "Export Data" to download the results as an Excel file.

**4. Scatter Plot and Volcano Plot**

- Define Groups to be compared. Choose the fold-change boundary (and p-value for Volcano Plot) of interest. Click "Update".
- Click on symbols to identify gene and fold-change (and p-value for Volcano Plot); mouse over table entries to point to symbol on plot.
- Click check boxes to remove genes from or add genes to plot. Click "Update".
- Click "Export Data" to download the results as an Excel file.

**5. Clustergram**

- Sort samples by "Array" or by "Group".
- Select "Join Type". The default of "Average" is recommended.
- Cluster in "1-D" by genes only or in "2-D" by genes and samples.
- Color code the graph by "Genes", "Samples" or "Entire Dataset". The default of "Genes" is recommended.
- Choose whether or not to display the gene symbols and array or group names.
- Click check boxes to remove genes from or add genes to plot.
- Click "Update".

**6. Multigroup Plot**

# Defining Experimental Design

RT<sup>2</sup> Profiler PCR Array Data Analysis

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Upload Readout | Readout | Analysis Result | Scatter Plot | Volcano Plot | Clustergram | Multigroup Plot | Export Data

Basic Setup | View Housekeeping Genes | Data Overview | Data QC

Update | Undo All Changes

Position	Gene Symbol	Control Gene	Resting 6 h	Resting 6 h	Resting 6 h	6-h Stimulation	6-h Stimulation	6-h Stimulation	Resting 24 h	Resting 24 h	Resting 24 h	24-h Stimulation	Stin
			Control Group	Control Group	Control Group	Group 1	Group 1	Group 1	Group 2	Group 2	Group 2	Group 3	Group
A01	BMP1	<input type="checkbox"/>	29.08	29.02	29.27	29.89	29.56	29.60	28.86	28.68	28.68	27.39	
A02	BMP2	<input type="checkbox"/>	32.02	32.13	31.9								
A03	BMP3	<input type="checkbox"/>	33.83	34.22	33.0								
A04	BMP4	<input type="checkbox"/>	33.95	33.26	32.85	31.30	32.24	32.80	33.03	32.43	32.12	29.21	
A05	BMP5	<input type="checkbox"/>	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	Unde
A06	BMP6	<input type="checkbox"/>	29.00	28.84	28.53	26.67	26.27	26.16	29.38	29.37	29.10	26.32	
A07	BMP7	<input type="checkbox"/>	Undetermined	37.05	Undetermined	37.28	35.35	35.37	Undetermined	36.76	37.76	34.30	
A08	BMP8B	<input type="checkbox"/>	27.33	27.11	27.3						28.94	31.00	
A09	CSF1	<input type="checkbox"/>	25.52	25.60	25.8						23.63	20.57	
A10	CSF2	<input type="checkbox"/>	27.12	27.21	27.12	16.77	16.80	16.77	26.82	26.80	26.89	15.49	
A11	FAM3B	<input type="checkbox"/>	35.53	36.21	37.66	33.49	36.02	33.52	34.99	35.55	34.92	32.88	
A12	FASLG	<input type="checkbox"/>	23.03	23.28	23.16	21.00	20.94	20.77	24.26	24.15	24.06	21.57	
B01	FIGF	<input type="checkbox"/>	34.10	34.36	32.92	35.17	35.06	34.44	32.48	31.85	33.04	32.53	
B02	GDF10	<input type="checkbox"/>	33.13	36.08	34.10	33.10	33.11	33.13	28.91	29.24	29.24	33.24	
B03	GDF11	<input type="checkbox"/>	25.30	25.36	25.30	25.02	25.00	24.89	25.39	25.23	25.21	26.10	
B04	GDF2	<input type="checkbox"/>	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined	36.38	Undetermined	Undetermined	Undetermined	Undetermined	Unde
B05	GDF3	<input type="checkbox"/>	Undetermined	36.13	Undetermined	Undetermined	Undetermined	Undetermined	34.89	Undetermined	Undetermined	37.85	Unde
B06	GDF5	<input type="checkbox"/>	Undetermined	38.61	37.43	Undetermined	Undetermined	35.93	Undetermined	Undetermined	Undetermined	Undetermined	Unde
B07	MSTN	<input type="checkbox"/>	33.12	36.53	Undetermined	Undetermined	35.88	37.31	33.41	32.53	33.00	36.29	
B08	GDF9	<input type="checkbox"/>	33.37	33.47	31.59	31.74	31.72	32.22	32.33	31.91	32.38	32.08	
B09	IFNA1	<input type="checkbox"/>	Undetermined	38.27	Undetermined	35.93	35.75	34.06	34.09	33.89	33.13	32.99	
B10	IFNA2	<input type="checkbox"/>	38.33	Undetermined	Undetermined	39.28	35.81	33.55	35.74	32.73	35.95	39.11	
B11	IFNA4	<input type="checkbox"/>	35.14	36.71	34.83	39.70	35.10	34.40	Undetermined	36.00	34.20	34.25	

1. Define Array Group (Control or Group X)

2. Select Housekeeping Gene



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Basic Setup | View Housekeeping Genes | Data Overview | **Data QC**

Select Group: Group 1

RT<sup>2</sup> Nano PreAMP cDNA Synthesis Kit used ☐

RT<sup>2</sup> FFPE PreAMP cDNA Synthesis Kit used ☐

### 1. PCR Array Reproducibility:

Array	6-h Stimulation	6-h Stimulation	6-h Stimulation	AVG	ST DEV
Average Ct (PPC)	18.4	18.13	18.14	18.22	0.15
ST DEV C <sub>t</sub> (PPC)	0.23	0.02	0.08	0.11	--
Average C <sub>t</sub> (RTC)	20.03	20.24	20.23	20.17	0.12
ST DEV C <sub>t</sub> (RTC)	0.05	0.04	0.18	0.09	--

### 2. Reverse Transcription Control (RTC):

Array	6-h Stimulation	6-h Stimulation	6-h Stimulation
Delta C <sub>t</sub> (AVG RTC - AVG PPC)	1.63	2.11	2.09
RT Efficiency	Pass	Pass	Pass

**Criteria:** If Delta C<sub>t</sub> (AVG RTC - AVG PPC) ≤ 5, RT Efficiency reports 'Pass'; otherwise, RT Efficiency reports 'Inquiry'.  
See the Troubleshooting Guide of the PCR Array User Manual or Contact Technical Support at 888-503-3187.

### 3. Genomic DNA Contamination (GDC):

Array	6-h Stimulation	6-h Stimulation	6-h Stimulation
C <sub>t</sub> (GDC)	35	35	35
Genomic DNA	Pass	Pass	Pass

**Criteria:** If C<sub>t</sub>(GDC) ≥ 35, then the GDC QC reports 'Pass'; if C<sub>t</sub>(GDC) < 35, then the GDC QC reports 'Inquiry'.  
See the Troubleshooting Guide of the PCR Array User Manual or Contact Technical Support at 888-503-3187.

1. Check PPC

2. Check RTC

3. Check GDC

# Select Housekeeping Gene

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Basic Setup | **View Housekeeping Genes** | Data Overview | Data QC

Normalized by: Arithmetic Mean Update

Position	Gene Symbol	Control Gene	Group 1			Group 2			Group 3			Control Group		
			6-h Stimulation	6-h Stimulation	6-h Stimulation	Resting 24 h	Resting 24 h	Resting 24 h	24-h Stimulation	24-h Stimulation	24-h Stimulation	Resting 6 h	Resting 6 h	Resting 6 h
H01	B2M	<input checked="" type="checkbox"/>	14.21	14.67	14.65	13.19	13.24	13.14	14.09	13.99	14.02	14.08	14.02	14.13
H02	HPRT1	<input checked="" type="checkbox"/>	25.01	24.19	24.09	24.87	24.39	24.65	22.39	22.23	22.59	24.52	24.44	24.52
H03	RPL13A	<input checked="" type="checkbox"/>	18.92	18.96	18.85	18.89	18.96	18.92	19.18	19.31	19.16	18.56	18.35	18.74
H04	GAPDH	<input checked="" type="checkbox"/>	18.20	18.31	18.20	17.52	17.50	17.61	16.86	17.00	16.97	17.89	17.77	18.01
H05	ACTB	<input checked="" type="checkbox"/>	17.20	17.29	17.12	15.57	15.59	15.65	17.53	17.89	17.73	17.30	17.13	17.48
Arithmetic Mean			18.71	18.68	18.58	18.01	17.94	17.99	18.01	18.08	18.09	18.47	18.34	18.58
Avg. of Arithmetic Mean			18.66			17.98			18.06			18.46		
Geometric Mean			18.39	18.44	18.34	17.60	17.56	17.60	17.80	17.88	17.88	18.17	18.05	18.29
Avg. of Geometric Mean			18.39			17.59			17.85			18.17		

Set Ct Cut-off: 35

# Analyze Results

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Position	Gene Symbol	Average $\Delta C_t$	$2^{-\Delta\Delta C_t}$	Fold Change	p-value	Fold Regulation	Heat Map
Fold Regulation (comparing to control group)							
		Group 1		Group 2		Group 3	
A01	BMP1	-1.29		-1.07		2.49	
A02	BMP2	2.26		-3.12		1.09	
A03	BMP3	5.50		-2.78		-3.22	
A04	BMP4	2.58		1.20		14.86	
		1.14		-1.40			
		6.14		-1.97			
		1.14		-1.40			
		-3.76		-3.36			
A09	CSF1	23.73		2.48		24.25	
A10	CSF2	1494.38		-1.14		2379.87	
A11	FAM3B	2.28		-1.37		3.94	
A12	FASLG	5.46		-2.80		2.32	
B01	FIGF	-1.77		1.81		1.95	
B02	GDF10	2.23		22.06		1.66	
B03	GDF11	1.46		-1.36		-2.19	
B04	GDF2	1.14		-1.40		-1.32	
B05	GDF3	1.14		-1.36		-1.32	
B06	GDF5	1.14		-1.40		-1.32	
B07	MSTN	-1.35		1.88		-1.16	
B08	GDF9	2.16		1.09		-1.10	
B09	IFNA1	1.42		1.76		3.80	
B10	IFNA2	1.60		1.21		-1.31	
B11	IFNA4	1.26		-1.21		1.12	
B12	IFNA5	-12.89		1.02		-3.85	
C01	IFNA8	1.14		-1.15		-1.32	
C02	IFNB1	-3.58		-2.73		-51.86	
C03	IFNG	525.91		-4.78		269.35	
C04	IFNK	-1.60		1.72		-3.32	
C05	IL10	62.77		5.25		1.36	
C06	IL11	136.74		-13.12		41.36	
C07	IL12A	1.14		-1.39		-1.31	
C08	IL12B	-4.25		5.19		2.11	
C09	IL13	3961.96		2.98		431.53	
C10	TXLNA	1.02		-1.25		1.42	
C11	IL15	-3.28		1.67		-3.12	
C12	IL16	-2.11		1.73		-7.45	
D01	IL17A	7601.14		2.76		9280.58	
D02	IL17B	1.14		-1.40		-1.32	
D03	IL17C	-1.55		-1.14		-19.79	
D04	IL25	1.14		-1.40		-1.32	

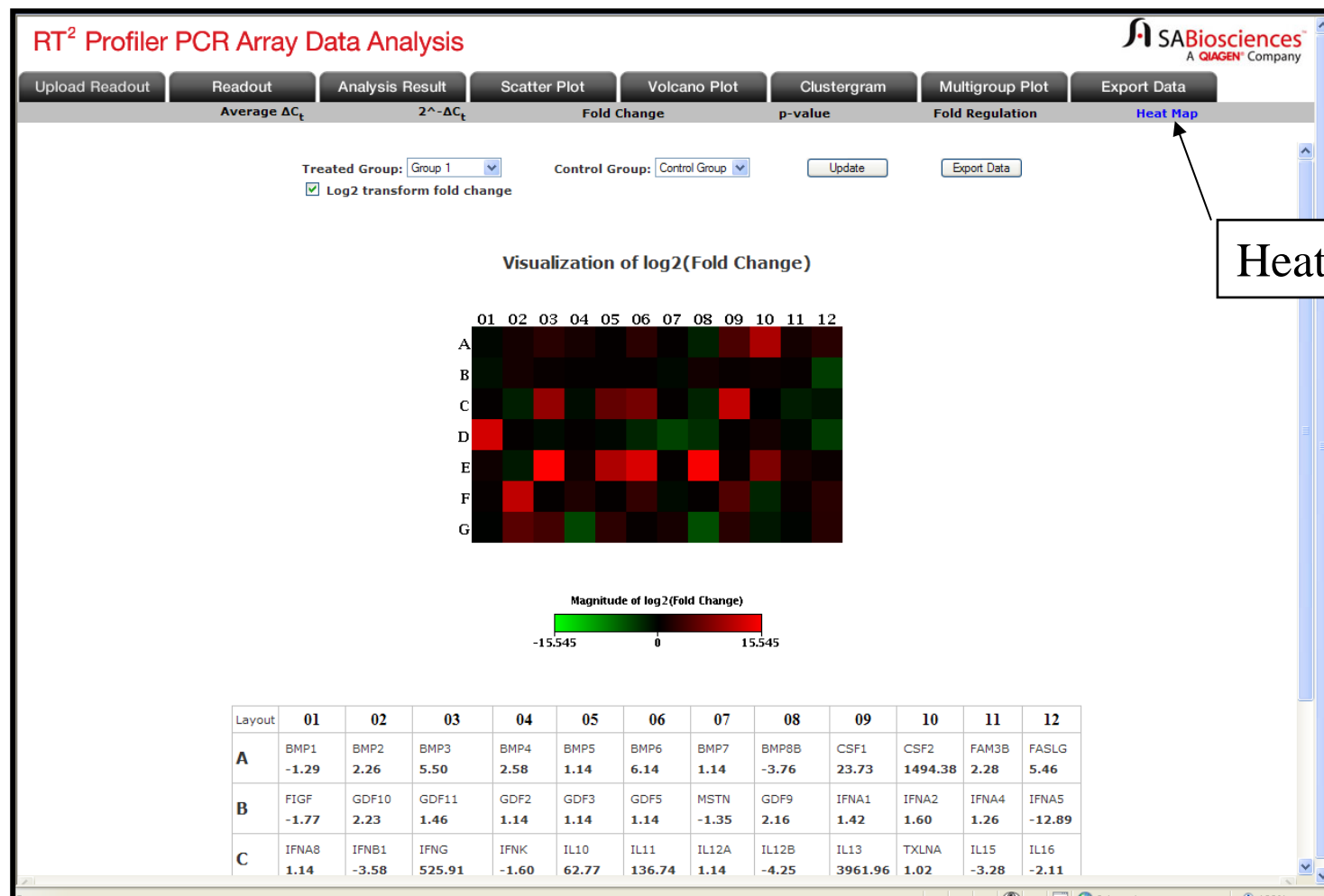
Done

Internet 100%

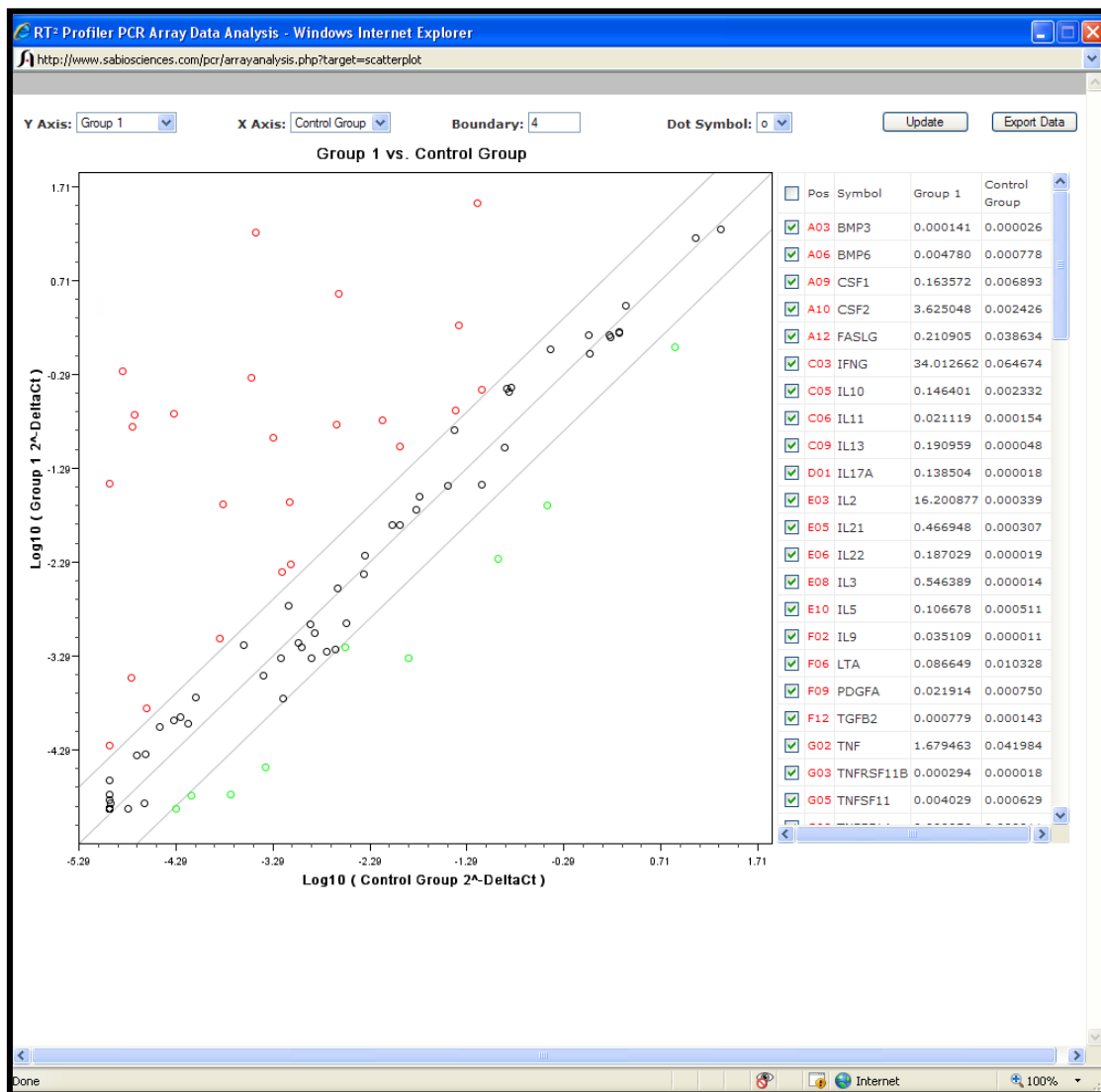
Average  $\Delta C_t$

Fold Change

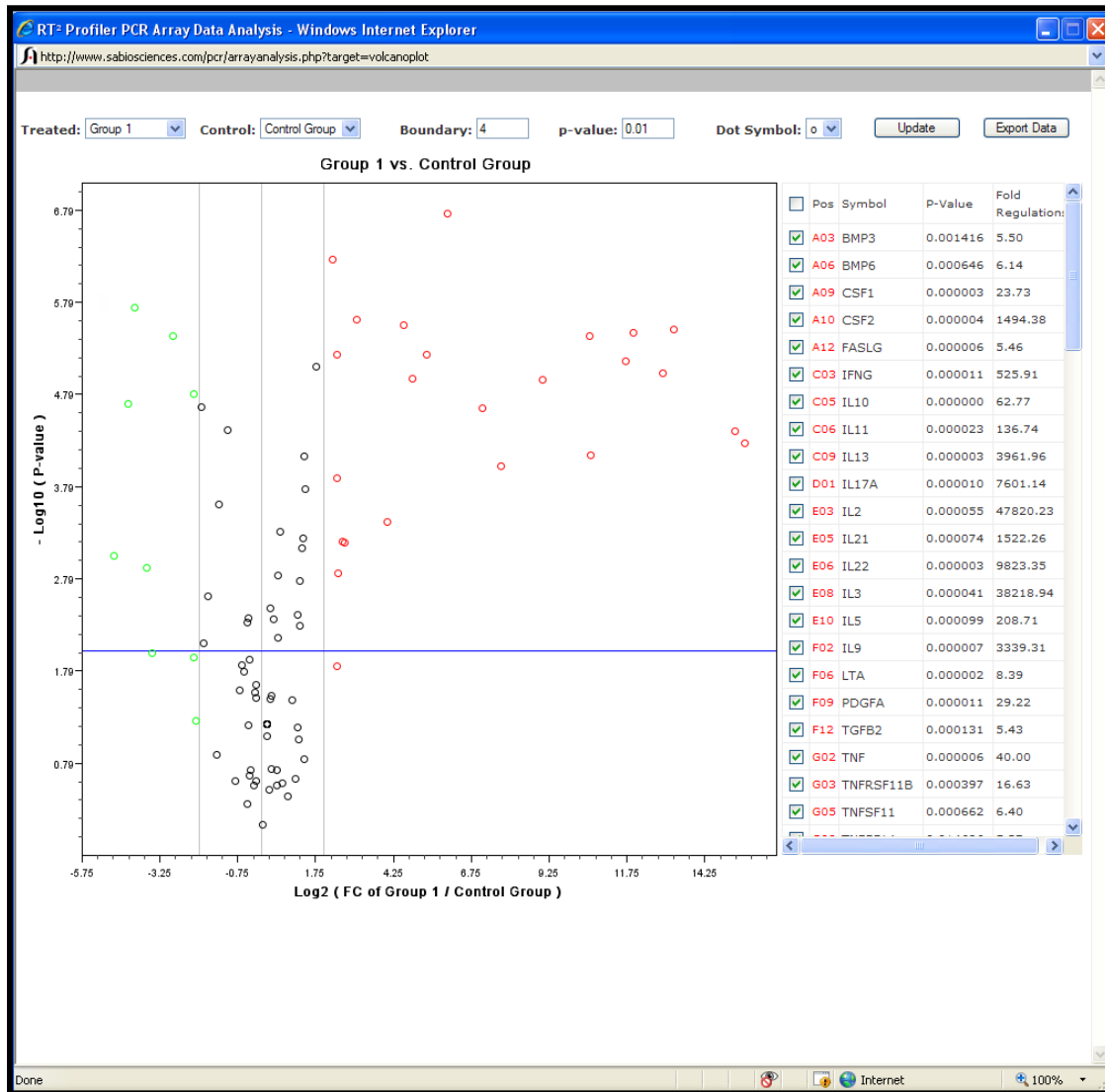
Fold Regulation



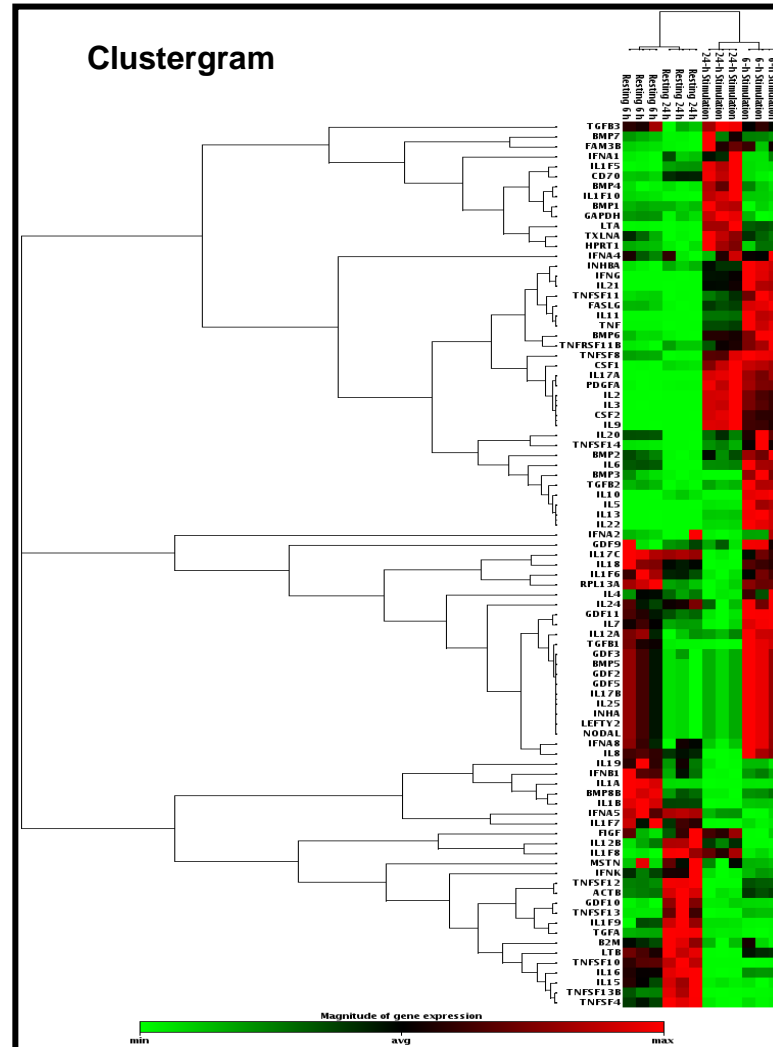
## Scatter Plot



## Volcano Plot







# Clustergram

# Summary

## **1. Set machine in Absolute Quantification / or Standard Curve Mode**

## **2. Set baseline: (ABI, Stratagene, Bio-Rad and Eppendorf\*)**

A. Adaptive (use auto)

## **3. Set threshold:**

A. Lower 2/3<sup>rd</sup>s of amplification plot in log view

B. Use same threshold for all PCR Arrays

\*Roche LC480 use Second Derivative Maximum

## **4. Export data into excel spreadsheet. Paste raw C(t) values into correct form:**

(sample row 1, C(t)s according to well location.)

## **5. Upload data to SABiosciences web portal, or download excel data analysis spreadsheets**

## **6. Analyze Data**

A. Group Biological/Technical Replicates

B. QC criteria (PPC, RTC, GDC)

C. Focus on Stable Housekeeping Genes

D. Fold Change Data

E. Export Data and Publish results

# Select Up-Coming Webinars. What do I do Next?

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