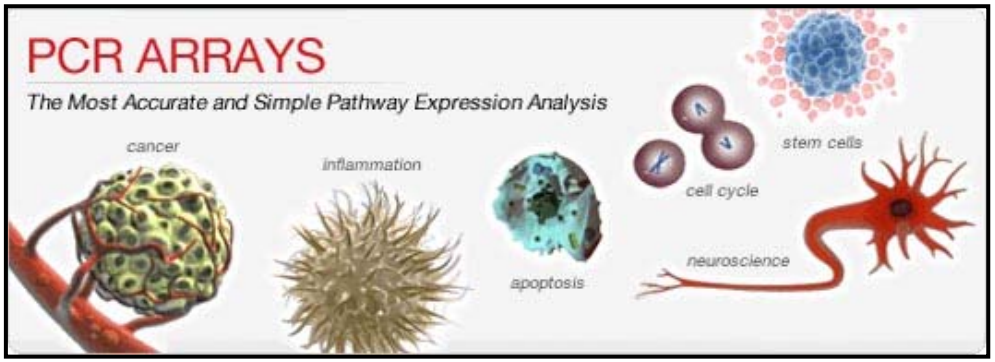
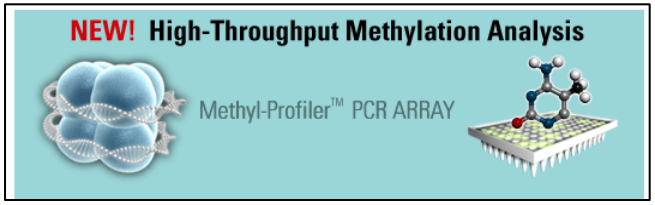
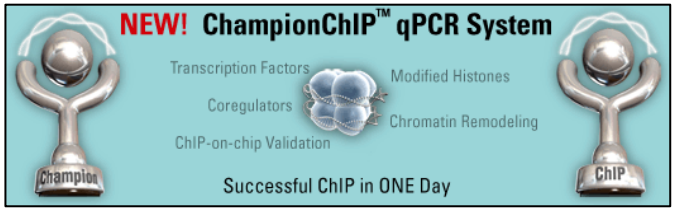
RT²*Profiler* PCR Array: Web-Based Data Analysis Tutorial

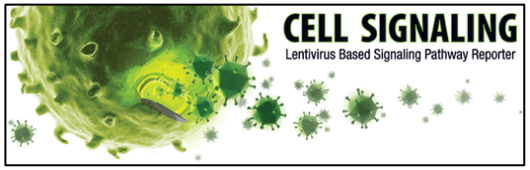


Samuel J. Rulli, Jr., Ph.D.
qPCR-Applications Scientist
Samuel.Rulli@QIAGEN.com

Pathway Focused Research from Sample Prep to Data Analysis!







Topics to be Covered

Topic I:

Brief Technology and Protocol Overview

Offered every Month! (Next RT^2 PCR Array Webinar)

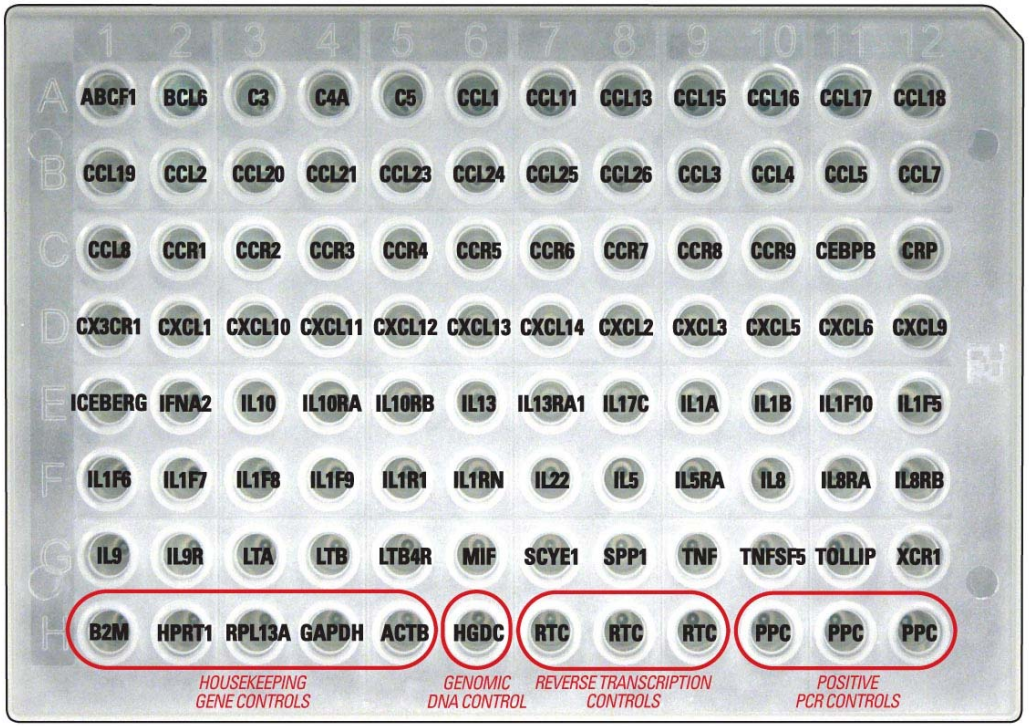
Topic II (Today):

PCR Array Data Analysis

- Defining Baseline and Threshold
- Web Portal Location / Address
- Uploading Raw C_t Data
- Analyzing Data & Controls
- Exporting Data

Anatomy of a PCR Array

Human Inflammatory Cytokines & Receptors RT^2 Profiler PCR Array



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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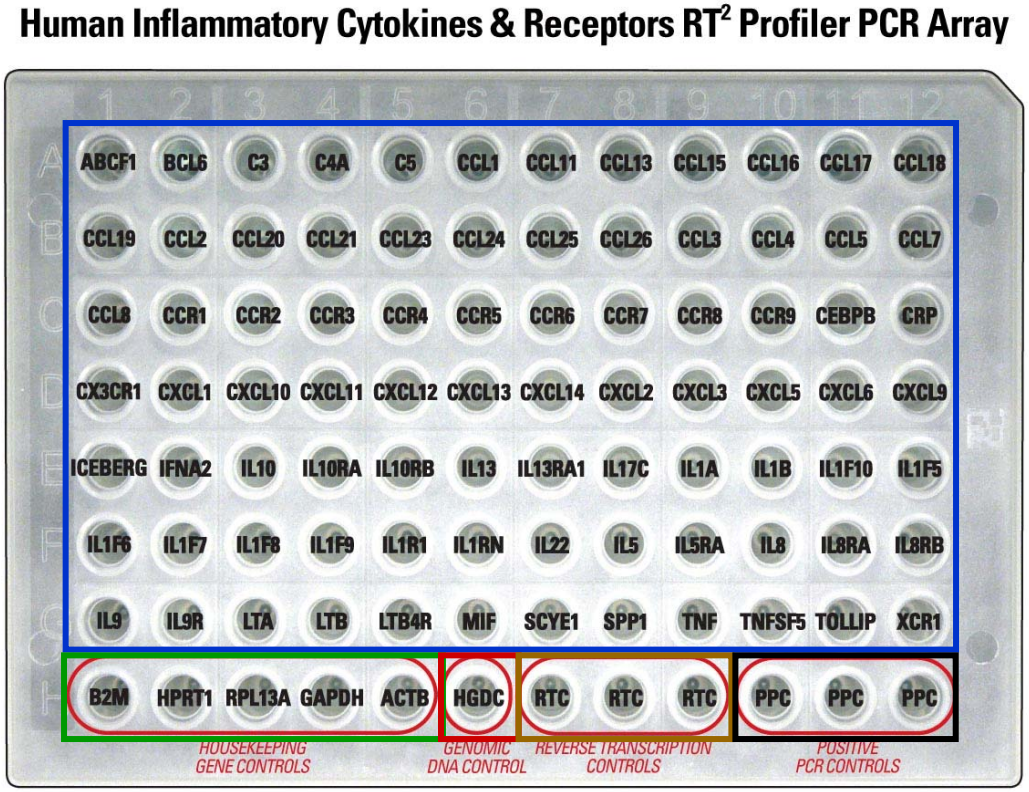

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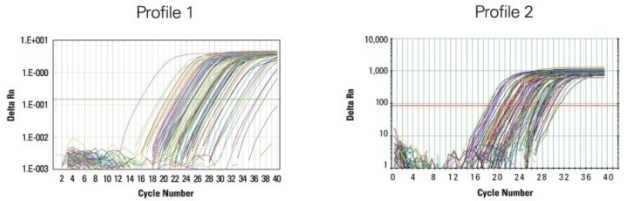

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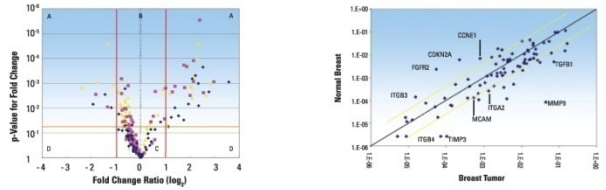

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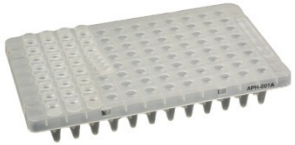

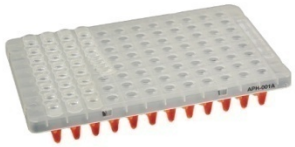

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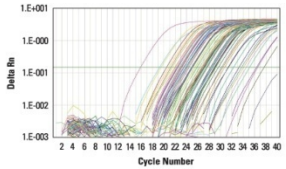

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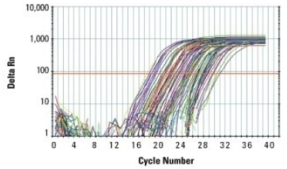

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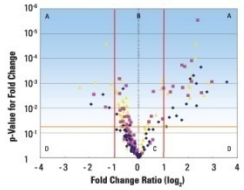

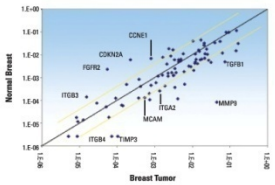

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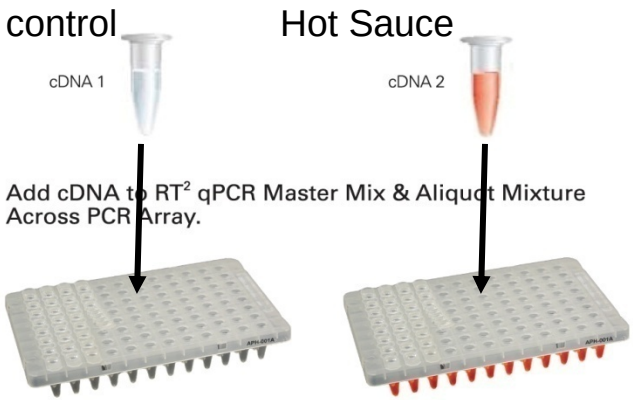

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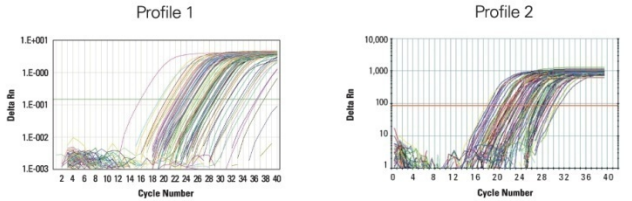

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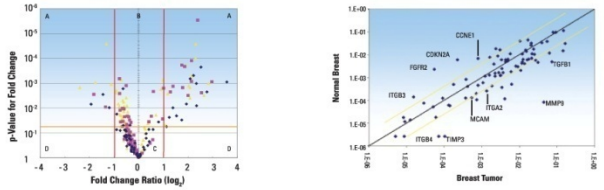

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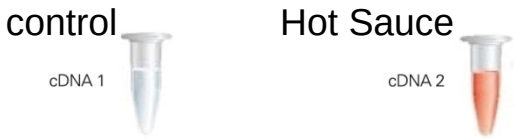

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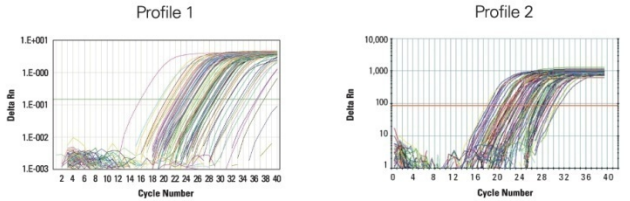

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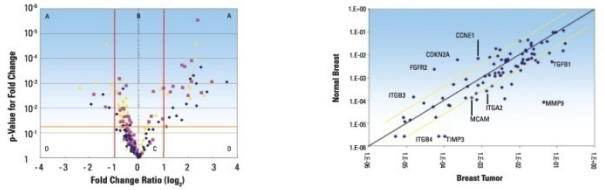

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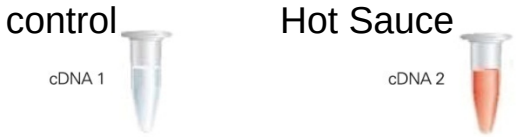

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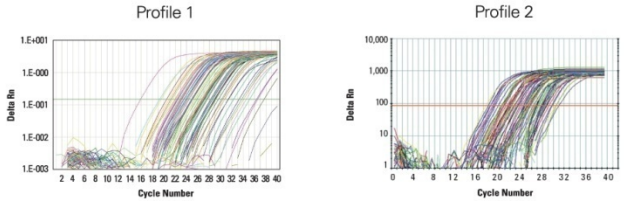

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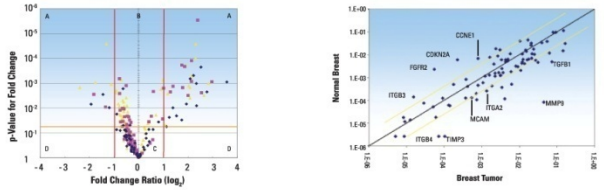

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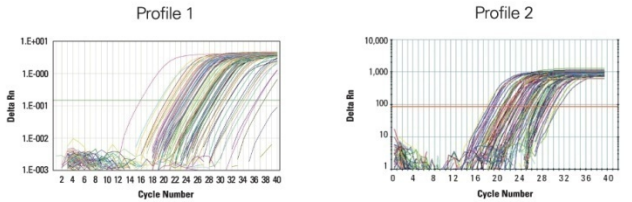

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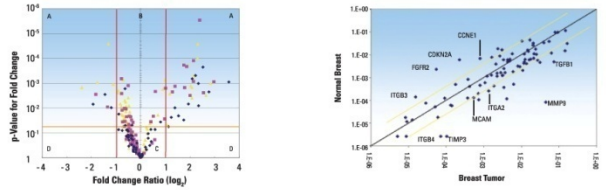

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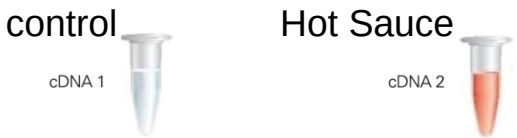

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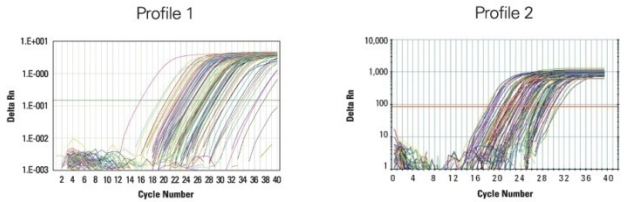

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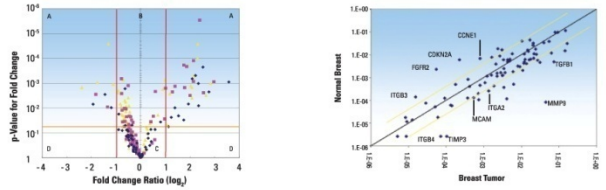

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- Using ΔΔC(t) calculations
- ABL1 is up/down regulated by x fold

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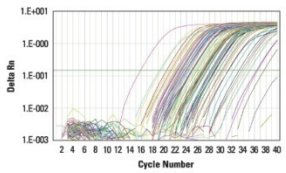

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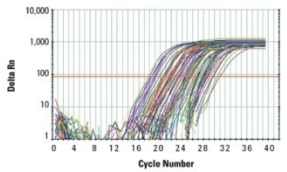

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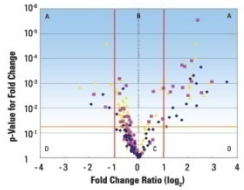

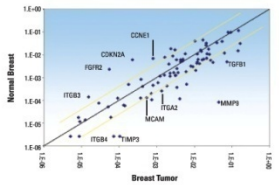

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For ABI, Stratagene, Bio-Rad, and Eppendorf Real-Time PCR Instruments*:

Baseline

- Use Automated Baseline
  -(if your instrument has Adaptive Baseline function) OR
- Manually Set Baseline
  -Using Linear View:
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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).

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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Setting Baseline

iCycler

Services Help

Library

Workshop

Run-Time Central

Data Analysis

View/Save Data

Allelic Discrimination N/A

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

Threshold Position: 70.5 — Auto Calculated / User Defined

Recalculate Threshold Cycles

Log View

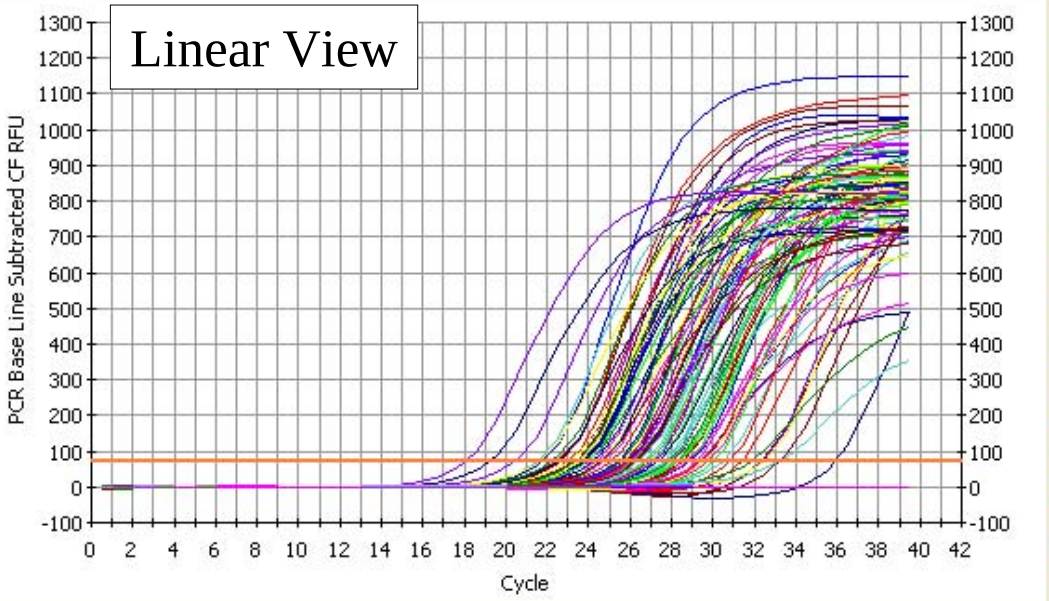
SYBR-490

Select a Reporter: SYBR-490

Save for X-axis Allelic Analysis

Save for Y-axis Allelic Analysis

Linear View



	Threshold Cycle	Identifier
	PCR threshold cycle and well identifier.	
F9	27.0	
F10	23.5	
F11	25.8	
F12	28.2	
G1	22.2	
G2	27.9	
G3	24.1	
G4	28.7	
G5	26.1	
G6	22.5	
G7	20.5	
G8	29.6	
G9	28.5	
G10	23.9	
G11	23.3	
G12	26.3	
H2	24.7	

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Threshold Position: 70.5 — Auto Calculated / User Defined

Select "AUTO CALCULATED"

Recalculate Threshold Cycles

Log View

SYBR-490

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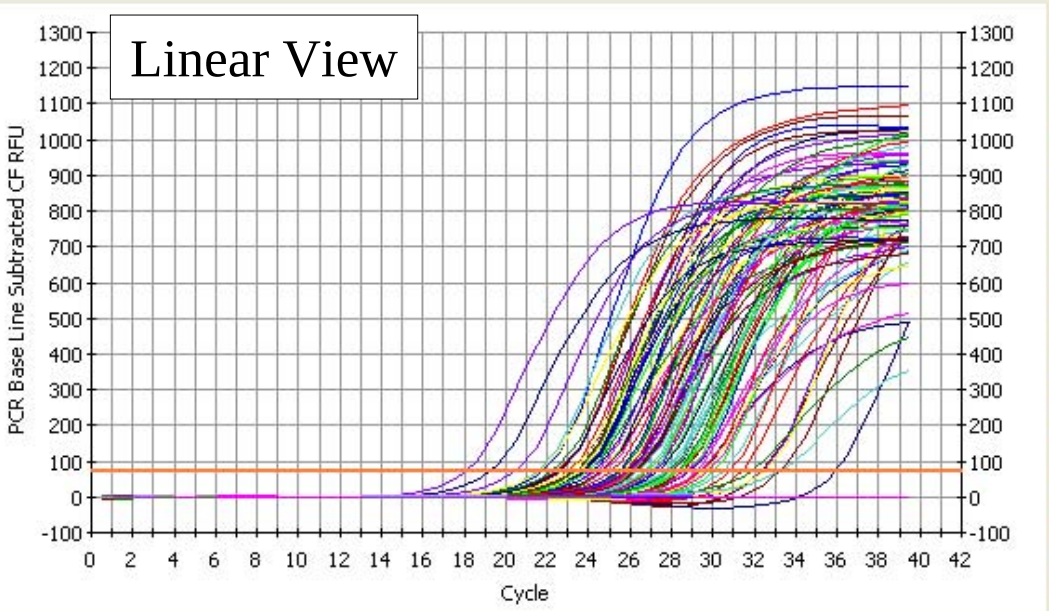
Save for X-axis Allelic Analysis

Save for Y-axis Allelic Analysis

Linear View



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G7	20.5	
G8	29.6	
G9	28.5	
G10	23.9	
G11	23.3	
G12	26.3	
H2	24.7	

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Threshold Cycle Calculation

Baseline Cycles: 2 through 15 — Auto Calculated / User Defined

Threshold Position: 22.6 — Auto Calculated / User Defined

Recalculate Threshold Cycles

Normal View

SYBR-490

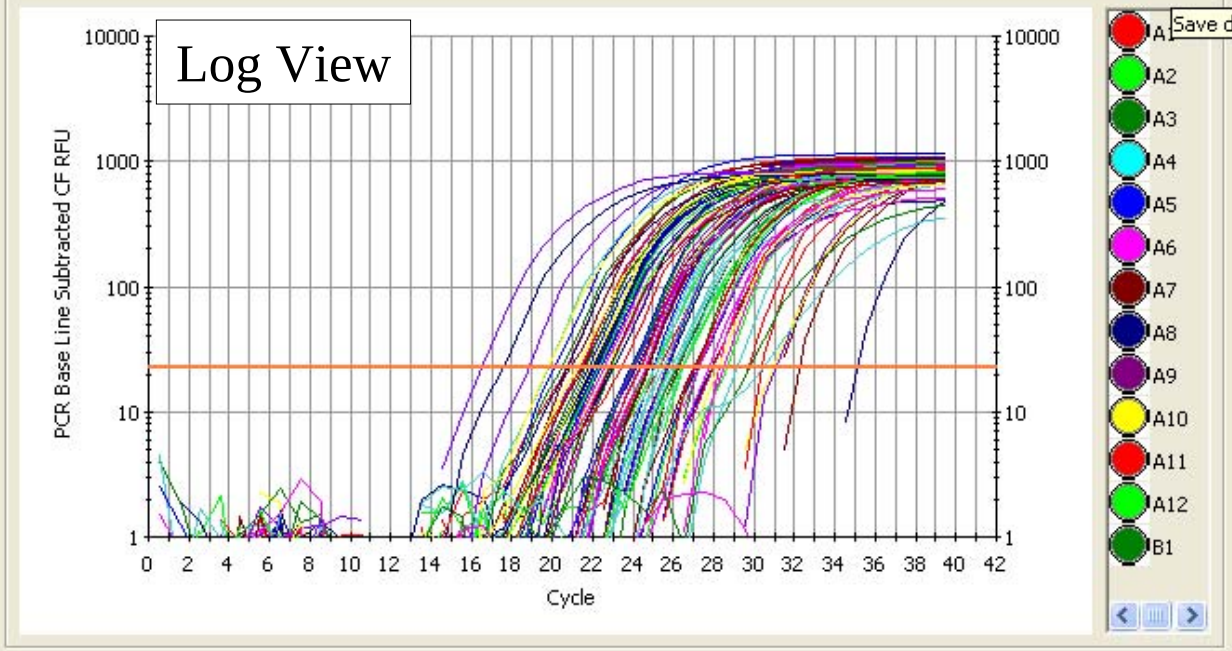
Select a Reporter: SYBR-490

Save for X-axis Allelic Analysis

Save for Y-axis Allelic Analysis

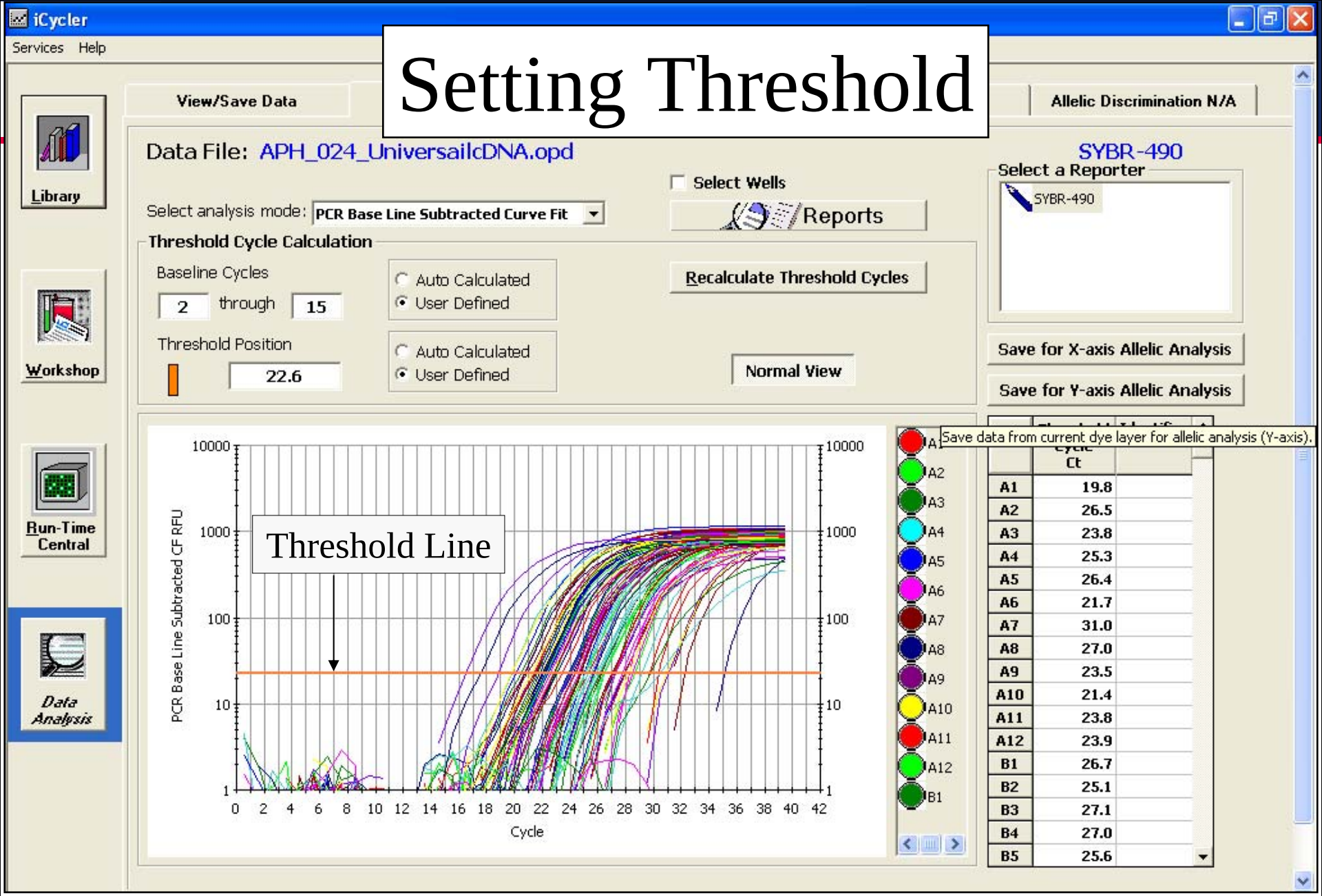
Save data from current dye layer for allelic analysis (Y-axis).

Log View

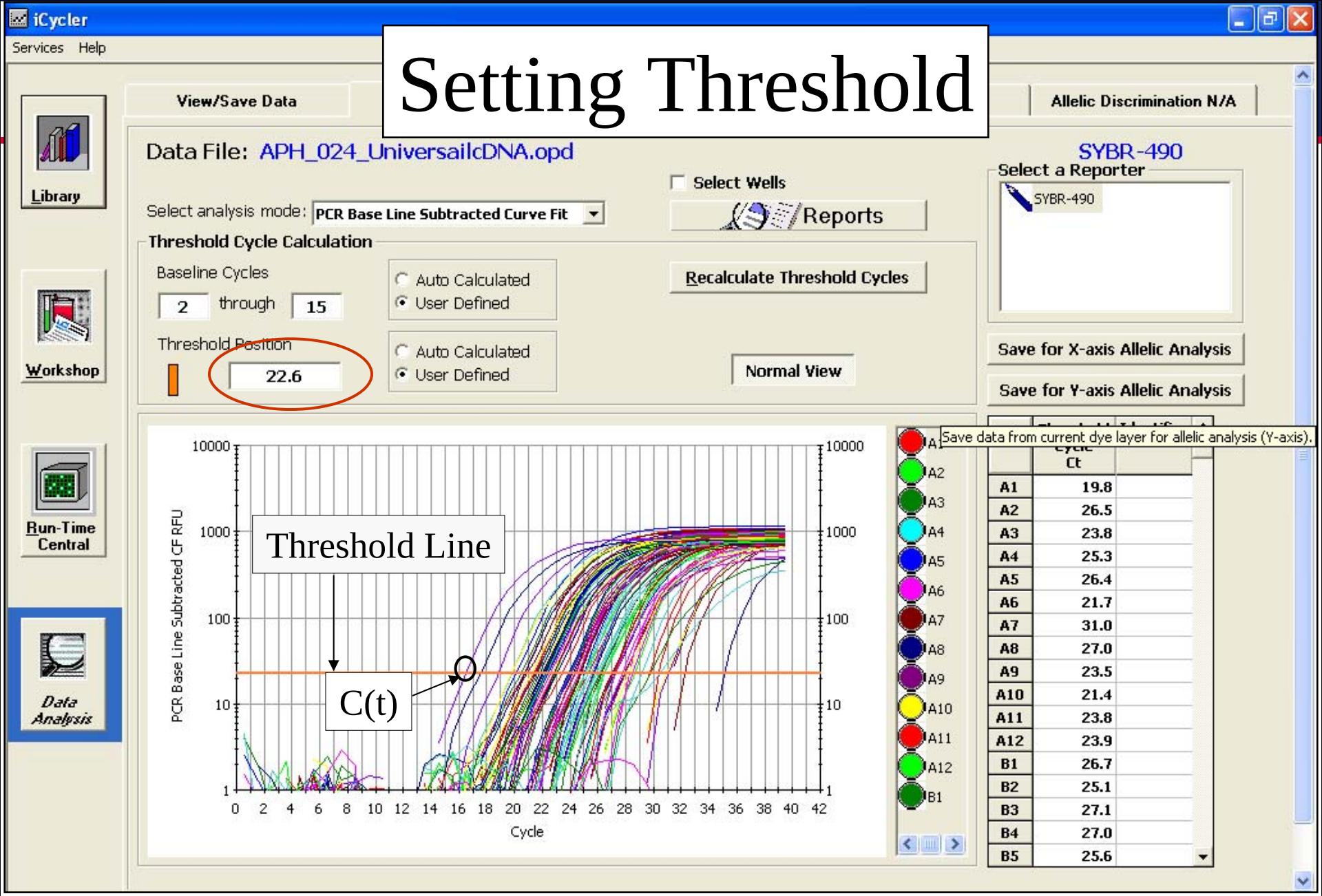


	Ct
A1	19.8
A2	26.5
A3	23.8
A4	25.3
A5	26.4
A6	21.7
A7	31.0
A8	27.0
A9	23.5
A10	21.4
A11	23.8
A12	23.9
B1	26.7
B2	25.1
B3	27.1
B4	27.0
B5	25.6

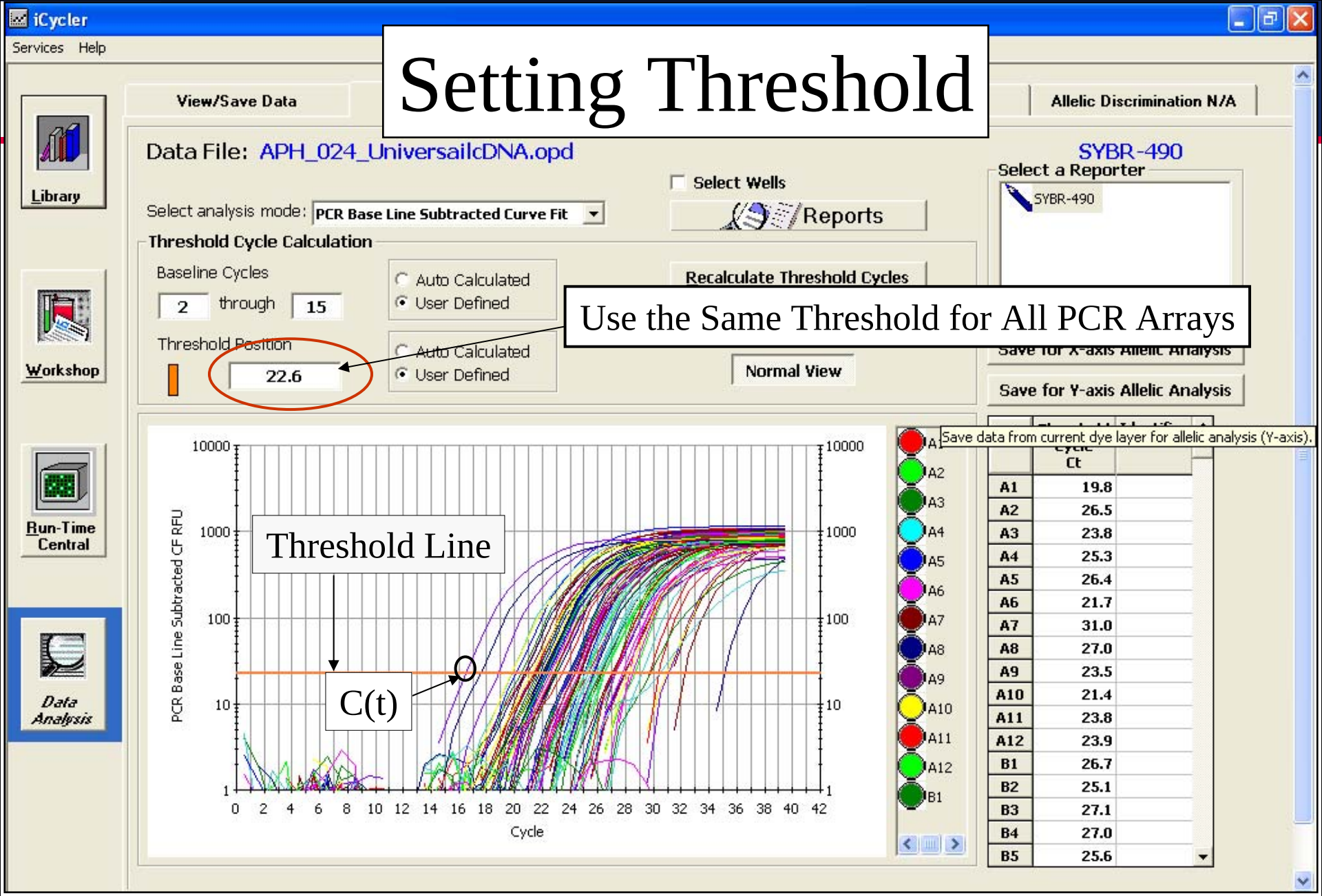
Setting Threshold



Setting Threshold



Setting Threshold



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

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- Good for 11 Group Comparisons (Control + 10 Experimental)
- 255 PCR Arrays Total

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Organizing Raw C(t) values

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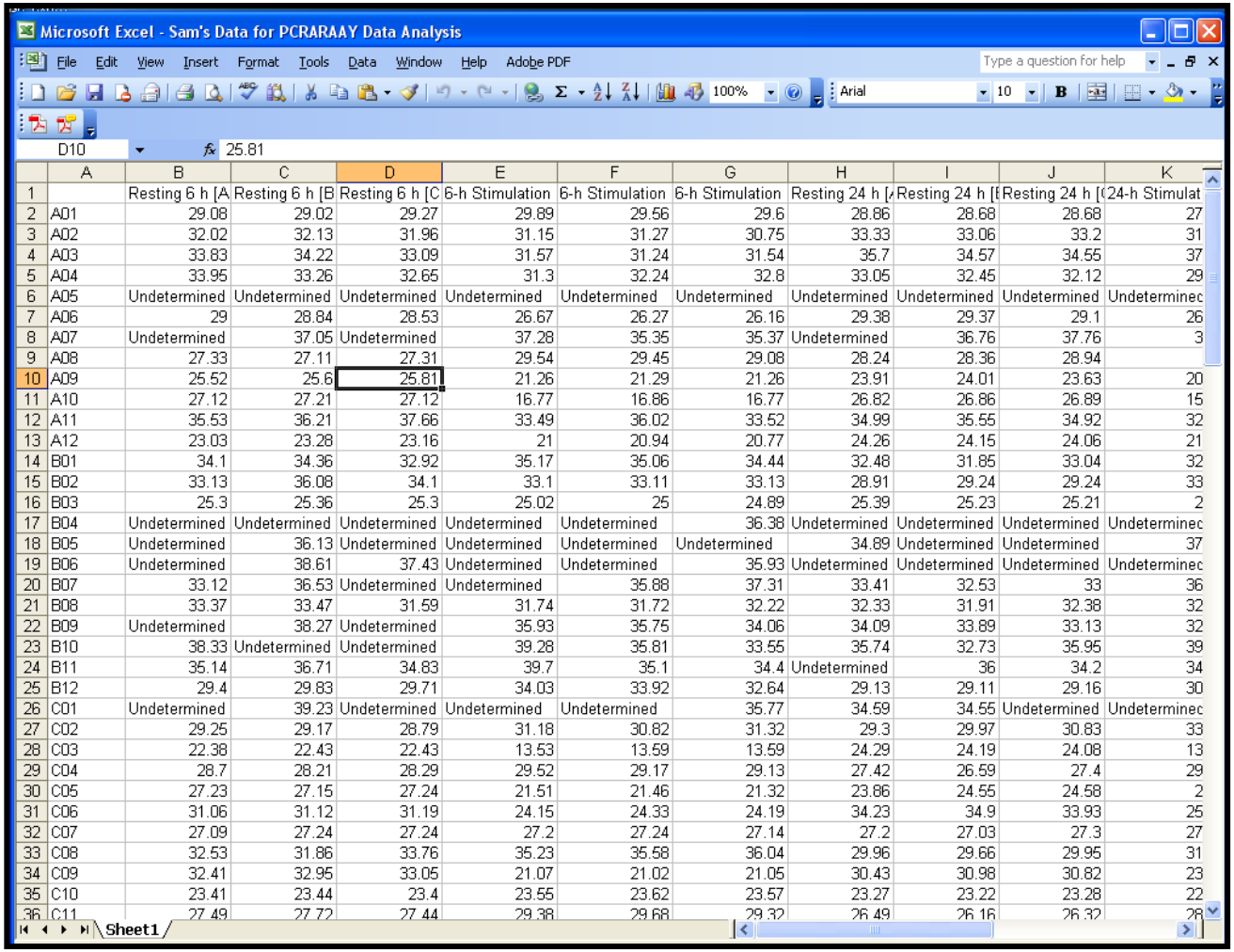

	A	B	C	D	E	F	G	H	I	J	K
1		Resting 6 h [A	Resting 6 h [B	Resting 6 h [C	6-h Stimulation	6-h Stimulation	6-h Stimulation	Resting 24 h [A	Resting 24 h [B	Resting 24 h [C	24-h Stimulat
2	A01	29.08	29.02	29.27	29.89	29.56	29.6	28.86	28.68	28.68	27
3	A02	32.02	32.13	31.96	31.15	31.27	30.75	33.33	33.06	33.2	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	Undeterminec
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	Undeterminec
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	Undeterminec
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	Undeterminec
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	34.9	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

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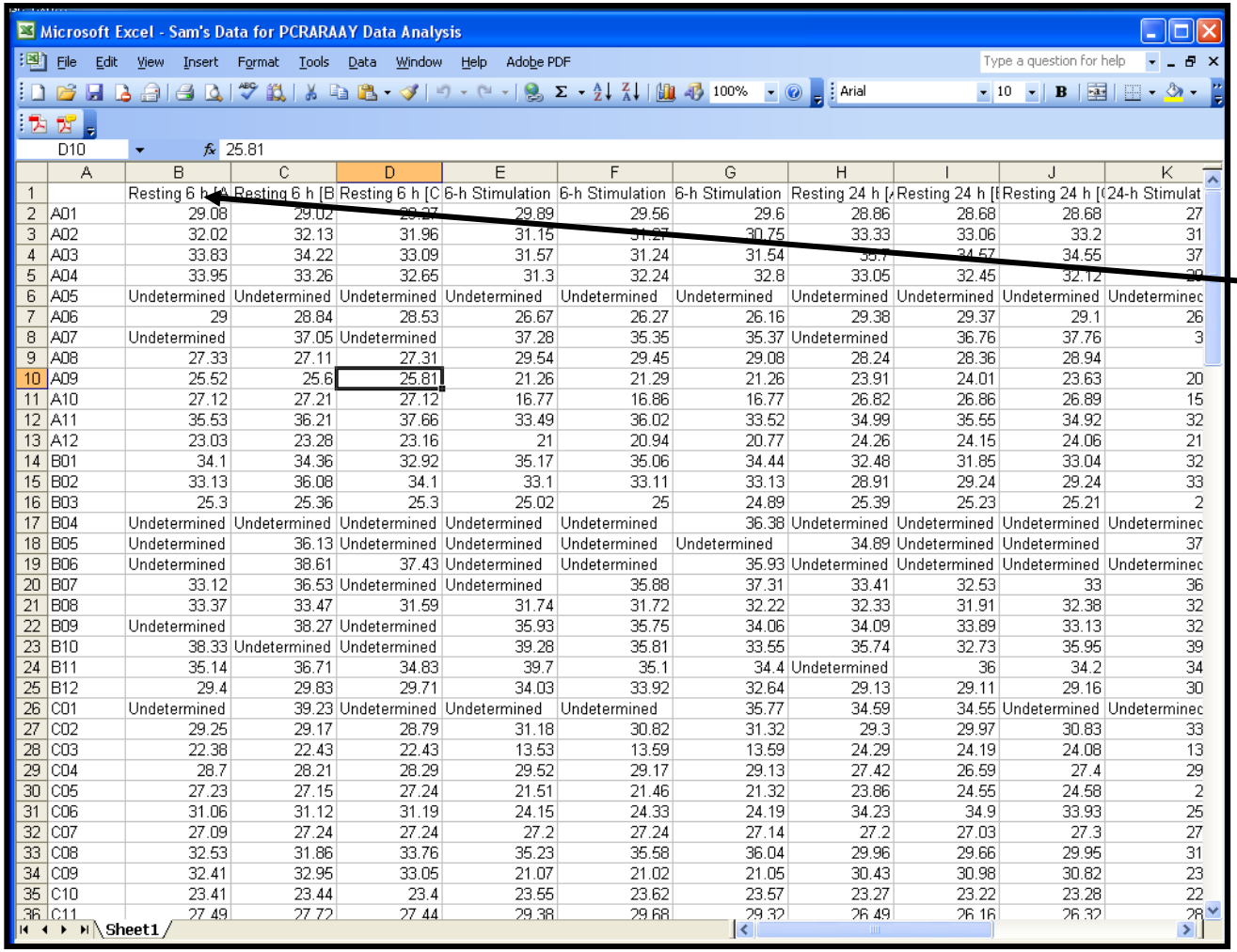

	A	B	C	D	E	F	G	H	I	J	K
1		Resting 6 h [A	Resting 6 h [B	Resting 6 h [C	6-h Stimulation	6-h Stimulation	6-h Stimulation	Resting 24 h [A	Resting 24 h [B	Resting 24 h [C	24-h Stimulat
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4	A03	33.83	34.22	33.09	31.57	31.24	31.54	35.7	34.57	34.55	37
5	A04	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	Undeterminec
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	Undeterminec
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	Undeterminec
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	Undeterminec
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	34.9	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
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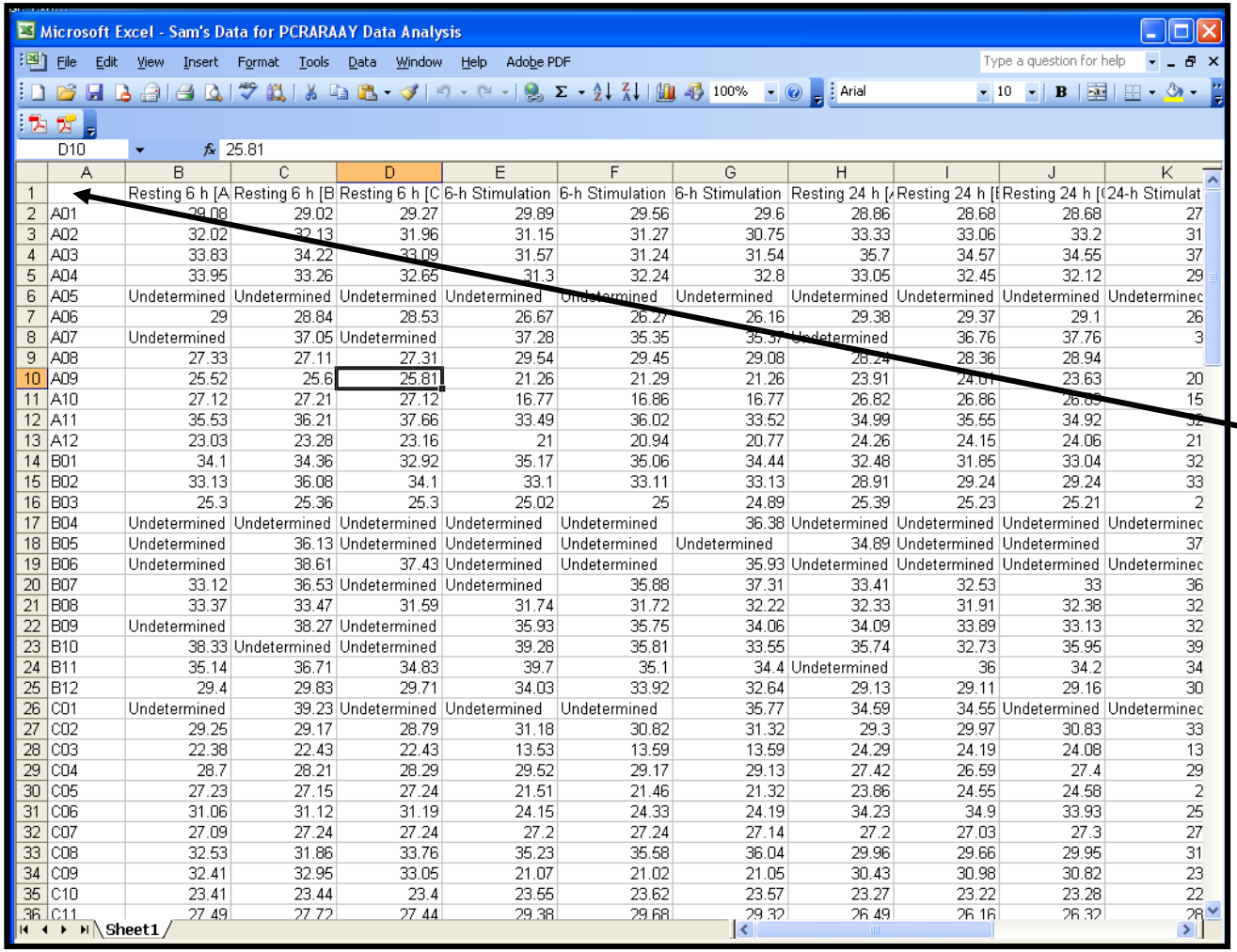

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1		Resting 6 h [A	Resting 6 h [B	Resting 6 h [C	6-h Stimulation	6-h Stimulation	6-h Stimulation	Resting 24 h [A	Resting 24 h [B	Resting 24 h [C	24-h Stimulat
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3	A02	32.02	32.13	31.96	31.15	31.27	30.75	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	Undeterminec
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	
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11	A10	27.12	27.21	27.12	16.77	16.86	16.77	26.82	26.86	26.83	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	Undeterminec
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	Undeterminec
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	Undeterminec
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	34.9	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

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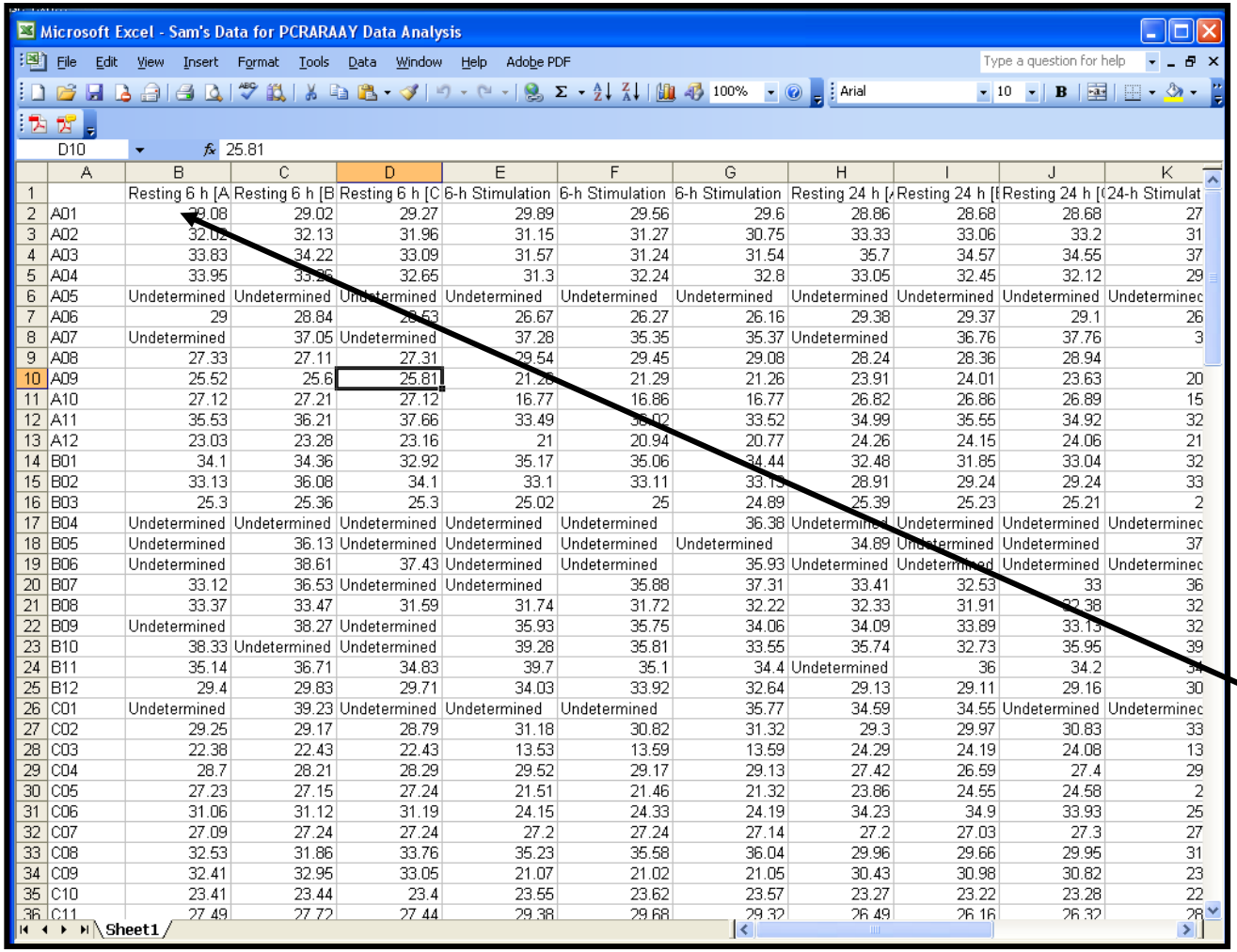

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13	A12	23.03	23.28	23.16	21	20.94	20.77	24.26	24.15	24.06	21
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25	B12	29.4	29.83	29.71	34.03	33.92	32.64	29.13	29.11	29.16	30
26	C01	Undetermined	39.23	Undetermined	Undetermined	Undetermined	35.77	34.59	34.55	Undetermined	Undeterminec
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	34.9	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

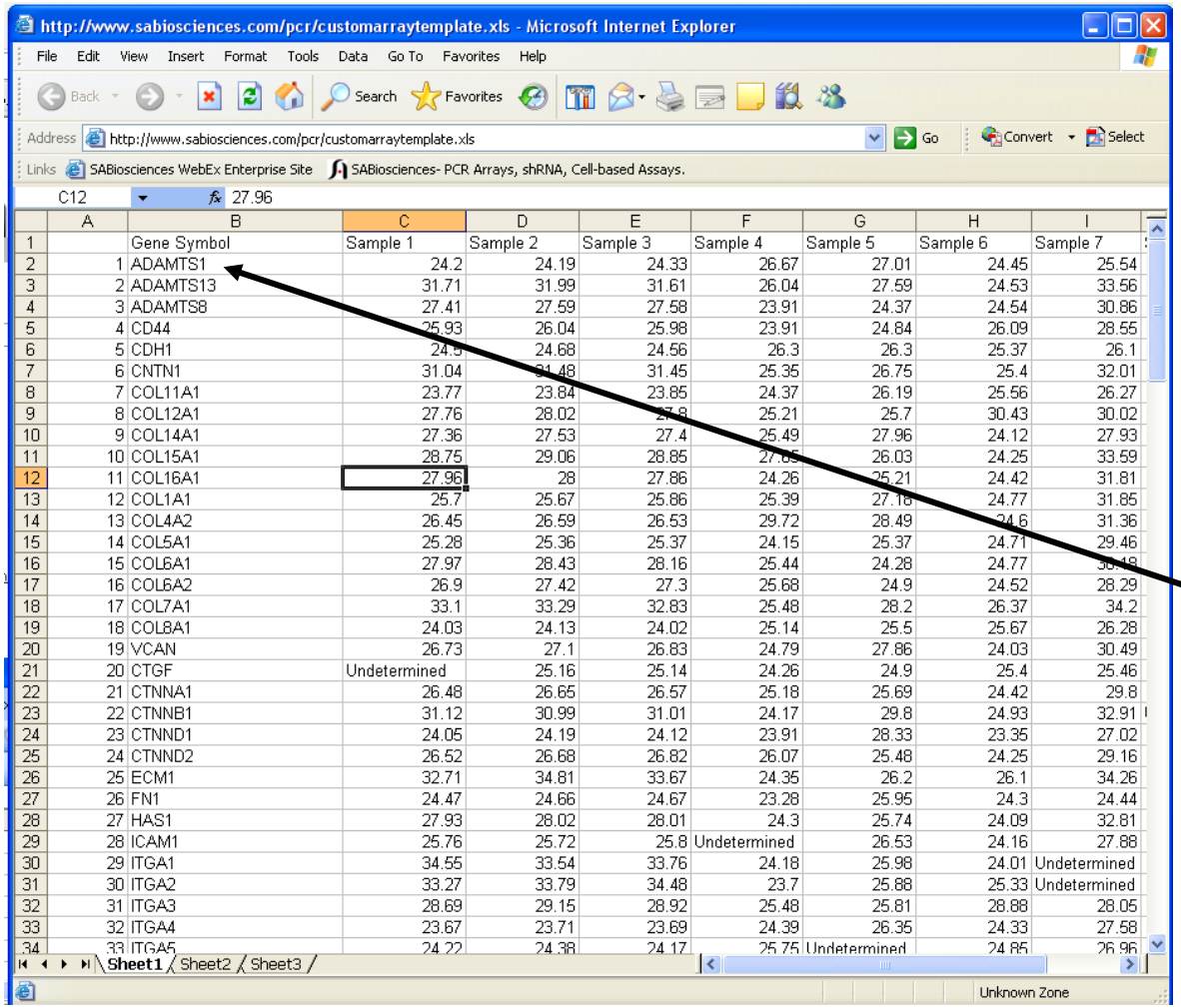
Column A:
Well Location

Column B-??:

Raw C(t) Values

Organizing Raw C(t) values

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



	A	B	C	D	E	F	G	H	I
1		Gene Symbol	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
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	24.5	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	27.18	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	26.65	26.57	25.18	25.69	24.42	29.8
23	22	CTNNB1	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

Custom Array

Row 1
Sample Name

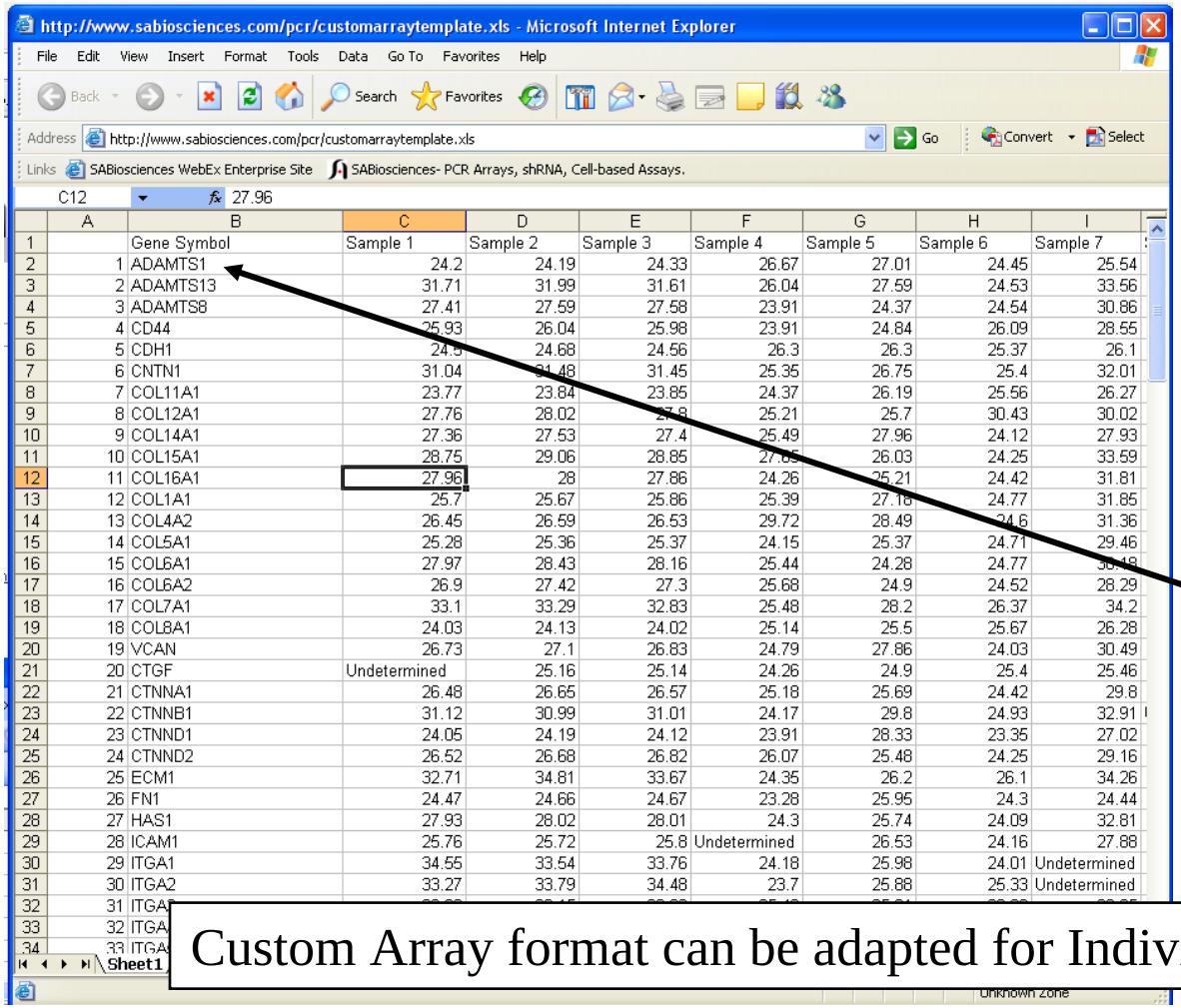
Column A:
Well Location

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.



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5	4	CD44	25.93	26.04	25.98	23.91	24.84	26.09	28.55
6	5	CDH1	24.5	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	27.18	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
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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
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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	ITGA							
33	32	ITGA							
34	33	ITGA							

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

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
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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

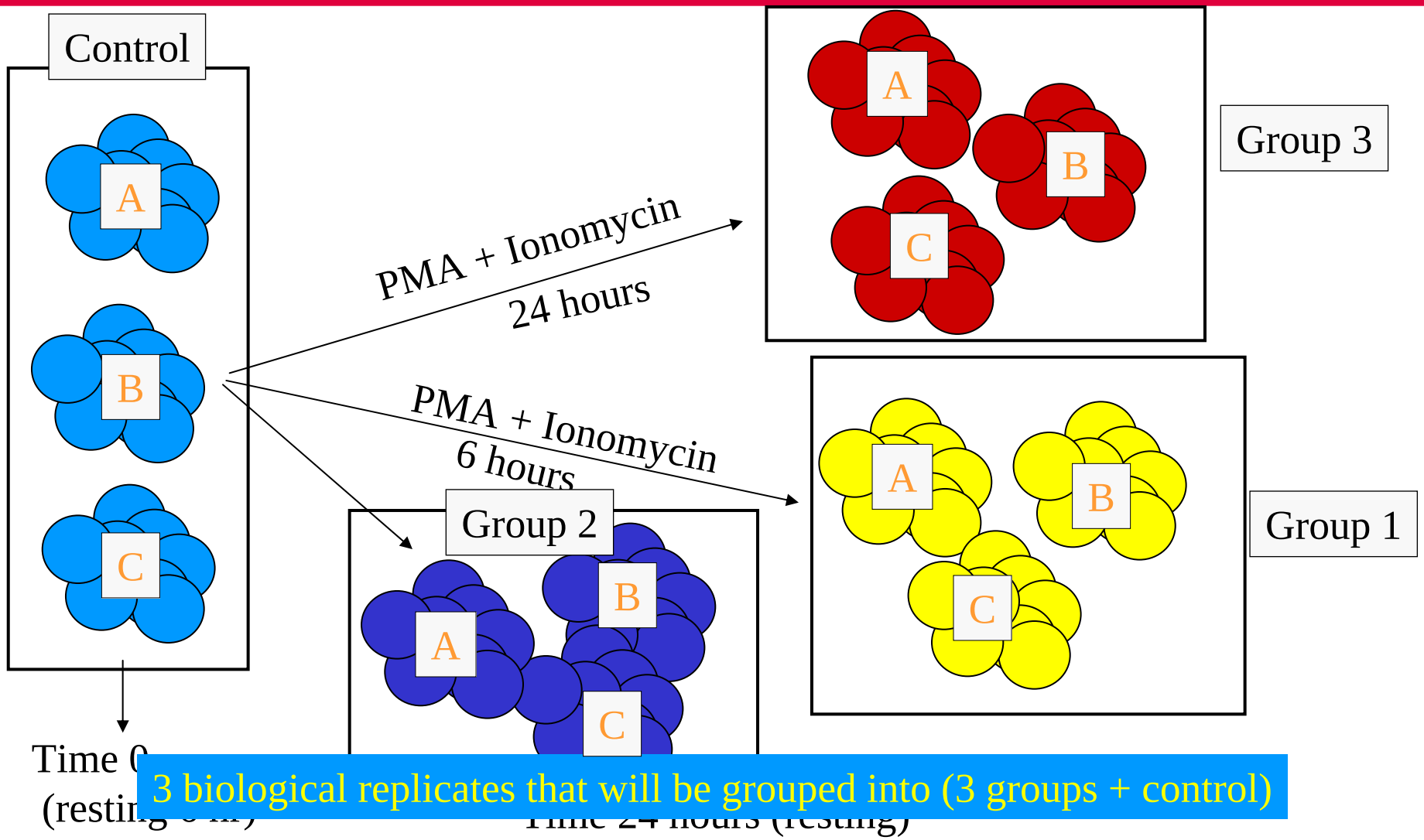
Column A:
Well Location

Column B:
Gene Name

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

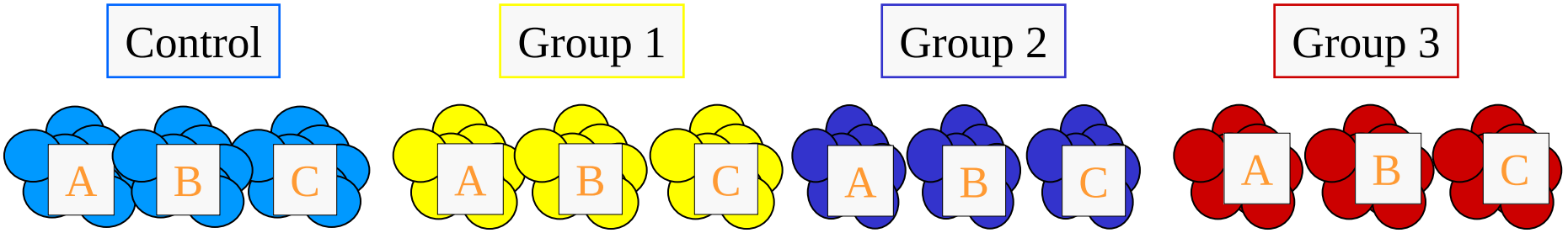
Custom Array format can be adapted for Individual PCR Assays

Our Experiment



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

Our Experiment-Data Analysis Overview



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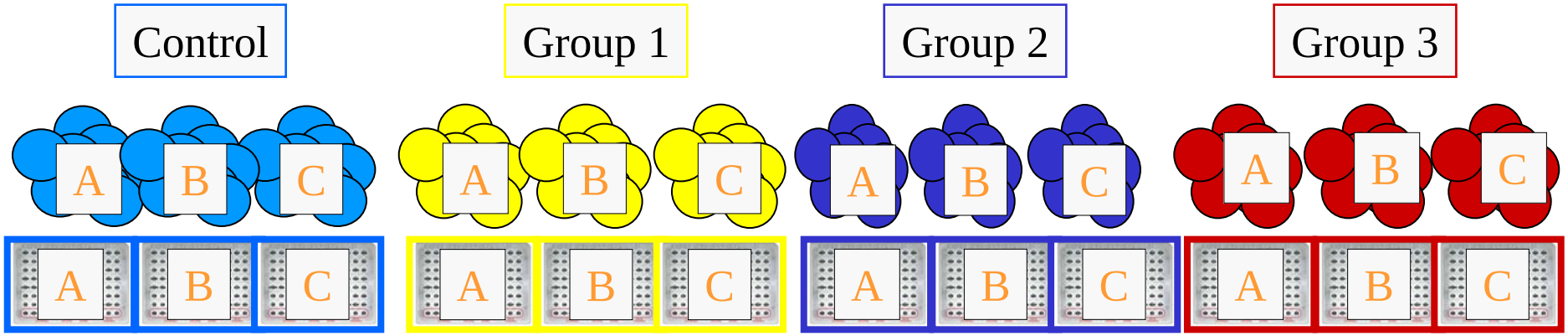
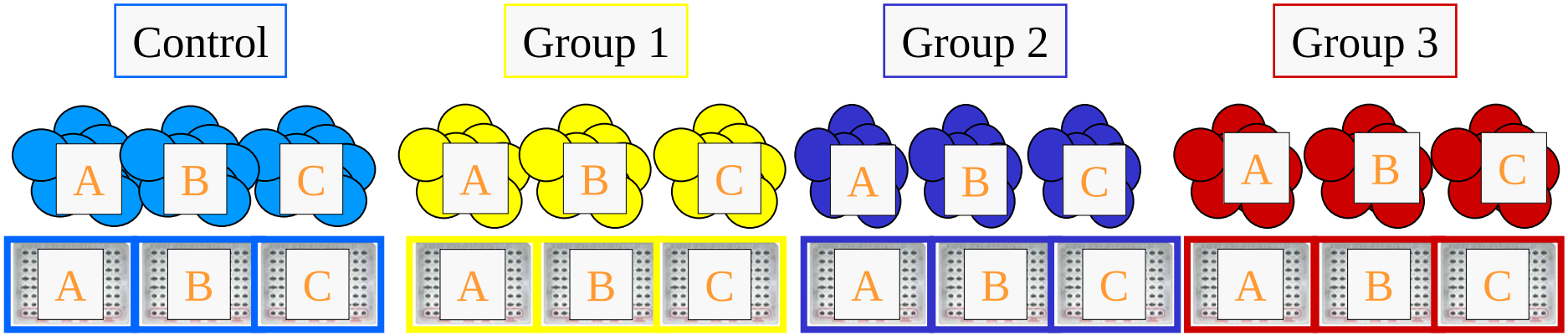


1 PCR Array for Each Sample (12 PCR Arrays Total)

Our Experiment-Data Analysis Overview

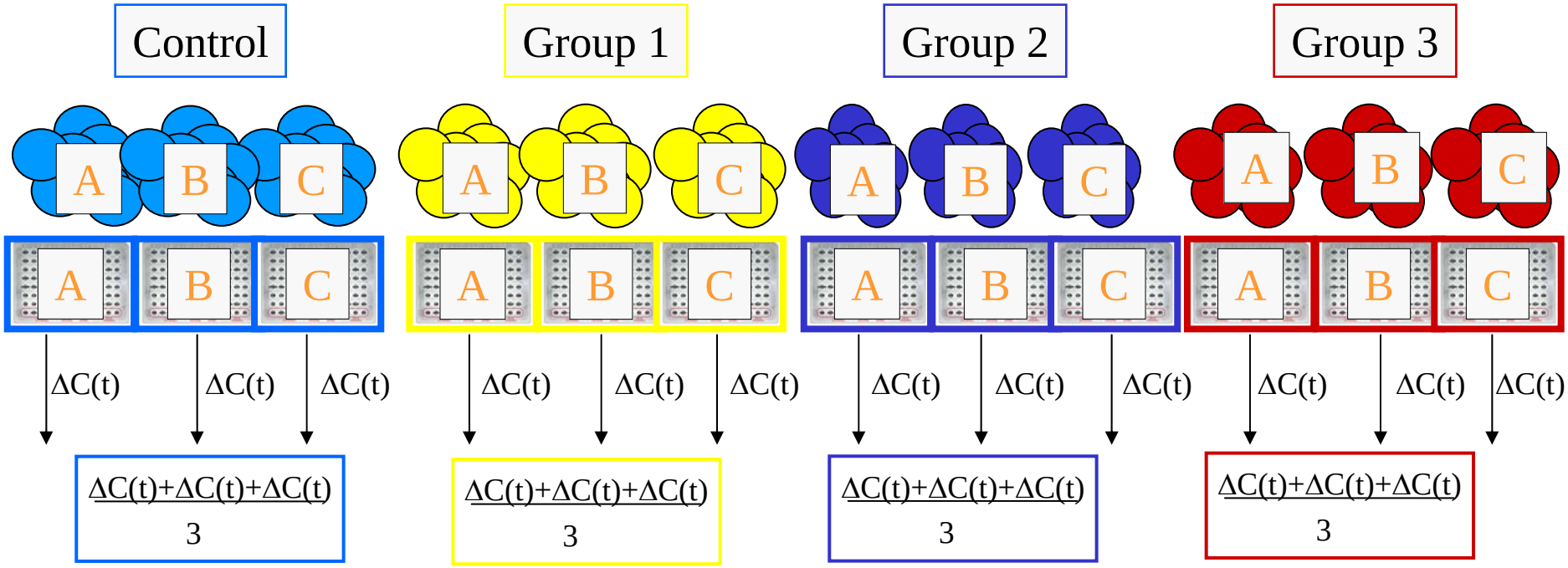


$\Delta C(t)$

$C(t)_{GOI} - C(t)_{HKG}$

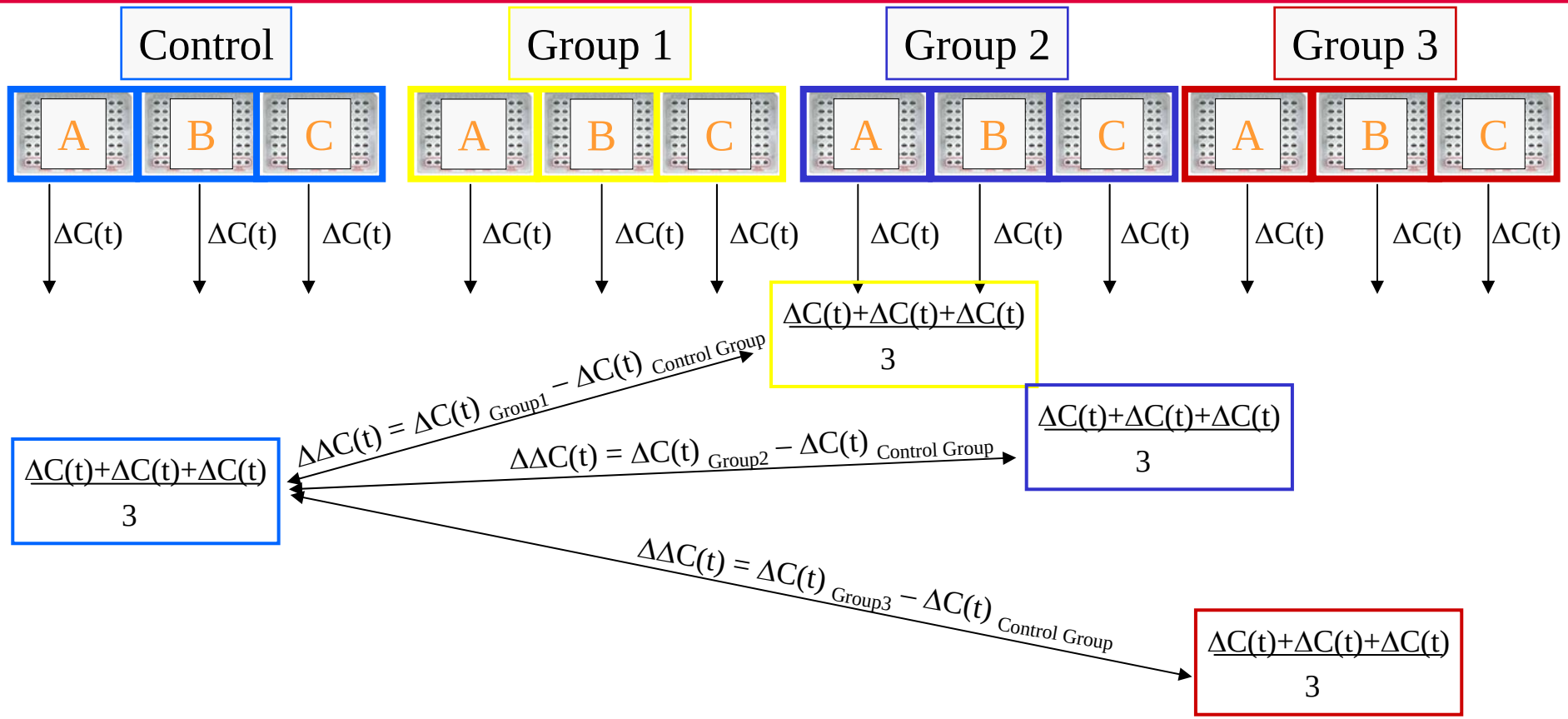
1. **Calculate Δ C(t) for on each array for each GOI (Gene Of Interest)**

Our Experiment-Data Analysis Overview



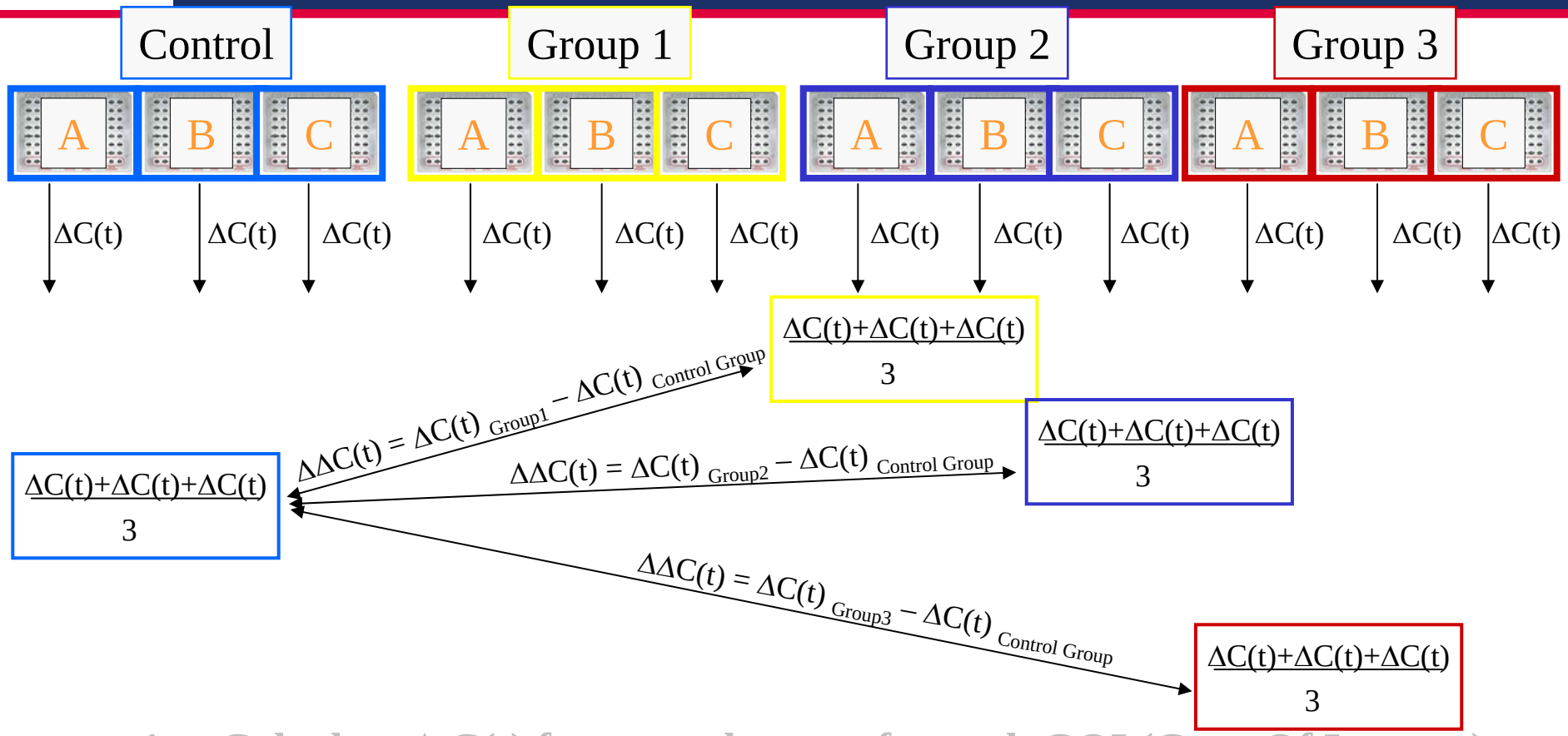
1. Calculate Δ C(t) for on each array for each GOI (**G**ene **O**f **I**nterest)
2. **Calculate Average Δ C(t) for each gene within a Group**

Our Experiment-Data Analysis Overview



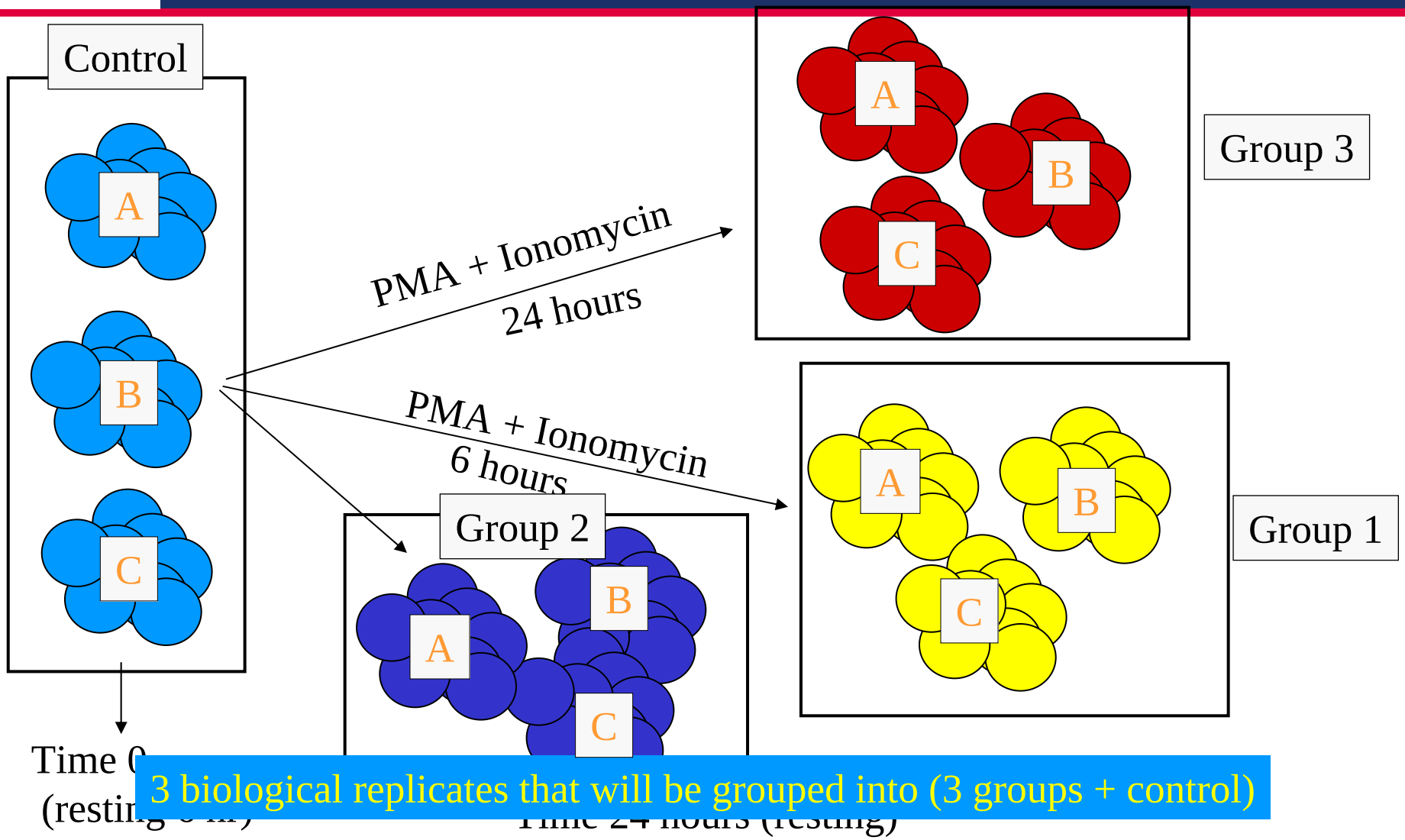
1. Calculate Δ C(t) for on each array for each GOI (Gene Of Interest)
2. Calculate Average Δ C(t) for each gene within a Group
3. **Calculate ΔΔ C(t) for each gene between Groups**

Our Experiment-Data Analysis Overview



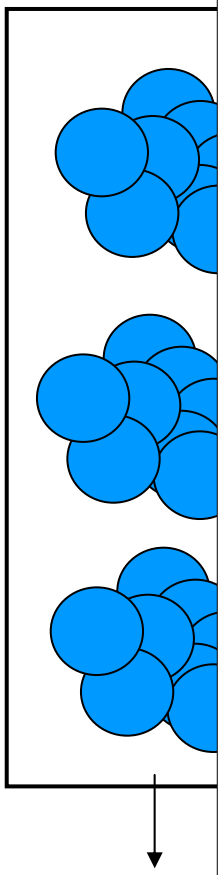
1. Calculate Δ C(t) for on each array for each GOI (Gene Of Interest)
2. Calculate Average Δ C(t) for each gene within a Group
3. Calculate ΔΔ C(t) for each gene between Groups
4. **Calculate Fold Change: $2^{(-\Delta\Delta Ct)}$**

Our Experiment



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

Our Experiment



Control
(resting

SABiosciences™

TECHNICAL ARTICLE

RT² 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
Email: support@SABiosciences.com

Abstract: The RT² 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 RT² 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

http://www.sabiosciences.com/manuals/PCRArrayWhitePaper_App.pdf

Topics to be Covered

Topic I:

Brief Technology and Protocol Overview

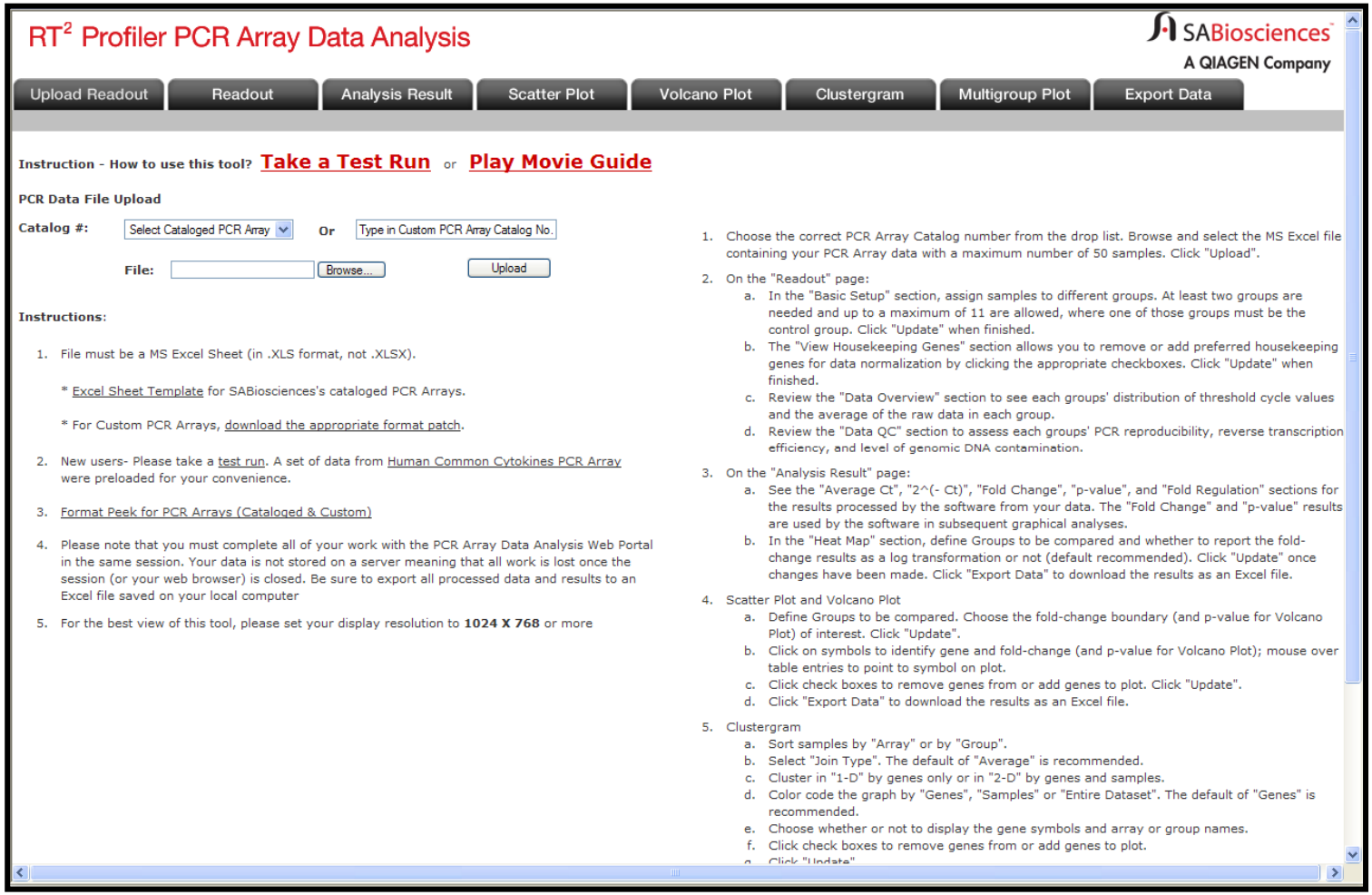
Monday November 15th @ 1pm EST (Next RT² PCR Array Webinar)

Topic II (Today):

PCR Array Data Analysis

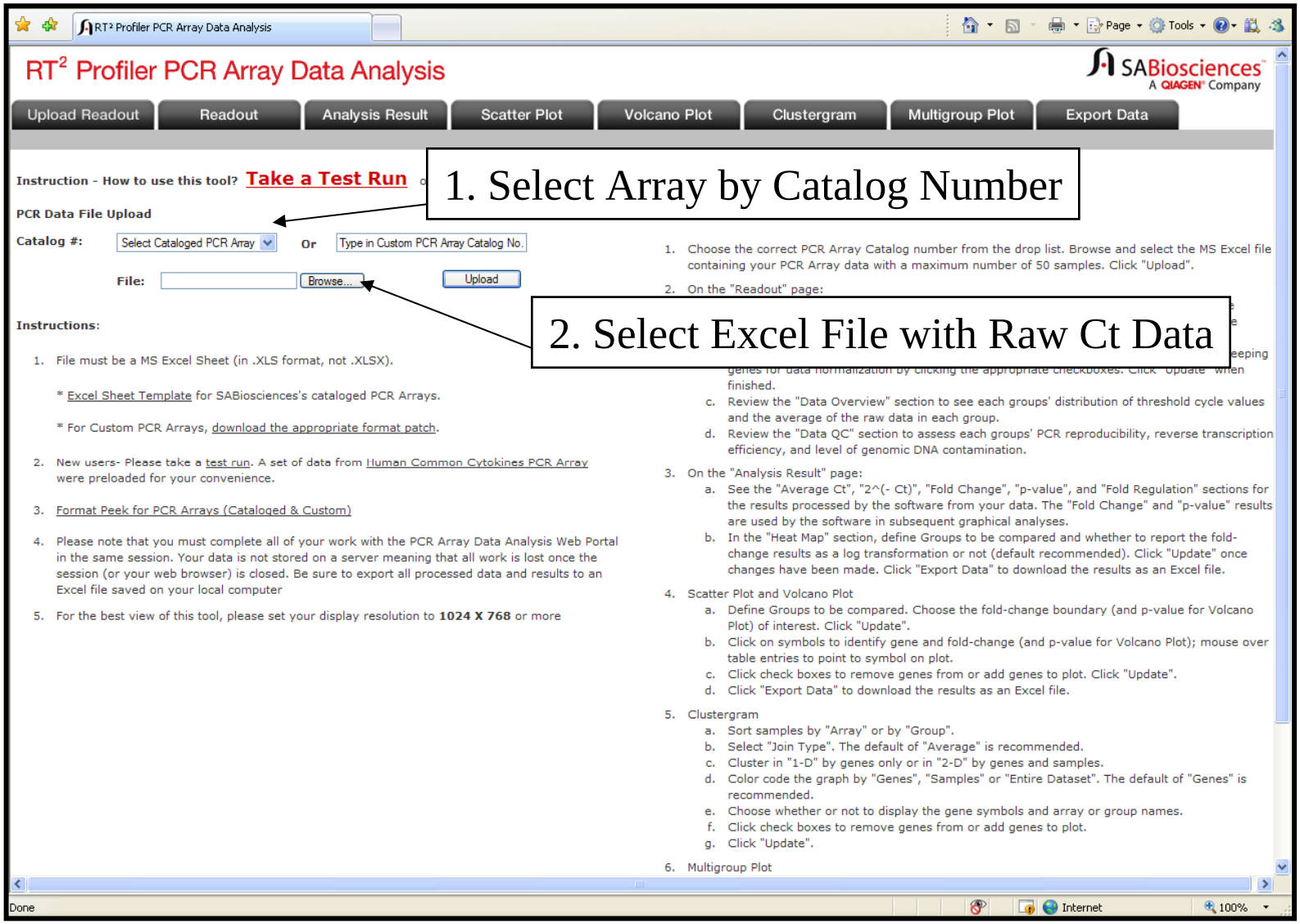
- Defining Baseline and Threshold
- Web Portal Location / Address
- Uploading Raw C_t Data
- Analyzing Data & Controls
- Exporting Data

Web Portal Location

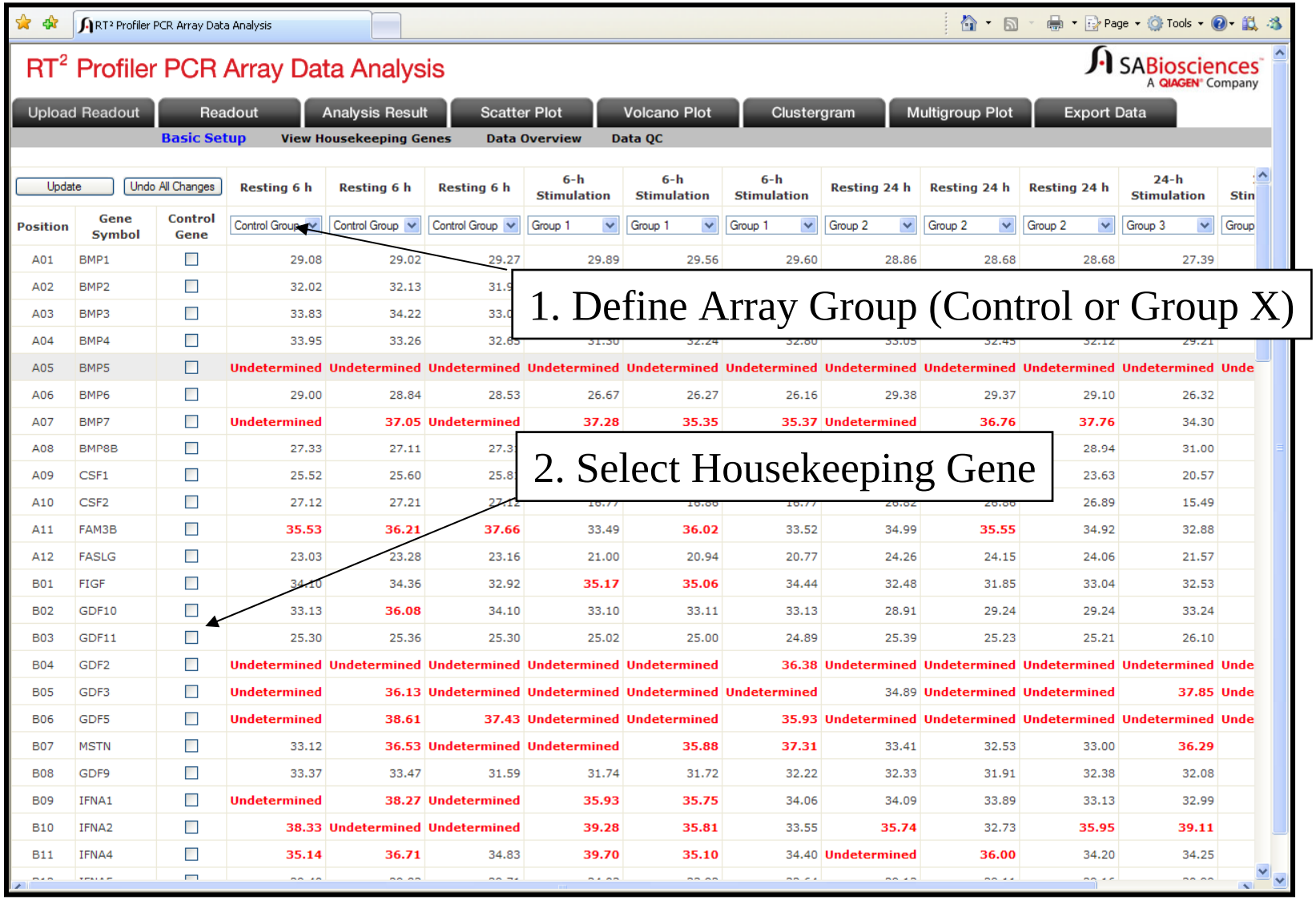


http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php

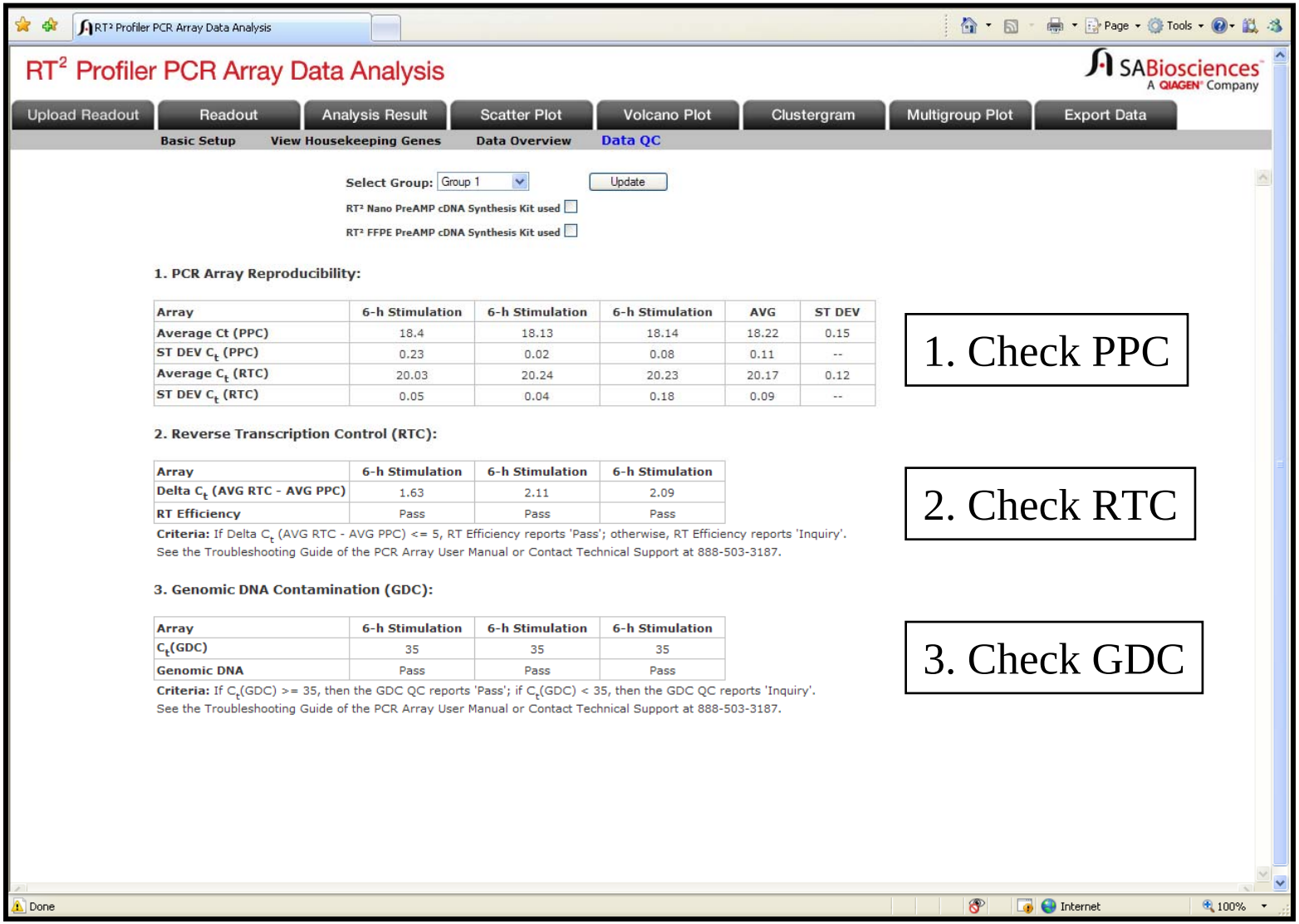
Uploading Raw Ct Data



Defining Experimental Design



PCR Array Reproducibility and Sample Quality



RT² Profiler PCR Array Data Analysis

SABiosciences A QIAGEN Company

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

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

Select Group: Group 1 Update

RT² Nano PreAMP cDNA Synthesis Kit used

RT² 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_t (PPC)	0.23	0.02	0.08	0.11	--
Average C_t (RTC)	20.03	20.24	20.23	20.17	0.12
ST DEV C_t (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_t (AVG RTC - AVG PPC)	1.63	2.11	2.09
RT Efficiency	Pass	Pass	Pass

Criteria: If Delta C_t (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_t(GDC)	35	35	35
Genomic DNA	Pass	Pass	Pass

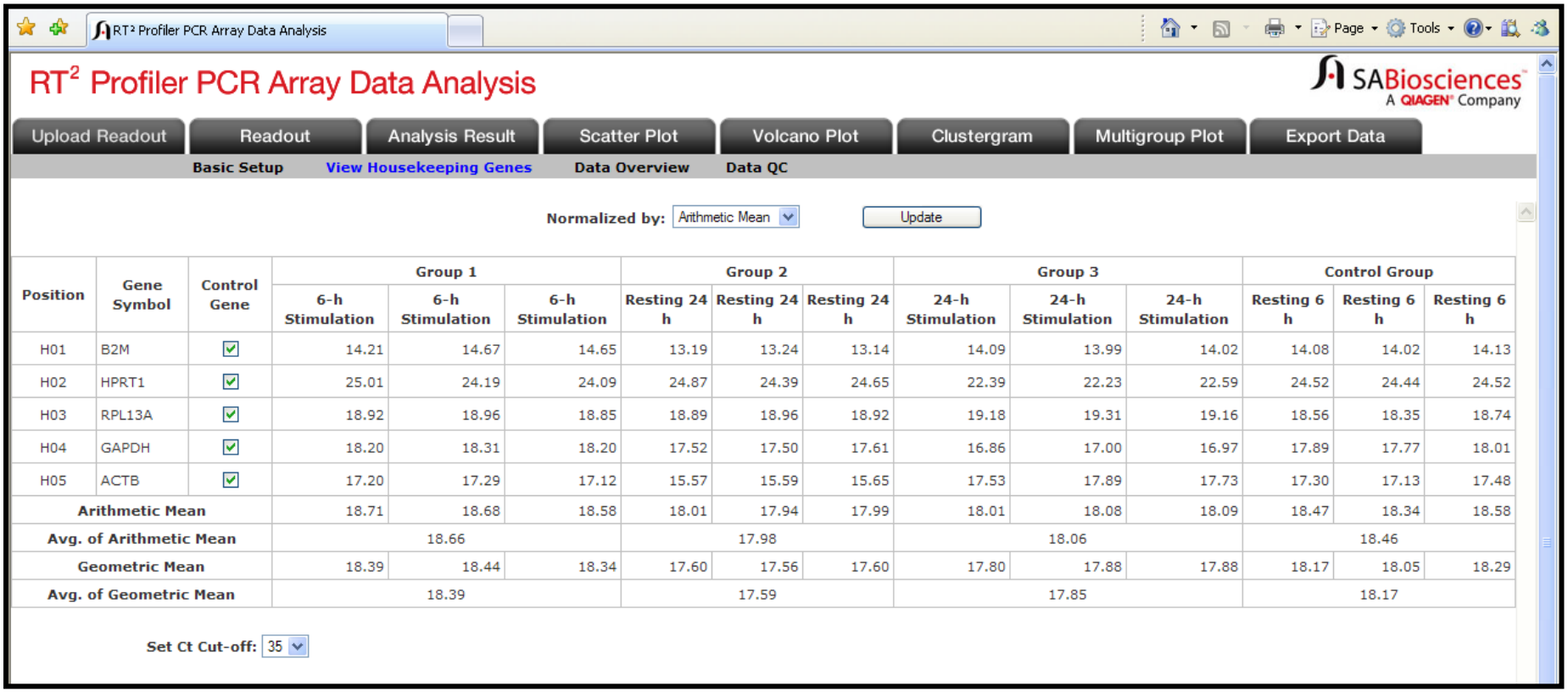
Criteria: If C_t(GDC) >= 35, then the GDC QC reports 'Pass'; if C_t(GDC) < 35, then the GDC QC reports 'Inquiry'.
See the Troubleshooting Guide of the PCR Array User Manual or Contact Technical Support at 888-503-3187.

1. Check PPC

2. Check RTC

3. Check GDC

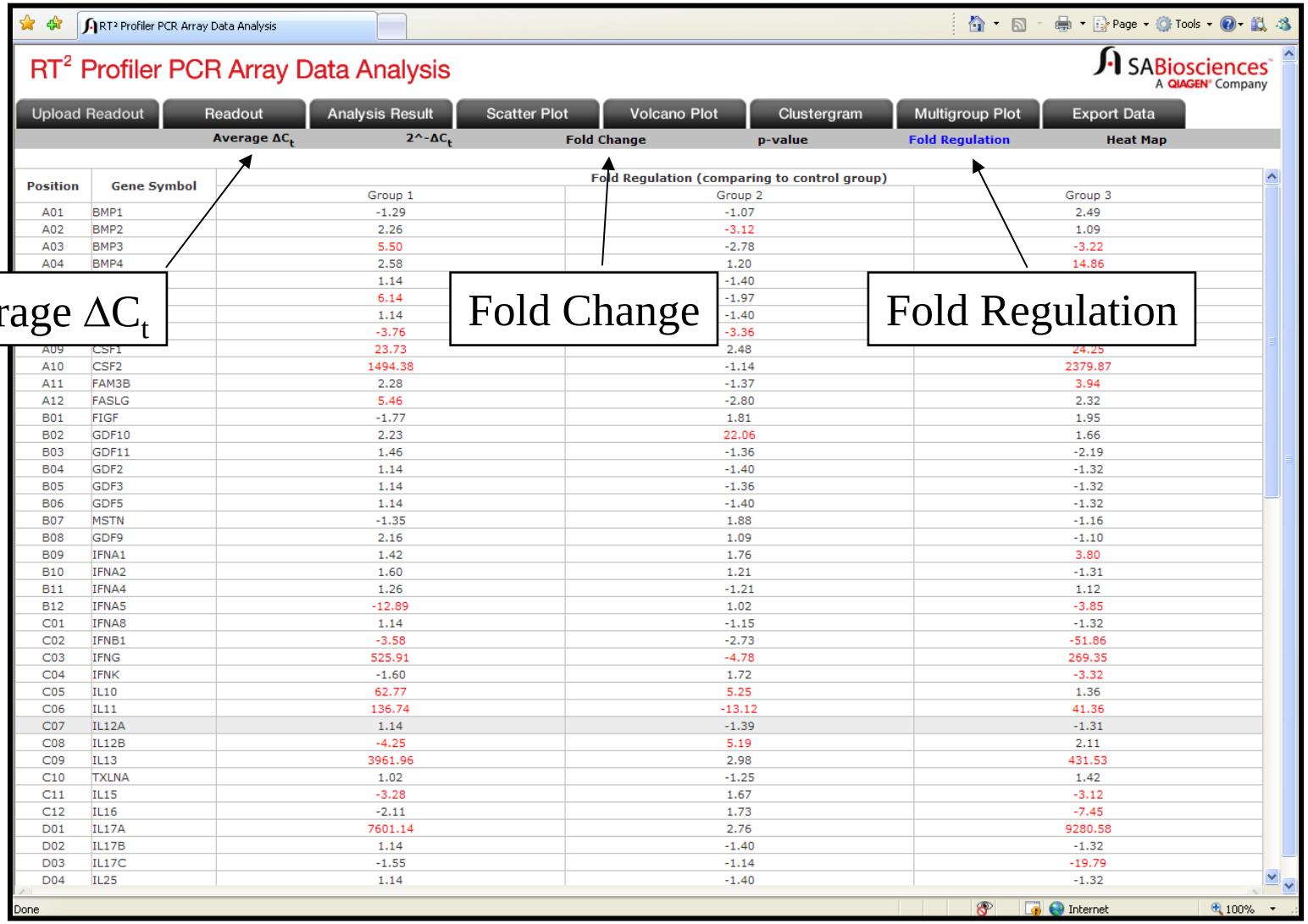
Select Housekeeping Gene



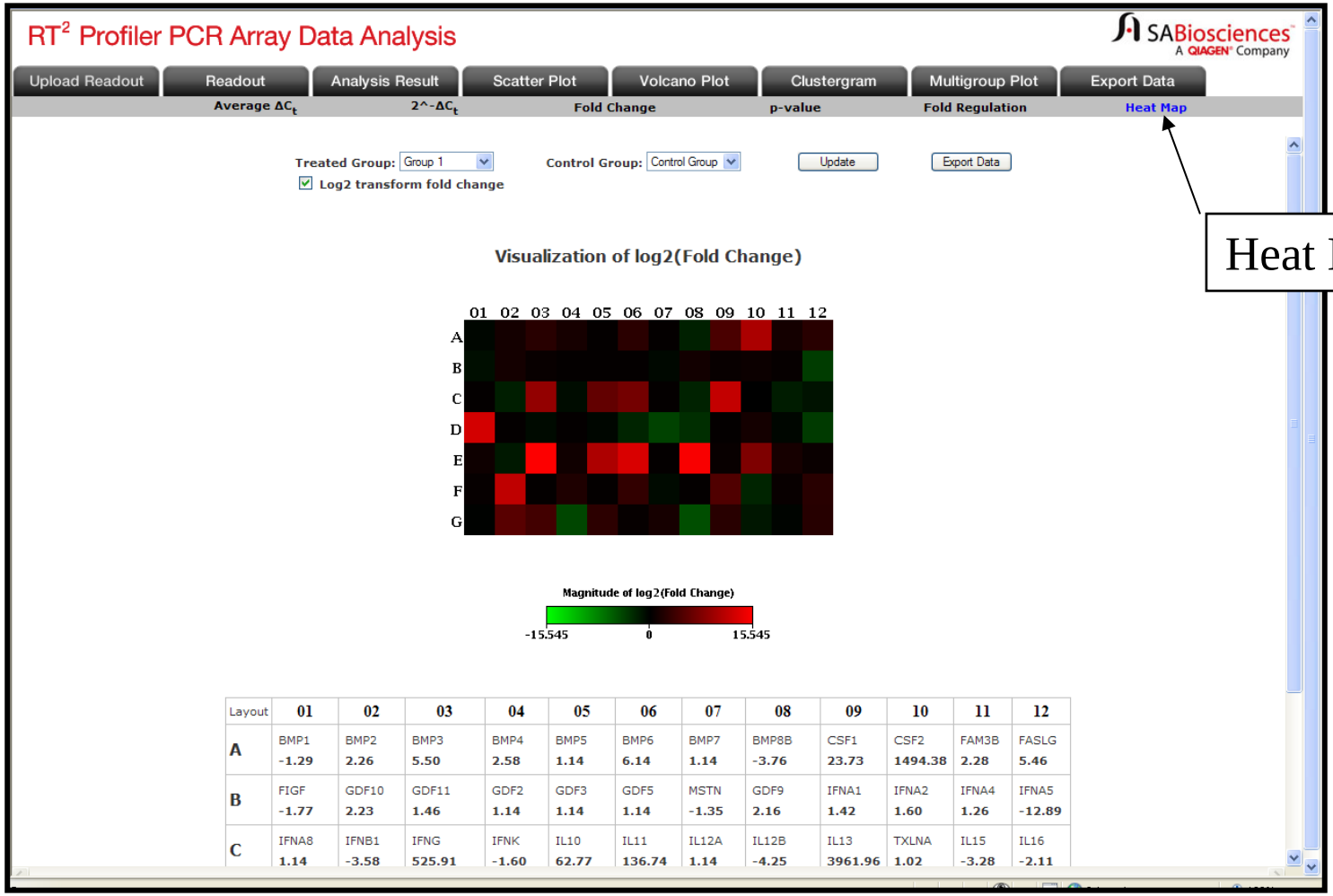
Position	Gene Symbol	Control Gene	Group 1			Group 2			Group 3			Control Group		
			6-h Stimulation	6-h Stimulation	6-h Stimulation	Resting 24 h	Resting 24 h	Resting 24 h	24-h Stimulation	24-h Stimulation	24-h Stimulation	Resting 6 h	Resting 6 h	Resting 6 h
H01	B2M	☑	14.21	14.67	14.65	13.19	13.24	13.14	14.09	13.99	14.02	14.08	14.02	14.13
H02	HPRT1	☑	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	☑	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	☑	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	☑	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



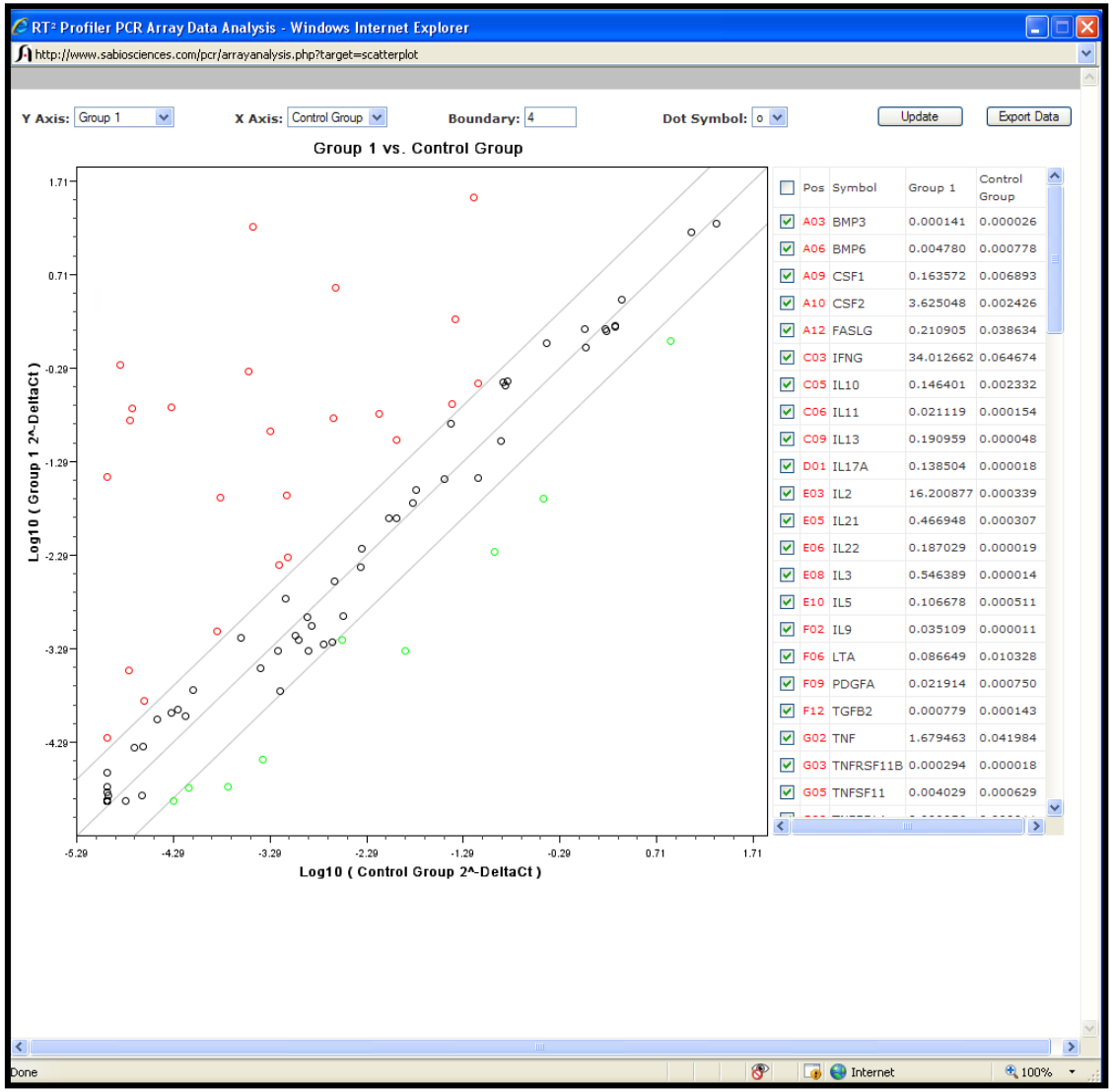
Visualization of Data



Heat Map

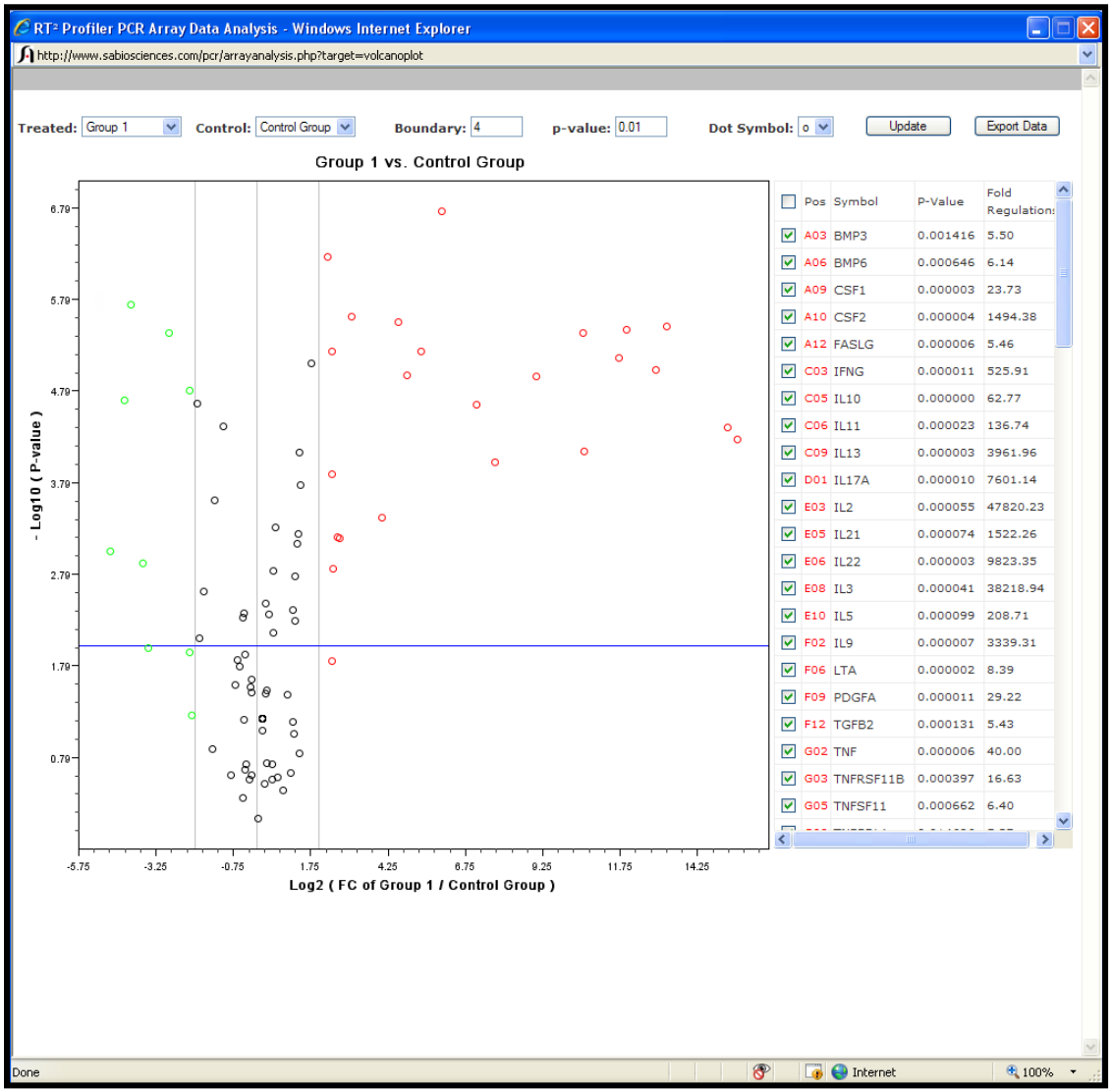
Layout	01	02	03	04	05	06	07	08	09	10	11	12
A	BMP1 -1.29	BMP2 2.26	BMP3 5.50	BMP4 2.58	BMP5 1.14	BMP6 6.14	BMP7 1.14	BMP8B -3.76	CSF1 23.73	CSF2 1494.38	FAM3B 2.28	FASLG 5.46
B	FIGF -1.77	GDF10 2.23	GDF11 1.46	GDF2 1.14	GDF3 1.14	GDF5 1.14	MSTN -1.35	GDF9 2.16	IFNA1 1.42	IFNA2 1.60	IFNA4 1.26	IFNA5 -12.89
C	IFNA8 1.14	IFNB1 -3.58	IFNG 525.91	IFNK -1.60	IL10 62.77	IL11 136.74	IL12A 1.14	IL12B -4.25	IL13 3961.96	TXLNA 1.02	IL15 -3.28	IL16 -2.11

Visualization of Data



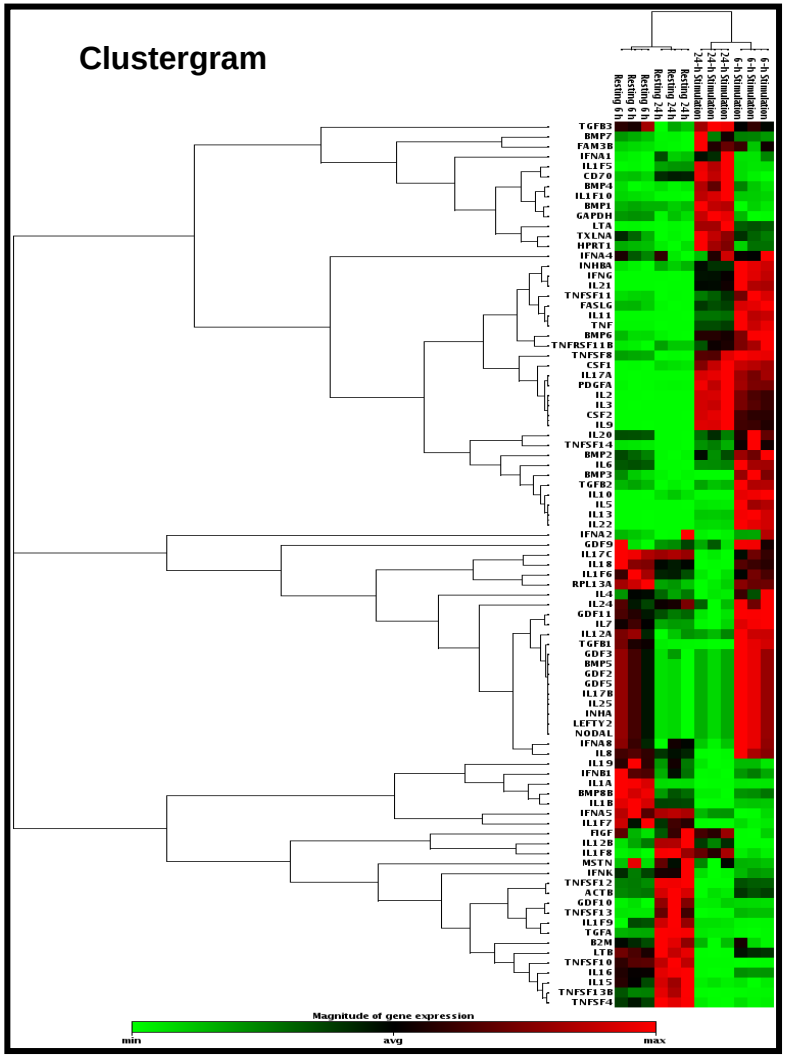
Scatter Plot

Visualization of Data



Volcano Plot

Visualization of Data



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^{rds} 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?

Profile the Methylation Status of Multiple Genes Simultaneously Without Bisulfite

The DNA methylation profiles of promoter CpG islands are important epigenetic biomarkers to both cancer and development research. Traditional bisulfite-based methods are too tedious and time-consuming to effectively keep pace with the needed rate of biomarker discovery and validation. In this 45-minute seminar, learn about the new Methyl-Profiler DNA Methylation PCR Arrays, a faster, easier, and higher-throughput DNA methylation profiling method that combines the reliability of well-controlled restriction digestions with quantitative PCR detection

NEW! Pilot Study Offers: http://www.sabiosciences.com/promotion/MethylProfilerPilotStudy.php

New Epigenetic Technologies for Research: microRNA, Chromatin IP, and DNA Methylation PCR Arrays

Epigenetics has grabbed the attention of many researchers by providing new insights into cell differentiation and oncogenesis. These rapidly expanding studies examine how heritable factors not coded within genomic DNA sequences regulate gene expression. Such factors include DNA methylation, chromatin remodeling due to histone modification, and even, in some cases, microRNA. The experimental methods designed to discover and correlate these factors with biological phenotypes have evolved. However, many still suffer from poor sensitivity and reliability, low-throughput, and unnecessary complexity. This seminar introduces advanced yet easy-to-use real-time PCR Array technologies offered by SABiosciences that analyze microRNA as well as mRNA expression profiles, CpG island DNA methylation, and chromatin immunoprecipitation fractions.

Download the Webinar!

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