

RT² Profiler™ PCR Array

Protocol Overview

RT² Profiler PCR Arrays

Microarray Profiling Capabilities with Real-Time PCR Performance



George J. Quellhorst, Jr., Ph.D.

Product Manager, Gene Expression Analysis

Topics to be Covered

PCR Array Protocol Tutorial

- Requirements: Equipment and Reagents
- RNA Isolation
- First Strand cDNA Synthesis (Reverse Transcription)
- RNA QC Plate
- Set-up and PCR Cycling Conditions
- Data Acquisition

Gene Expression Research Continuum

Discovery

High-Density,
Genome-Wide

Screening

Low-Density,
Pathway-Focused

Confirmation

Gene-By-Gene
RT-PCR Validation

Gene Function

Protein Function

- **1** – Low Sample Number, High Gene Content
 - What genes are involved in the biology under study?
- **2** – Higher Sample Number, Lower Gene Number
 - How do genes change expression under wider variety of conditions?
- **3** – Very Low Number of Very High Value Genes
 - Do these genes really change their expression?
- **4** – RNA Interference & Functional Biology
 - What role do these gene play in the biology under study?

RT² Profiler PCR Array Applications

Discovery

High-Density,
Genome-Wide

Screening

**Low-Density,
Pathway-Focused**

Confirmation

Gene-By-Gene
RT-PCR Validation

Gene
Function

Protein Function

- 1 – Low Sample Number, High Gene Content
- 2 – Higher Sample Number, Lower Gene Number
 - **RT² Profiler™ PCR Array**
- 3 – Very Low Number of Very High Value Genes
- 4 – RNA Interference & Functional Biology

PCR Array Data

Benefits of the PCR Array System

- **Combine microarray profiling capabilities with real-time PCR performance.**
- **Pathway-Focused and Customizable**
 - Profile the expression of a panel of genes relevant to a pathway or disease state.
- **Simple and Accurate**
 - Simple real-time PCR method provides high level of sensitivity, reproducibility, and specificity.
- **Designed for Routine Use**
 - Bring expression profiling to almost any lab with a real-time PCR instrument.

RT² Profiler™ PCR Array Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	G01	G02	G3	G04	G05	G06	G07	G08	G09	G10	G11	G12
B	G13	G14	G15	G16	G17	G18	G19	G20	G21	G22	G23	G24
C	G25	G26	G27	G28	G29	G30	G31	G32	G33	G34	G35	G36
D	G37	G38	G39	G40	G41	G42	G43	G44	G45	G46	G47	G48
E	G49	G50	G51	G52	G53	G54	G55	G56	G57	G58	G59	G60
F	G61	G62	G63	G64	G65	G66	G67	G68	G69	G70	G71	G72
G	G73	G74	G75	G76	G77	G78	G79	G80	G81	G82	G83	G84
H	HK1	HK2	HK3	HK4	HK5	GDC	RTC	RTC	RTC	PPC	PPC	PPC

- Wells A1-G12 (G01-G84): 84 Pathway-Focused Genes
- Wells H1-H5 (HK1-HK5): 5 Housekeeping Genes
- Wells H6 (GDC): Genomic DNA Control
- Well H7-H9 (RTC): Reverse Transcription Control
- Well H10-H12 (PPC): Positive PCR Control

Requirements for Complete Assay

Equipment

- **RNase/DNase-free pipette tips and tubes**
- **Calibrated Pipettors**
 - Multi-channel preferable for seeding plate
 - “Single Channel” for RT and Cocktails
- **Real-time PCR Instrument**
 - Almost all instruments supported
 - ABI, BioRad, Stratagene, Roche, Eppendorf
 - 96-well and 384-well instruments
 - Cannot support rotor-based instruments

Requirements for Complete Assay

RNA Isolation Kit:

- **RT² qPCR-Grade Total RNA Isolation Kit (PA-001)**
 - Includes DNase for on-column treatment
- **RT² First Strand Kit (C-03)**
 - Built-in Genomic DNA Elimination & External RNA Control
 - High positive call rate from ng to μg RNA amounts

Requirements for Complete Assay

- **RT² RNA QC PCR Array (PAXX-999Y)**
 - Test RNA quality & integrity before wasting reagents
 - Troubleshoot RNA that previously performed poorly
 - XX = HS, MM, or RN
 - Y = Code for Plate Format for Instrument
- Buyer's Guide in a few slides

Requirements for Complete Assay

- **RT² Real-Time™ SYBR Green PCR Master Mix**
 - HotStart enzyme minimizes primer dimers
 - 2X solution; one tube for every two arrays or two samples
 - Sold in sets of two or twelve (2, 12, or 24)
 - Instrument-Specific Formulations with Reference Dyes

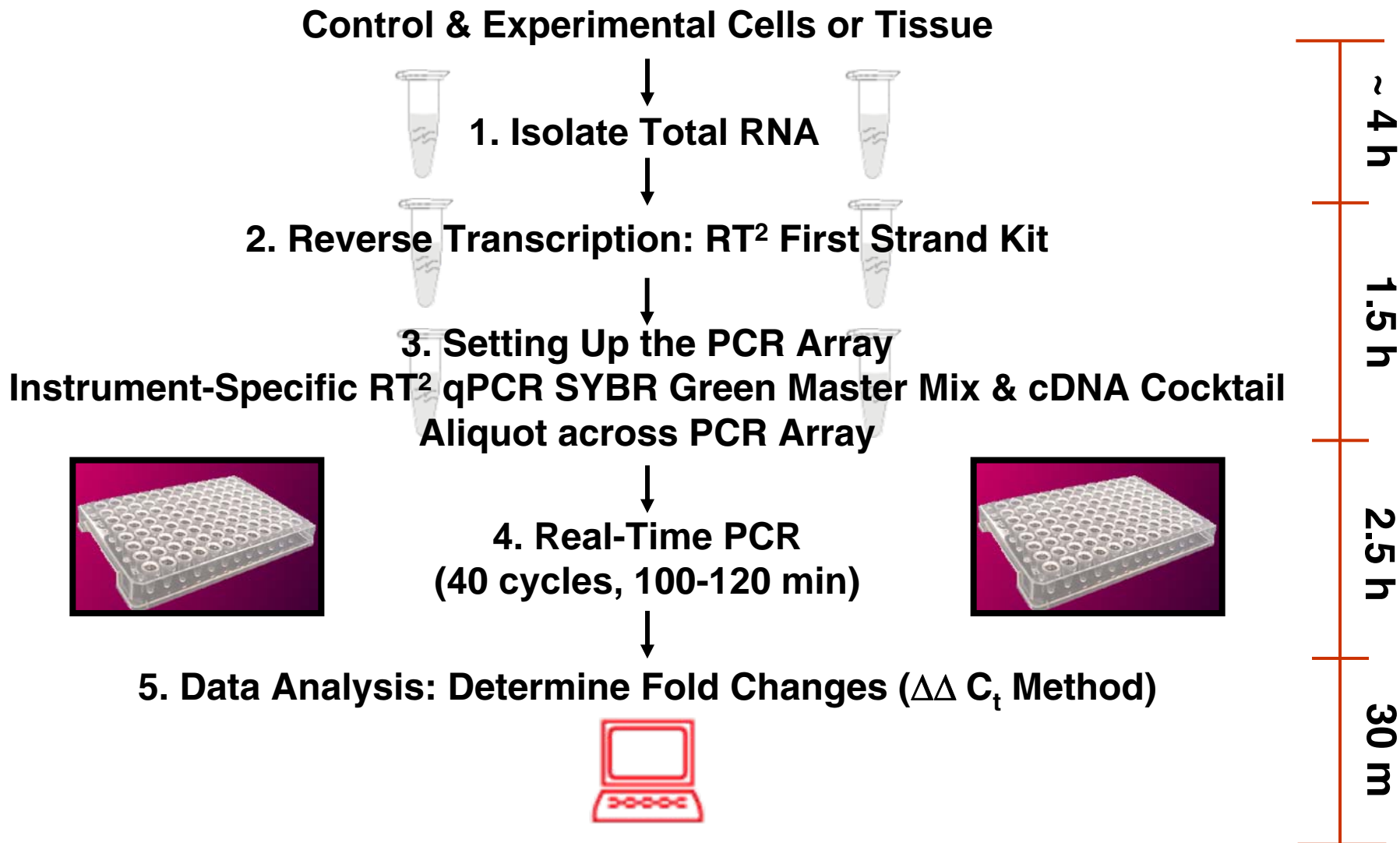
Requirements for Complete Assay

- **Pathway-Specific RT²Profiler™ PCR Array**
 - One for each experimental RNA sample
 - Sold in sets of two or twelve (2, 12, or 24)
 - Instrument-Specific Plate Formats
- **See website for PCR Array and Master Mix Buyer's Guide:**
<http://www.SABiosciences.com/manuals/PCRArrayGuide.pdf>

PCR Array Buyer's Guide

Instrument Model		Plate Format	Master Mix
ABI	5700, 7000, 7300, 7700	A	PA-012
	7500 Standard 96-well	A	
	7500 FAST 96-well	C	
	7900HT Standard 96-well	A	
	7900HT FAST 96-well	C	
	ABI 7900HT 384-well	E	
Bio-Rad	iCycler, iQ5, MyiQ	A	PA-011
	Chromo4 (MJ Research)	A	PA-010
	Opticon (2) (MJ Research)	D	
Stratagene	Mx3000p, Mx3005p	B	PA-012
	Mx4000p	D	
Roche	LightCycler 480 96-well	F	PA-010
	LightCycler 480 384-well	G	
Eppendorf	Mastercycler ep realplex	A	Inquire

Ease-of-Use: How the PCR Array Works



RNA Isolation Recommendations

- **Cultured Cells:**
 - Spin-Column RNA Isolation Kit
- **Tissue Samples:**
 - TRIzol® protocol (Invitrogen, Catalog # 15596-026)
 - Then, Spin-Column RNA Isolation Kit
- **Remember to use the on-column DNase treatment**
- **Others? See User Manual or Call Technical Support**
- **Quality Control:**
 - $A_{260}/A_{280} > 2.0$ and $A_{260}/A_{230} > 1.7$ in Tris pH 8.0
 - For Total RNA, 28S:18S agarose gel band intensity ~ 2:1
 - Agarose Gel or BioAnalyzer Electropherogram

Reverse Transcription (RT) Reaction

Using RT² First Strand Kit (C-03)

1. Genomic DNA Elimination Mix:

For each RNA sample, combine the following in a sterile PCR tube:

RNA	25.0 ng to 5.0 µg total RNA (0.5 to 1.0 µg recommended)
GE (gDNA Elimination Buffer)	2.0 µl
RNase-free H₂O	Adjust to ...
Final Volume	10 µl

Incubate 42 °C for 5 min. Immediate chill on ice for at least 1 min.

Reverse Transcription (RT) Reaction

Using RT² First Strand Kit (C-03)

2. Prepare the RT Cocktail:

Combine the following in a sterile PCR tube:

5X RT Buffer (BC)	4 μ l
RNase-free H ₂ O	3 μ l
Primer & External Control Mix (P2)	1 μ l
RT Enzyme Mix (RE2)	2 μ l
Final Volume	10 μ l

3. Perform RT Reaction:

Add 10 μ l RT Cocktail to each 10- μ l Genomic DNA Elimination Mixture. Inc. 42 °C for 15 min. Heat at 95 °C for 5 min. Dilute with 91 μ l ddH₂O. Store on ice or -20 °C.

RT² RNA QC PCR Array

Cocktail Preparation

Experimental Cocktail #1: 1X Master Mix + cDNA

Mix the following components in a 1.5-ml tube:

2X SABiosciences PCR master mix	75 μ l
Diluted first strand cDNA synthesis reaction	6 μ l
ddH ₂ O	69 μ l
Total volume	150 μ l

Experimental Cocktail #2: 1X Master Mix + H₂O

45 μ l each 2X SABiosciences PCR master mix and ddH₂O

Experimental Cocktail #3: 1X Master Mix + RNA

1 μ l 1:100 dilution of RNA sample + 24 μ l Cocktail #2

RT² RNA QC PCR Array LAYOUT

Samples 1 -12

+ cDNA	HK1	HK1	HK1	HK1	HK1	HK1	HK1	HK1	HK1	HK1	HK1	HK1
	HK2	HK2	HK2	HK2	HK2	HK2	HK2	HK2	HK2	HK2	HK2	HK2
	RTC	RTC	RTC	RTC	RTC	RTC	RTC	RTC	RTC	RTC	RTC	RTC
	PPC	PPC	PPC	PPC	PPC	PPC	PPC	PPC	PPC	PPC	PPC	PPC
	GDC	GDC	GDC	GDC	GDC	GDC	GDC	GDC	GDC	GDC	GDC	GDC
+ RNA	NRT	NRT	NRT	NRT	NRT	NRT	NRT	NRT	NRT	NRT	NRT	NRT
+ H ₂ O	PPC	PPC	PPC	PPC	PPC	PPC	PPC	PPC	PPC	PPC	PPC	PPC
	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC

- HK1, HK2 High and Low Level Housekeeping Genes
- RTC Reverse Transcription Control
- PPC Positive PCR Control ± Template
- GDC Genomic DNA Control
- NRT No Reverse Transcription Control
- NTC No Template Control

Experimental Cocktail Preparation

Pathway-Focused PCR Arrays

Mix the following components in a 5-ml tube or a multi-channel pipettor reservoir:

	Plate Format:	96-well A, C, D, & F	384-well E & G
2X SABiosciences PCR master mix		1275 μ l	550 μ l
Diluted first strand cDNA synthesis reaction		102 μ l	102 μ l
ddH ₂ O		1173 μ l	448 μ l
Total volume		2550 μ l	1100 μ l

NOTE: *This recipe only provides an excess volume of ONLY ~ 140 μ l. Very carefully add the cocktail to the PCR Array to insure that each well receives the required volume.*

Experimental Cocktail Preparation

PCR Array 384HT

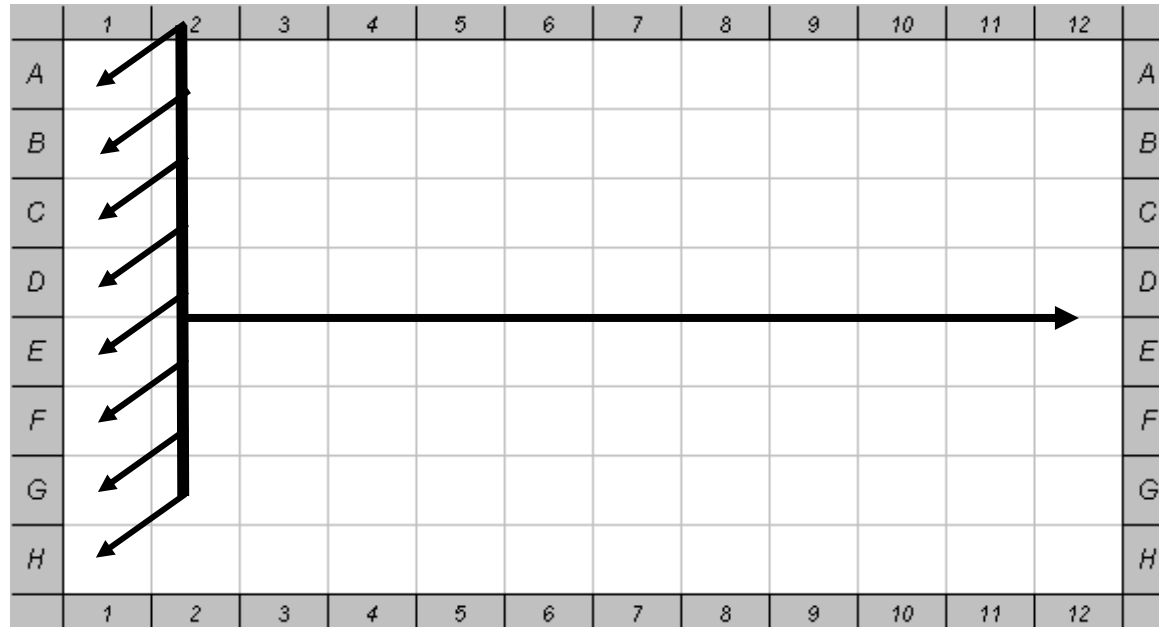
Mix the following components in a 5-ml tube or a multi-channel pipettor reservoir:

Plate Format:	<i>PCR Array 384HT</i> Formats E & G
2X SABiosciences PCR master mix	2000 μ l
Diluted first strand cDNA synthesis reaction	102 μ l
ddH ₂ O	1898 μ l
Total volume	4000 μ l

NOTE: *This recipe only provides an excess volume of ONLY ~ 140 μ l. Very carefully add the cocktail to the PCR Array to insure that each well receives the required volume.*

Loading Cocktail into PCR Array

Pathway-Focused 96-Well Formats A, C, D & F



Preferably, use all tips of a 8-channel pipettor to load all wells of each column one at a time with the same cocktail of master mix and template. Add 25 μ l per well.

Carefully seal Formats A & D with 8-well optical cap strips OR
Carefully seal Formats C & F with an optical film seal.

Loading Cocktail into PCR Array

Pathway-Focused 384-Well Formats E & G

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
A	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	A
B	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	B
C	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	C
D	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	D
E	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	E
F	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	F
G	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	G
H	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	H
I	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	I
J	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	J
K	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	K
L	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	L
M	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	M
N	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	N
O	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	O
P	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	P

Preferably, use all tips of a 8-channel pipettor to load all the appropriate wells with the correct cocktail of master mix and template. Add 10 μ l per well.

Carefully seal Formats E & G with an optical film seal.

Loading Cocktail into PCR Array

Pathway-Focused 384-Well Formats E & G

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
A	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	A
B	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	B
C	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	C
D	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	D
E	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	E
F	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	F
G	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	G
H	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	H
I	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	I
J	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	J
K	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	K
L	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	L
M	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	M
N	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	N
O	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	O
P	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	P
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	

Preferably, use all tips of a 8-channel pipettor to load all the appropriate wells with the correct cocktail of master mix and template. Add 10 μ l per well.

Carefully seal Formats E & G with an optical film seal.

Loading Cocktail into PCR Array

Pathway-Focused 384-Well Formats E & G

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
A	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	A
B	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	B
C	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	C
D	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	D
E	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	E
F	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	F
G	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	G
H	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	H
I	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	I
J	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	J
K	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	K
L	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	L
M	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	M
N	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	N
O	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	O
P	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	P
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	

Preferably, use all tips of a 8-channel pipettor to load all the appropriate wells with the correct cocktail of master mix and template. Add 10 μ l per well.

Carefully seal Formats E & G with an optical film seal.

Loading Cocktail into PCR Array

Pathway-Focused 384-Well Formats E & G

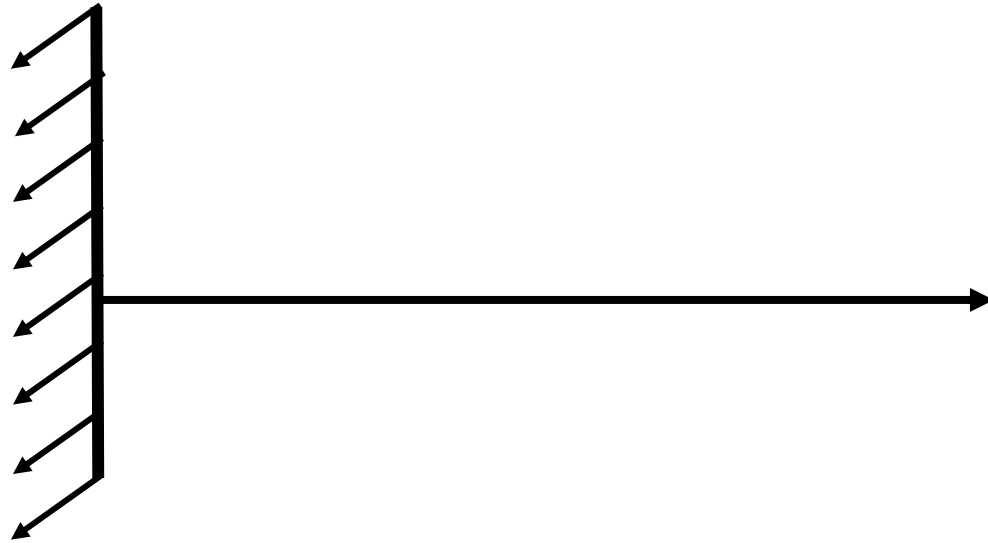
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
A	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	A
B	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	B
C	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	C
D	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	D
E	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	E
F	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	F
G	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	G
H	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	H
I	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	I
J	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	J
K	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	K
L	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	L
M	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	M
N	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	N
O	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	O
P	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	P

Preferably, use all tips of a 8-channel pipettor to load all the appropriate wells with the correct cocktail of master mix and template. Add 10 μ l per well.

Carefully seal Formats E & G with an optical film seal.

Loading Cocktail into PCR Array 384HT

PCR Array 384HT Formats E & G



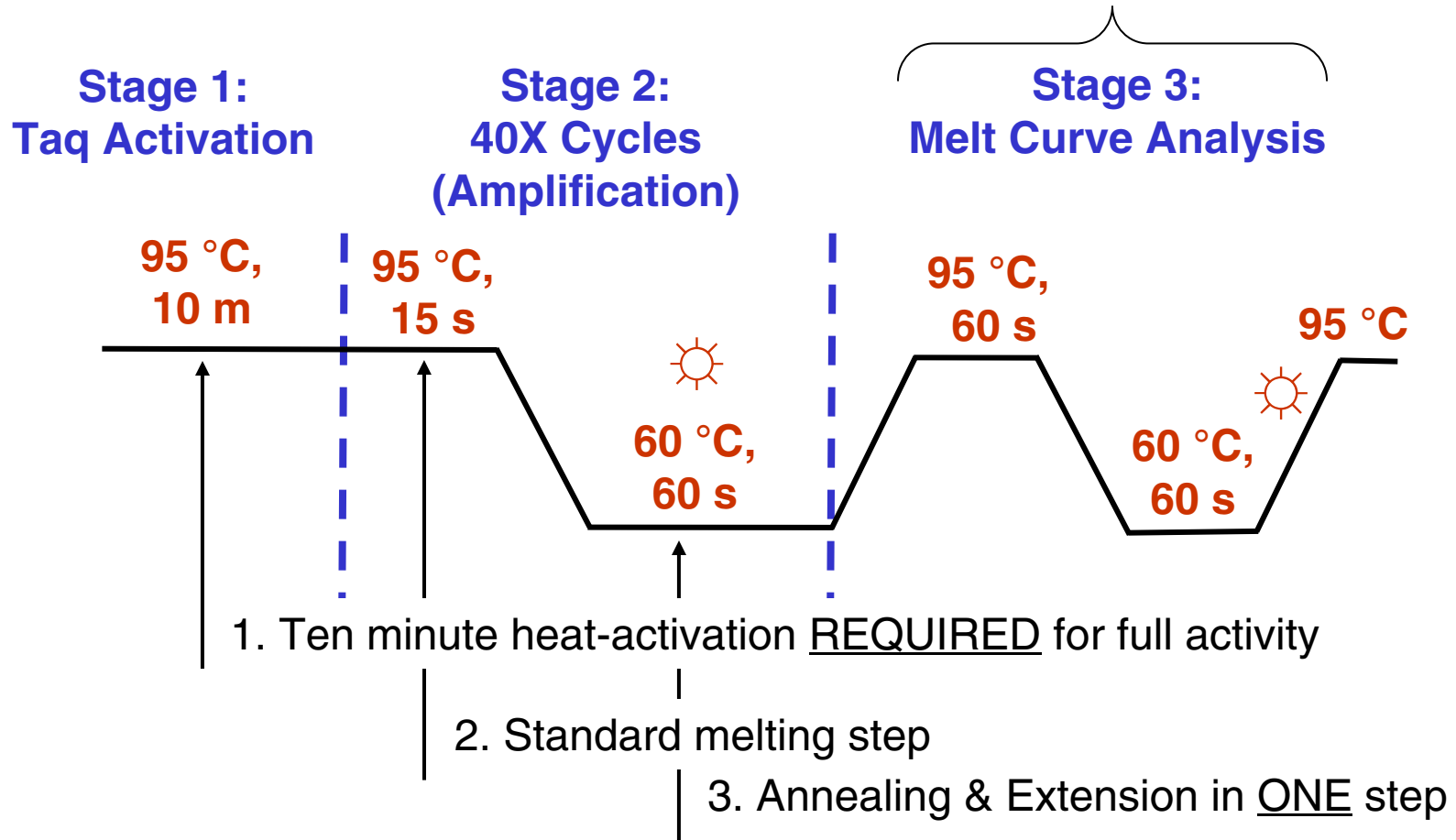
Preferably, use all tips of a 8-channel pipettor to load all wells of each column with the same cocktail of master mix and template. Add 10 μ l per well.

Carefully seal Formats E & G with an optical film seal.

PCR Cycle Parameters

4. Melt (Dissociation) Curve

OR use instrument's default melt curve program
Analyzes specificity of each assay well



Baseline and Threshold: Definitions

Baseline Value

During PCR, changing reaction conditions and environment can influence fluorescence. The background signal is most evident during the initial cycles of PCR prior to significant accumulation of the target amplicon. During these early PCR cycles, background signal in all wells is used to determine the “baseline fluorescence” across the entire reaction plate. The goal of data analysis is to determine when target amplification is sufficiently above the background signal, facilitating more accurate measurement of fluorescence.

Threshold

The threshold is the numerical value assigned for each run, statistically significant point above the calculated baseline.

C_t Value

The Threshold Cycle (C_t) reflects the cycle number at which the fluorescence generated within a reaction crosses the threshold.

From Applied Biosystems Technical Note

Post-Run: Baseline and Threshold

- **Baseline**
 - Use Automated baseline
(if your instrument has Adaptive Baseline function) OR
 - 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
 - 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**

Post-Run: Baseline Definition

View/Save Data
PCR Quantification
PCR Standard Curve
Melt Curve
Allelic Discrimination N/A

Data File: **A549.opd**

SYBR-490

Select analysis mode: **PCR Base Line Subtracted Curve Fit**

Threshold Cycle Calculation

Baseline Cycles

Threshold Position: **71.2**

Select Wells

Auto Calculated ←

Auto Calculated

User Defined

Auto Calculated

User Defined

Log View

Select a Reporter

SYBR-490

Save for X-axis Allelic Analysis

Save for Y-axis Allelic Analysis

Linear View

- A1
- A2
- A3
- A4
- A5
- A6
- A7
- A8
- A9
- A10
- A11
- A12
- B1

	Threshold Cycle Ct	Identifier
A1	24.0	
A2	22.5	
A3	29.2	
A4	34.8	
A5	23.7	
A6	30.8	
A7	31.3	
A8	27.8	
A9	28.8	
A10	24.9	
A11	29.0	
A12	27.1	
B1	34.8	
B2	24.5	
B3	36.9	
B4	28.6	
B5	24.5	

ACTION:
Data Window: Last 95%
Filter: Weighted Mean
Analysis: Post-Run

Post-Run: Threshold Definition

SAME number for EACH sample

The screenshot displays the 'PCR Quantification' tab of a software interface. The 'Data File' is 'APH_024_UniversailcDNA.opd'. The 'Select analysis mode' is 'PCR Base Line Subtracted Curve Fit'. Under 'Threshold Cycle Calculation', 'Baseline Cycles' are set from 2 to 15. The 'Threshold Position' is set to 22.6, which is circled in red. The 'User Defined' radio button is selected for both 'Auto Calculated' and 'User Defined' options. A red arrow points to the 'User Defined' option with the text 'User Defined'. The 'Log View' is selected, showing a graph of 'PCR Base Line Subtracted CF RFU' vs 'Cycle' on a log scale. A red bracket highlights the threshold line at 22.6. A table on the right shows Ct values for samples A1 through B5.

Sample	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

Fold Change Calculation

Due to the inverse proportional relationship between threshold cycle and the original gene expression level, and the doubling of the amount of product of every cycle, the original expression level (L) for each gene of interest is expressed as:

$$L = 2^{-C_t}$$

To normalize the expression level of a gene of interest (GOI) to a housekeeping gene (HKG), the expression levels of the two genes are divided:

$$\frac{2^{-C_t(\text{GOI})}}{2^{-C_t(\text{HKG})}} = 2^{-[C_t(\text{GOI}) - C_t(\text{HKG})]} = 2^{-\Delta C_t}$$

To determine fold change in gene expression, the normalized expression of the GOI in the experimental sample is divided by the normalized expression of the same GOI in the control sample:

$$\frac{2^{-\Delta C_t \text{ expt}}}{2^{-\Delta C_t \text{ control}}} = 2^{-\Delta \Delta C_t} \quad \text{Where } \Delta \Delta C_t \text{ is equal to } \Delta C_t \text{ expt} - \Delta C_t \text{ control}$$

The complete calculation is as follows:

$$\frac{\frac{2^{-C_t(\text{GOI}) \text{ expt}}}{2^{-C_t(\text{HKG}) \text{ expt}}}}{\frac{2^{-C_t(\text{GOI}) \text{ control}}}{2^{-C_t(\text{HKG}) \text{ control}}}} = \frac{2^{-[C_t(\text{GOI}) - C_t(\text{HKG})] \text{ expt}}}{2^{-[C_t(\text{GOI}) - C_t(\text{HKG})] \text{ control}}} = \frac{2^{-\Delta C_t \text{ expt}}}{2^{-\Delta C_t \text{ control}}} = 2^{-\Delta \Delta C_t}$$

RT² Profiler™ PCR Array

Protocol Overview

RT² Profiler PCR Arrays

Microarray Profiling Capabilities with Real-Time PCR Performance

