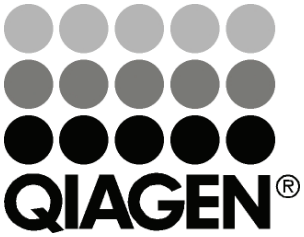
March 2011

qBiomarker PCR Array Handbook

qBiomarker Screening PCR Array

qBiomarker Validation PCR Array

For induced pluripotent stem cell screening, validation, and differentiation



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Product Use Limitations

qBiomarker Screening PCR Arrays and qBiomarker Validation PCR Arrays are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

CONTENTS

I. Background and Introduction

iPSC (**I**nduced **P**luripotent **S**tem **C**ells) is a breakthrough technology accessible to molecular and cell biology researchers. The iPSC technology was first developed by the Yamanaka lab at Kyoto University and quickly repeated by the Hochedlinger lab at Harvard University and the Jaenisch lab at MIT. By using retroviral vectors to express four transcription factors (Oct4, Sox2, c-Myc and Kif4), these groups were able to reprogram adult mouse fibroblast into iPSCs that successfully made it to the germ line in the chimerical animals after their injection into early stage mouse embryos. Later, many labs have induced iPSC from various human cells using a variety of expression systems and different combination of transcription factors. To date, iPSCs have been induced from virtually all human tissue and the induced iPSC have been further differentiated into many cell types.

Since iPSC avoids the ethical controversy of human embryonic stem cell research and the technology does not require any special equipment beyond a basic molecular biology lab with the capability of cell culture, it enables molecular biologists to adopt the technology in three major applications: regenerative medicine, disease specific modeling and basic research associated with stem cell and cell differentiation.

A standard iPSC induction takes about 16-20 weeks. After forced expression of the reprogramming transcription factors (Yamanaka factors: Oct4, Sox2, c-Myc and KLf4; Thomson factors: Oct4, Sox2, NANOG and Lin28) in parental somatic cells (typically fibroblast) by various vectors (typically lentivirus), stem cell like colonies appear around weeks 2-3. The appearance of the embryonic stem cell like colonies is the first sign of a successful cell reprogramming. Morphologically these colonies are the densely round cells with a well defined edge. Some of the colonies are fully de-differentiated pluripotent stem cells. Some are partially reprogrammed, thus will lack the full potential to differentiate into desired cell types.

A typical proceeding is to pick >20 colonies, based on colony morphology, to expand. Some of the colonies would not be able to expand. The expanded colonies would be screened for the expression of pluripotency markers, and hopefully some of them will be a true iPSC. Around weeks 4-6, the iPSC colonies that show all the pluripotency markers will be selected for more rigorous validation experiments which include parental gene and transgene silencing, epigenetic markers, in vitro differentiation and teratomas formation. Normally one to four colonies are selected for the above mentioned validations. The validation process completes when the teratomas formation results are produced, normally around weeks 16-20. An iPSC line is established when all the validation criteria are met. An established iPSC line is ready for subsequently applications (e. g. differentiated into desired progenitors and terminal differentiated cell types).

The qBiomarker iPSC PCR Array System is composed of 8 qPCR arrays for a typical iPSC researcher to screen or to validate cell lines at various stage of the iPSC induction process. Brief descriptions of the arrays are shown in Table 1.

Table 1: Description of the qBiomarker iPSC PCR Array System.

Product (PCR array)	Specification	Application
1. iPSC colony screening Cat.# 337221 IPHS-001	Includes eight 8 pluripotency markers and 4 sample controls (see Product Specification Sheets for details), able to analyze simultaneously 8 samples in the same 96-well plate (Fig. 2, plate layout I) or 32 samples in the same 384-well plate (Fig.2, plate layout III)	Analyze multiple samples and able to distinguish fully reprogrammed colonies from partially reprogrammed
2. Yamanaka reprogramming factors expression Cat.# 337221 IPHS-002	Measures endogenous and total expression level of OCT4, SOX2, KLF4 and c-MYC. Includes 4 sample controls (see Product Specification Sheets for details). Analyze simultaneously 8 samples in the same 96-well plate (Fig. 2, plate layout I)	Analyze multiple samples for comparing total and endogenous expression levels of 4 reprogramming transcription factors (OCT4, SOX2, KLF4 and c-MYC) to determine the degree of exogenous gene silencing and endogenous gene induction.
3. Thomson reprogramming factors expression Cat.# 337221 IPHS-003	Measures endogenous and total expression level of OCT4, SOX2, Nanog and Lin28. Includes 4 sample controls (see Product Specification Sheets for details), Analyze simultaneously 8 samples in the same 96-well plate (Fig. 2, plate layout I)	Analyze multiple samples for comparing total and endogenous expression levels of 4 reprogramming transcription factors (OCT4, SOX2, Nanog and Lin28) to determine the degree of exogenous gene silencing and endogenous gene induction.
4. iPSC pluripotency validation Cat.# 337221 IPHS-100	Includes 17 pluripotency markers, 1 parental cell markers, 1 differentiation markers and 5 controls (see Product Specification Sheets for details) to allow comparative gene expression analysis of 4 samples in the same 96-well plate (Fig. 2 , plate layout II) or 16 samples in the same 384-well plate (Fig. 2, plate layout IV)	Able to distinguish iPSC line from partially programmed cell lines, adult parental cell line and spontaneously differentiated iPSC
5. Embryoid body differentiation Cat.# 337321 IPHS-101	Includes 16 markers for all three germ layer, 3 pluripotency markers and 5 controls (see Product Specification Sheets for details) to allow comparative gene expression analysis of 4 samples (including parent iPSC line) simultaneously in the same 96-well plate (Fig. 2 , plate layout II) or 16 samples in the same 384-well plate (Fig. 2, plate layout IV)	Analyze multiple samples and able to determine successful differentiation of iPSC to embryoid bodies
6. Cardiomyocytes differentiation Cat.# 337321 IPHS-102	Includes 19 cardiomyocyte markers and 5 controls (see Product Specification Sheets for details) to allow comparative gene expression analysis of 4 samples (including parent iPSC line) simultaneously in the same 96-well plate (Fig. 2 , plate layout II) or 16 samples in the same 384-well plate (Fig. 2, plate layout IV)	Analyze multiple samples and able to determine successful differentiation of iPSC to cardiomyocytes

7. Neuronal lineage differentiation Cat.# 337321 IPHS-103	Includes 19 neuronal lineage markers and 5 controls (see Product Specification Sheets for details) to allow comparative gene expression analysis of 4 samples (including parent iPSC line) simultaneously in the same 96-well plate (Fig. 2 , plate layout II) or 16 samples in the same 384-well plate (Fig. 2, plate layout IV)	Analyze multiple samples and able to determine successful differentiation of iPSC to neuronal lineage
8. Reprogramming factors expression Cat.# 337221 IPHS-104	Measures endogenous and total expression level of 6 commonly used reprogramming factors (OCT4, SOX2, NANOG, Lin-28, KLF4 and c-MYC). Includes 4 sample controls. Analyze simultaneously 24 samples in the same 384-well plate (Fig. 2 , plate layout V).	Analyze multiple samples for comparing total and endogenous expression levels of 6 commonly used reprogramming transcription factors to determine the degree of exogenous gene silencing and endogenous gene induction.

The biomarkers on each array in the qBiomarker PCR Array System are selected from intensive literature study and are biologically validated for their robust up- or down-regulation in iPSCs, embryoid bodies from the iPSCs, and terminally differentiated cells. House keeping gene(s) and three sample integrity controls are also included on the arrays to assist data analysis and to assess final data quality.

The end user is strongly encouraged to include a negative control and a positive control when using these arrays. For arrays 1-4 and 8, a typical negative control would be the parental somatic cell line and a typical positive control would be an established human embryonic stem cell line. For arrays 5-7, a typical negative control would be the starting iPSC line and a typical positive control would be a differentiated cell line or a validated tissue sample.

Benefits of the qBiomarker PCR Arrays:

- **Validated Biological Relevance:** Each of the biomarkers is biologically validated for its robust up- or down- regulation upon cell reprogramming.
- **Easy Interpretation of Results:** each array has a companion Data Analysis Template in Excel to produce results and graphs by simply copying and pasting the raw data from any PCR instrument, making interpretation easy and direct.
- **Increased Throughput:** each array in 96-well PCR plat format can analyze 8-19 marker genes of 4-8 samples on a 96-well plate or of 16-32 samples on a 384-well plate simultaneously.
- **Simple and Accurate:** Routinely used real-time PCR technology provides accurate and reproducible results on any qPCR instrument.

Typical Experimental Workflow

Isolate Total RNA from your Experimental Samples
Use QIAGEN RNeasy® Mini Kit (cat. no. 74104) and optional DNase step
Start with as little as 120 ng of total RNA for each sample
(amount depends on type of qBiomarker PCR Array Used)

↓

Prepare cDNA from your RNA Samples
(Use RT^2 First Strand Kit cat. no. 330401)

↓

Add cDNA to qBiomarker SYBR Green Mastermix

↓

Aliquot the Mixtures to the corresponding rows of the qPCR Array
Refer to the plate layout to add your samples.

↓

Perform Thermal Cycling on qPCR Instrument

↓

Analyzing Changes in Gene Expression
Download qBiomarker Data Analysis Templates or
Upload your Data to the qBiomarker Data Analysis Website

Figure 1: Overview of the qBiomarker PCR Array Workflow.

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

Plate Layout (I)

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

Plate Layout (II)

	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	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	12
B	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	12
C	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	12
D	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	12
E	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	12
F	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	12
G	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	12
H	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	12
I	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	12
J	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	12
K	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	12
L	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	12
M	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	12
N	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	12
O	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	12
P	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	12

Plate Layout (III)

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

Plate Layout (IV)

	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	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
C	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
D	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
E	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
F	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
G	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
H	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
I	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
J	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
K	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11
L	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
M	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
N	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14
O	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
P	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16

Plate Layout (V)

Figure 2: Plate Layout of the qBiomarker PCR Array System.

Plate layout I: 12 genes X 8 samples' 96-well plate layout. Wells 1 to 8 are selected biomarker genes, well 9 is a housekeeping gene and wells10-12 are sample integrity controls. Rows A through H are for 8 samples per plate.

Plate layout II: 24 genes X 4 samples 96-well plate layout. Wells 1-19 are biomarker genes, wells 20-21 are house keeping genes and wells 22-24 are sample integrity controls. All 24 wells in columns 1-3 are for one sample. Columns 4-6, 7-9 and 10-12 are replica of columns 1-3.

Plate layout III: 12 genes X 32 samples 384-well plate layout. Wells 1 to 8 are selected biomarker genes, well 9 is a house keeping gene and wells 10-12 are sample integrity controls. Each row is designed for 2 samples in alternative columns.

Plate layout IV: 24 genes X 16 samples 384-well plate layout. Wells 1-19 are biomarker genes, wells 20-21 are house keeping genes and wells 22-24 are sample integrity controls. Each sample is added to a different row of the plate. Up to 16 samples can be assayed.

Plate layout V: 16 genes X 24 samples 384-well plate layout. Wells 1-12 are biomarker genes, well 13 is a house keeping gene and wells 14-16 are sample integrity controls. Each sample is added to one column of the plate. Up to 24 samples can be analyzed at one time.

Well Contents:

Each well contains a biologically selected, wet-bench validated qPCR assay which is validated using the qBiomarker SYBR® Green Mastermixes. Using any other mastemixes may compromise the results.

WARNING: USING OTHER MASTERMIXES WILL POTENTIALLY COMPROMISE THE SENSITIVITY, SPECIFICITY OR AMPLIFICATION EFFICIENCY OF THESE qPCR ASSAYS.

Biomarkers: See the specification sheet of each qBiomarker PCR Array for details.

Housekeeping Assay: Array formats I, III and V contain a single housekeeping gene for sample normalization and data analysis. Array formats II, and IV contain two housekeeping genes. One or both can be used for sample normalization and data analysis.

Genomic DNA Controls (GDC): Specifically detects a non-transcribed genomic DNA region with a high level of sensitivity. This assay is used to assess genomic DNA contamination within the sample.

Reverse Transcription Controls (RTC): The Reverse Transcription Controls (RTC) provided with the RT^2 First Strand Kit (cat. no. 330401) is used to monitor the Reverse Transcription step. In conjunction with the PPC Assay a metric for reverse transcription reproducibility can be established.

Positive PCR Controls (PPC): During manufacturing of the PCR Arrays, an artificial DNA template and the qPCR Assays are directly added to the PCR Array. The PPC Assay is used to detect any potential PCR inhibitors in the samples and for assessing the reverse transcription efficiency.

II. Materials Provided:

The PCR Arrays are available in eight different plate formats, each tailored to a specific subset of real-time PCR instruments and associated blocks. Formats A, C, D, and F are 96-well plates, while Formats E and G are 384-well plates.

PLATE Format	For Real-Time Instruments	Plate
A	ABI "**standard**" blocks: 5700, 7000, 7300, 7500, 7700, 7900HT, ViiA™7, (96-block) Bio-Rad: iCycler®, iQ™5, MyiQ, MyiQ2, Chromo4™ (MJ Research) Eppendorf: MasterCycler® ep RealPlex® 2, 2s, 4, 4s Stratagene: Mx3005p®, Mx3000p® Takara: TP-800	96-well
C	ABI: 7500 **FAST** block, 7900HT **FAST** block, ViiA 7 **FAST** block, StepOnePlus™	96-well
D	Bio-Rad: CFX96™, Opticon® and Opticon 2 (MJ Research) Stratagene: Mx4000®	96-well
E	ABI: 7900HT (384-well block), ViiA7 (384-well block) Bio-Rad: CFX384™	384-well
F	Roche: LightCycler® 480 96-well block	96-well
G	Roche: LightCycler 480 384-well block	384-well

NOTE: *The format of the PCR Array is indicated by the last letter of the catalog number. Be sure that you have the correct PCR Array format for your instrument before starting the experiment.*

The 96-well PCR Arrays (Formats A, C, D, and F) are shipped in sets of 2, 12, or 24, while the 384-well PCR Arrays (Formats E and G) are shipped in sets of 4. The PCR Array Plate Format H is shipped in a Format A plate in solution (on ice). The R format is shipped with 2, 12 or 24 rotors.

Each PCR Array shipment includes the arrays and either 12 optical thin-wall 8-cap strips (Formats A and D) or 1 optical adhesive film (Formats C, E, F, and G, RG) per array.

Storage Conditions:
All components included in this kit, except for format H plates, are shipped at ambient temperature but must be stored at -20°C upon receipt. Format H plates are shipped at -20°C. When stored properly at -20°C, their quality is guaranteed for 6 months.

III. Additional Materials Required:

A. RNA Isolation Kit: Qiagen RNeasy® Mini Kit (Catalog # 74104) with optional DNase step. Follow the RNA Isolation Protocol provided with the kit. For more information on RNA quality, See Page **15**.

B. High-quality, nuclease-free H_2O. **Do NOT USE DEPC TREATED WATER**.

C. RT² First Strand Kit (Cat. No. 330401)
REQUIRED for a Complete and Successful Experiment. Contains Reverse Transcription Control Detection (RTC). Each kit contains enough reagents for 12 reactions. You need to order 2 kits (Plate Layout IV and V) or 3 kits (Plate Layout III) to maximize the sample load of a 384-well plate.

D. qBiomarker SYBR Green Mastermix
REQUIRED for a Complete and Successful Experiment
Please select the correct mastermix for the qPCR instrumentation you plan on using.

qBiomarker SYBR Green ROX™ Mastermix:
Specifically designed for:

- **ABI** 5700,7000, 7300, 7500 (Standard & FAST), 7700, 7900HT 96-well block (Standard & FAST) and 384-well block, StepOnePlus, ABI ViiA7
- **Eppendorf** Mastercycler ep realplex 2/2S/4/4S
- **Stratagene** Mx3000p, Mx3005p, Mx4000
- **TaKaRa** TP-800

Catalog Number	Component Name	qBiomarker PCR Arrays per order
337630	SYBR Green ROX qPCR Mastermix	For 2 96-well qBiomarker PCR Arrays
337632		For 12 96-well qBiomarker PCR Arrays
337633		For 24 96-well qBiomarker PCR Arrays
337631		For 4 384-well qBiomarker PCR Arrays

qBiomarker SYBR Green Fluor Mastermix:
Specifically designed for:

- **Bio-Rad** iCycler, iQ5, MyiQ, MyiQ2

Catalog Number	Component Name	qBiomarker PCR Arrays per order
337620	SYBR Green Fluor qPCR Mastermix	For 2 96-well qBiomarker PCR Arrays
337622		For 12 96-well qBiomarker PCR Arrays
337623		For 24 96-well qBiomarker PCR Arrays
337621		For 4 384-well qBiomarker PCR Arrays

qBiomarker SYBR Green Mastermix:
Specifically designed for instrumentation that does not require a reference dye:

- **Bio-Rad** CFX96, CFX384, Chromo4, Opticon 2
- **Roche** LightCycler 480 (96-well & 384-well)
- **All Others**

Catalog Number	Component Name	qBiomarker PCR Arrays per order
337610	SYBR Green qPCR Mastermix	For 2 96-well qBiomarker PCR Arrays
337612		For 12 96-well qBiomarker PCR Arrays
337613		For 24 96-well qBiomarker PCR Arrays
337611		For 4 384-well qBiomarker PCR Arrays

Equipment:

1. For recommendations on specific real-time instrumentation (thermal cyclers with fluorescent detection), see the list of master mixes and plate formats above.

NOTE: *The qBiomarker PCR Arrays can be used in any 96-well and 384-well real-time PCR instrument. PCR Arrays can not be used in the Cepheid SmartCycler®, or the Roche LightCycler 2.0.*

2. Calibrated Multi-Channel Pipette
3. RNase / DNase-free pipette tips and tubes

Complementary Products:

- **qBiomarker iPSC Expression Plasmids and Lentivirus**
  - Transduction-Ready Lentivirus for Oct4, cMyc, Klf4, Nanog, Sox2, Lin28
  - Transfection-Ready Plasmids for Oct4, cMyc, Klf4, Nanog, Sox2, Lin28

- **qBiomarker Screening PCR Arrays**
  - iPSC Colony Screening
  - iPSC Pluripotency Validation
  - Yamanaka Reprogramming Factors
  - Thomson Reprogramming Factors
  - Reprogramming Factors Expression

- **qBiomarker Validation PCR Arrays**
  - Embryonic Body Formation
  - Cardiomyocytes Differentiation
  - Neuronal Lineage Differentiation

IV. Before You Begin:

Please read through this entire protocol before beginning your experiment.
RNA samples are very sensitive to RNase digestion; therefore, wear gloves and maintain an RNase-free work area while performing this protocol.

NOTE: Master Mix and First Strand Synthesis Considerations

The performance of the qBiomarker PCR Arrays is only guaranteed with qBiomarker SYBR Green Mastermixes and the RT^2 First Strand Kit. Therefore, the use of the complete qBiomarker PCR Array System is absolutely essential for obtaining accurate, specific and sensitive real-time PCR results.

The chemically-modified and tightly controlled HotStart enzyme and other proprietary chemical components in our qBiomarker SYBR Green Mastermixes provide more accurate SYBR Green results by preventing the amplification of primer dimers and other non-specific products. They also help ensure high amplification efficiencies even for those genes that are the most difficult to amplify. When we test other sources of enzymes or mastermixes with our qBiomarker PCR Arrays, we frequently see primer dimers and other non-specific products that confound SYBR Green-based real-time PCR detection. Because each instrument uses a different reference dye to normalize their optics, be sure that you use the correct master mix for the instrumentation in your laboratory.

The RT^2 First Strand Kit includes a proprietary buffer to eliminate residual genomic DNA contamination in your RNA samples before it can be amplified into secondary products that would otherwise cause false positive signals. The Reverse Transcription Controls (RTC) on the PCR Array can only be evaluated with the built-in external RNA control of the RT^2 First Strand Kit. These controls do not yield results when used with other sources of reverse transcriptases or first strand synthesis kits. The buffer components and the magnesium concentration in our RT^2 First Strand Kit are also more compatible with our PCR master mixes than other enzymes or kits providing the PCR Arrays with maximum levels of sensitivity with ng to µg amounts of total RNA.

NOTE: Preparing a Workspace Free of DNA Contamination
For accurate and reproducible PCR Array results, it is very important to avoid contamination of the assay with foreign DNA. Any DNA contamination will artificially inflate the SYBR Green signal yielding skewed gene expression profiles and false positive signals. The most common sources of DNA contamination are the products of previous experiments spread into the air of your working environment. Please follow the recommendations below on how to set up and maintain a working environment free of DNA contamination.

1. Wear gloves throughout the procedure. Use only fresh PCR-grade reagents (H_20) and lab ware (tips and tubes).

2. Physically separate the workspaces used for PCR setup and post-PCR processing or non-PCR operations. Decontaminate your PCR workspace and lab ware (pipette barrels, tube racks, etc.) before each new use with UV light to render any contaminating DNA ineffective in PCR through the formation of thymidine dimers or with 10% bleach to chemically inactivate and degrade any DNA.

3. Close all tubes containing PCR products once you are finished adding or removing volumes. Before discarding any lab ware (tips or tubes) containing PCR products or other DNA, treat with 10% bleach.

4. Do not remove the PCR Array plate from its protective sealed bag until immediately ready to use. Do not leave lab ware (tubes and tip boxes) exposed to the air for long periods of time.

5. Do not open any previously run and stored PCR Array plate. Removing the thin-wall 8-cap strips or the adhesive film from PCR Arrays releases PCR product DNA into the air where it will contaminate and confound the results of future real-time PCR experiments.

RNA Preparation and Quality Control:

High quality RNA is <u>ESSENTIAL</u> for obtaining good real-time PCR results.
The most important prerequisite for any gene expression analysis experiment is consistent, high-quality RNA from every experimental sample. Therefore, the sample handling and RNA isolation procedures are critical to the success of the experiment. Residual traces of proteins, salts or other contaminants will either degrade the RNA or decrease the efficiency of (if not block completely) the enzyme activities necessary for optimal reverse transcription and real-time PCR performance.

1. Recommended RNA Preparation Methods:
High quality total RNA for your real-time PCR experiment must be prepared using QIAGEN RNeasy® Mini Kit (Catalog # 74104) with optional DNase step.

IMPORTANT: You must perform the recommended DNase treatment step.

2. RNA Quality Control:
For best results from the PCR Array, all RNA samples should also demonstrate consistent quality according to the following criteria:

a. RNA Concentration and Purity by UV Spectrophotometry

NOTE: *Prepare dilutions and measure absorbance in 10 mM Tris, pH 8.0 buffer. The spectral properties of nucleic acids are highly dependent on pH.*

i) A_{260}:A_{230} ratio should be greater than 1.7.
ii) A_{260}:A_{280} ratio should be 1.8 to 2.0.
iii) Concentration by A_{260} should be greater than 40 µg / ml total RNA

b. Ribosomal RNA band integrity

Electrophorese a fraction of each RNA sample on a denaturing agarose gel or on an Agilent BioAnalyzer using an RNA 6000 Nano LabChip® and verify that there is a sharp distinction at the small side of both the 18S and 28S ribosomal RNA (rRNA) bands or peaks. Any smearing or shoulder to the rRNA bands or peaks indicates that degradation has occurred in the RNA sample.

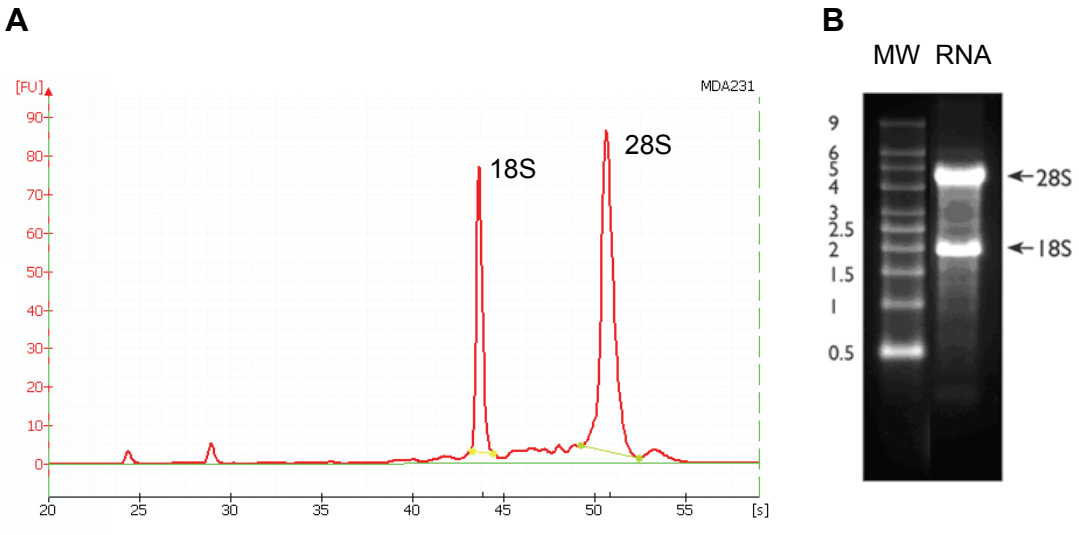

Figure 3: Good Ribosomal RNA Band Integrity Is Important for Optimal PCR Array Results.
Panel **A** displays an Agilent BioAnalyzer electropherogram of a high-quality total RNA preparation showing sharp peaks without shoulders (especially to the left of each peak) for the 18S and 28S ribosomal RNA (left to right). Panel **B**, right-hand lane, displays an analysis of the same high-quality total RNA preparation by agarose gel electrophoresis demonstrating sharp bands (especially at the bottom of each band) for the 28S and 18S ribosomal RNA (top to bottom).

Because some contaminants are difficult to detect by simply looking at RNA integrity and can be missed by UV spectrophotometry, it is essential to choose the proper RNA isolation method for your biological sample as described above.

2. Genomic DNA Contamination:

Eliminating genomic DNA contamination is essential for obtaining optimal real-time gene expression profiling results using the PCR Array. The Genomic DNA Control in each PCR Array specifically tests for genomic DNA contamination in each sample during each run. A GDC threshold cycle value less than 35 indicates the presence of a detectable amount of genomic DNA contamination that should be addressed.

We highly recommend performing the DNase treatment step with the Qiagen RNeasy® Mini Kit (Catalog # 74104) followed by using the RT^2 First Strand Kit (cat. no. 330401) to remove any and all residual contamination from your RNA samples.

3. Amount Considerations:

The PCR Array System yields robust results with as little as **5 ng** total RNA per well of a 96-well PCR plate or **2 ng** total RNA per well of a 384-well PCR plate. For a particular format (See Figure 2 for details), the recommended starting total RNA amount per sample is 250 ng to 1000 ng per sample for 96 well plate formats and 100 to 400 ng for 384 well plate formats. **Less sample may be used, but it is important to dilute the cDNA from the first strand kit to avoid buffer carryover that could affect the qPCR step.**

V. PROTOCOL

A. cDNA SYNTHESIS (RT^2 First Strand Kit).

!WARNING!: ***Do not use DEPC treated H_2O***. *Use high-quality, nuclease-free H_2O. If you are not sure whether your RNase, DNase-free water has been DEPC treated, please check with the supplier*

!!WARNING!!: *The use of the RT^2 First Strand Kit (Cat. No. 330401) is critical for detecting the Reverse Transcription Controls (RTC) and for obtaining the best results from the qBiomarker PCR Array*

NOTE: *RNA samples must meet the standards of integrity and purity from protein, organics, and genomic DNA contamination described on the previous two pages.*

NOTE: *Use the same amount of total RNA in this reaction for every sample. First time users are recommended to start with up to 1000 ng of total RNA for sample.*

NOTE: ***Carefully pipette reagents from reagent tubes,*** *starting with pipette tip at top of tube and working down slowly.*

1. Genomic DNA Elimination Step:

1. Briefly spin (10-15 seconds) down all reagents.
2. Prepare the **Genomic DNA Elimination Mixture****:
    a. For each RNA sample, combine the following in a sterile PCR tube:

Total RNA (96 well plate)	250 to1000	ng
Total RNA (384 well plate)	100 to 400	
Buffer GE** (5x gDNA elimination buffer)	2.0	µl
H_2O to a final volume of	10.0	µl

****The RT^2 First Stand Kit (cat. no. 330401) is not compatible with the chemicals in Ambion's DNA-*free*™ kits. If your RNA sample has been treated with Ambion's DNA-*free*™ reagents, please call SABiosciences Technical Support at 1-888-503-3187.**

    b. Mix the contents gently with a pipette followed by brief centrifugation.
    c. Incubate at 42°C for 5 min.
    d. Chill on ice immediately for at least one minute.

2. Reverse Transcription Step:

2. Prepare the RT Cocktail:

RT Cocktail	1 reaction	4 reactions	8 reactions	16 reactions	24 reactions	32 reactions
5x Buffer BC3	4 µl	16 µl	32 µl	64 µl	96 µl	128 µl
Control P2 (Primer and control mix)	1 µl	4 µl	8 µl	16 µl	24 µl	32µl
RE3 Reverse Transcriptase Mix	2 µl	8 µl	16 µl	32 µl	48 µl	64 µl
H_2O	3 µl	12 µl	24 µl	48 µl	72 µl	96 µl
Final Volume	10 µl	40 µl	80 µl	160 µl	240 µl	320 µl

3. First Strand cDNA Synthesis Reaction:

a. Add 10 µl of RT Cocktail to each 10-µl Genomic DNA Elimination Mixture.
b. Mix well but gently with a pipette.
c. Incubate at 42°C for ***exactly*** 15 min and then ***immediately*** stop the reaction by heating at 95°C for 5 minutes.
d. Add **91** µl of H_2O to each 20-µl of cDNA synthesis reaction. Mix well.
e. Hold the finished First Strand cDNA Synthesis Reaction on ice until the next step or store overnight at -20°C.

4. RNA Quality Control Check (Optional):

If desired, proceed to characterize a small aliquot (6 µl) of the diluted cDNA template on the correct species-specific and instrument-specific RT^2 RNA QC PCR Array following the instructions provided in its handbook. Save the remainder at – 20°C.

B. Setup and Performing Real-Time PCR Step:

NOTE: ***Do not use DEPC treated H_2O***. *Use high-quality, nuclease-free H_2O. If you are not sure whether your RNase, DNase-free water has been DEPC treated, please check with the supplier.*

NOTE: *An incorrectly chosen PCR Array plate format will not properly fit into your real-time PCR instrument, and its use will damage the instrument. Be sure you have the correct PCR Array format for your instrument before continuing with this protocol.*

NOTE: *If precipitates are present in the Master Mix tubes, please contact a technical applications scientist at 1-888-503-3187or* ***support@SABiosciences.com*** *for further instructions.*

NOTE: ***Carefully pipette reagents from reagent tubes,*** *starting with pipette tip at top of tube and working down slowly.*

1. Briefly (10-15 seconds) spin down all reagents.
2. **Experimental Cocktail** Preparation

Mix the following components in a 1.5-ml microcentrifuge tube for each sample

Plate Layout (see Figure 2)	I	II	III	IV	V
2x SYBR Green qPCR Mastemix	200 µl	350 µl	80 µl	140 µl	100 µl
Diluted First Strand cDNA Synthesis Reaction	16 µl	28 µl	16 µl	28 µl	20 µl
H_2O	184 µl	322 µl	64 µl	112 µl	80 µl
Total Volume	400 µl	700 µl	160 µl	280 µl	200 µl

NOTE: *This recipe provides an excess volume to allow for multiple pipetting. Very carefully add the cocktail to the PCR Array precisely as described below to insure that each well receives the required volume.*

Loading the PCR Arrays

Please select your PCR Array Format for loading instructions.

NOTE: *Change pipette tips following each addition to avoid any cross-contamination between the wells or reactions.*

a. **Loading the 96-Well PCR Array Formats A, C, D, or F**

i. **CAREFULLY** remove the PCR Array from its sealed bag.

ii. Add 25 µl of the Experimental Cocktail to the corresponding row (See plate layout shown in Fig. 2 to assist the sample loading) of the PCR Array.

iii. Proceed to the next section (**STEP 4**) on "Performing Real-Time PCR Detection".

b. **Loading the 384-Well PCR Array Formats E or G:**

i. **CAREFULLY** remove the PCR Array from its sealed bag.

ii. Add 10 μl of the Experimental Cocktail to the corresponding row (See plate layout shown in Fig. 2 to assist the sample loading) of the PCR Array.

iii. Proceed to the next section (**STEP 4**) on "Performing Real-Time PCR Detection".

4. Performing Real-Time PCR Detection:

ATTENTION Users of Bio-Rad and Eppendorf Real-Time Instruments: *Prior to initiating the run, please make sure your instrument has been calibrated to use clear, flat optical caps with PCR Array plates.*

NOTE: *Be sure to follow the manufacturer's instructions for the proper operation and maintenance of your real-time instrument.*

a. **CAREFULLY** but tightly seal the PCR Array with the optical thin-wall 8-cap strips (Formats A and D) or with the optical adhesive film (Formats C, E, F, and G).

b. **Centrifuge the plate** for 5 full minutes at room temperature at 3000 g to remove bubbles. Visually inspect the plate from underneath of the plate to ensure no bubbles are present in each well.

!!!WARNING!!!: Bubbles remaining in the bottom of the wells of a PCR Array will interfere with results.

c. Place the plate on ice while setting up the PCR cycling program below.

d. Place one plate in your real-time thermal cycler. If recommended by your instrument's user manual, use a compression pad with the optical film-sealed plate formats.

e. Enter and run the appropriate program for your real-time instrument (below). If prompted by your instrument software, select "Absolute Quantitation" to begin.

Use a **two-step cycling program** for the following instrumentation:

	Cycles	Duration	Temperature
ABI: 5700, 7000, 7300, 7500, 7700, 7900HT, ViiA 7, StepOnePlus **Bio-Rad**: iCycler, iQ5, MyiQ, MyiQ2, CFX96*, CFX384*, Opticon (MJ Research), Opticon 2 (MJ Research), Chromo 4 (MJ Research) **Eppendorf***: Mastercycler ep realplex 2, 2S, 4, 4S **Stratagene**: Mx3000p, Mx3005p, Mx4000p **Takara**: TP-800	1	10 minutes[1]	95°C
	40	15 seconds	95°C
		1 minute[2]	60°C
	1	Dissociation/Melt Curve Analysis (See Below)	

****Attention*** Bio-Rad CFX96 & CFX384 Users:

- **Adjust the ramp rate to 1°C/sec.**

*****Attention*** Eppendorf Mastercyler ep realplex 2, 2S, 4, and 4S Users:

- **For the Silver Thermoblock: Adjust the ramp rate to 26%.**
- **For the Aluminum Thermoblock: Adjust the ramp rate to 35%.**
- Please refer to the Instrument Setup Guide at **http://sabiosciences.com/pcrarrayprotocolfiles.php** for detailed setup instructions.

Use an **extended two-step cycling program** for the following instrumentation:

	Cycles	Duration	Temperature
Roche***: LightCycler 480	1	10 minutes[1]	95°C
	45	15 seconds	95°C
		1 minute[2]	60°C
	1	Disassociation/ Melt Curve Analysis (See Below)	

******Attention*** Roche LightCycler 480 Users: **Adjust the ramp rate to 1°C/sec.** Please refer to the Instrument Setup Guide at **http://sabiosciences.com/pcrarrayprotocolfiles.php** for more information on other **REQUIRED** changes to settings for Melt Curve Acquisition.

NOTE: *For additional help with instrument setup, see our Instrument-Specific Setup Instructions and Protocol Files at:* ***www.SABiosciences.com/pcrarrayprotocolfiles.php***

5. Dissociation (Melting) Curve

For instrument-specific melt curve analysis settings, please refer to the corresponding Instrument Setup Guide for your instrument at:

http://sabiosciences.com/pcrarrayprotocolfiles.php

General Protocol:

Run a melting curve program immediately after the above cycling program, and generate a first derivative dissociation curve for each well in the entire plate using your instrument's software. No more than one peak should appear in each reaction at temperatures greater than 80°C. If your instrument does not have a default melting curve program, run the following program instead:

95°C, 1 min; 65°C, 2 min (OPTICS OFF); 65°C to 95°C at 2°C / min (OPTICS ON).

NOTE: If you decide not to obtain the dissociation curve immediately, save the plates wrapped in aluminum foil at -20°C as is, in case you need to perform this operation at a later point in time for troubleshooting purposes. When ready, simply warm the plate to room temperature, place it into your real-time instrument, and run the melting program described above.

NOTE: *Be sure to visually inspect the plate after the run for any signs of evaporation from any of the wells. If evaporation is observed, make a note of which wells so that you may qualify your data analysis appropriately.*

NOTE: *DO NOT open any previously run and stored PCR Array plate. Removing the thin-wall 8-cap strips or the adhesive film from PCR Arrays releases PCR product DNA into the air where it will contaminate and confound the results of future real-time PCR experiments.*
See also the Note on "Preparing a Workspace Free of DNA Contamination

C. Data Analysis:

Using the software on your qPCR Instrument, you will need to define the baseline and set the threshold before exporting your raw Ct values:

1.) Calculate the threshold cycle (C_t) for each well using the instrument's software.

NOTE: For **Roche LightCycler 480** Users, there are two options available to analyze your data.

1. Use the second derivate maximum setting & there is no need to set a threshold, **OR**
2. Use "Fit Points" and follow instruction ii below.

i. **To define the Baseline**, choose the Automated Baseline option if your instrument has the Adaptive Baseline Function (check with instrument manual or manufacturer, if unsure). If it does not have the adaptive baseline function, you will need to set the baseline manually. Use the Linear View of the amplification plots to determine the earliest visible amplification. Set the instrument to use the readings from cycle number two (2) through two (2) cycles before the earliest visible amplification, but no more than cycle 15. The earliest amplifications usually will be visible between cycles 14 and 18.

ii. **Manually define the Threshold Value** by using the Log View of the amplification plots and place it above the background signal but within the lower one-third to lower one-half of the linear phase of the amplification plot.

***IMPORTANT:** Ensure that the thresholds are the same across all PCR Array runs in the same analysis. The absolute position of the threshold is less critical than its consistent position across arrays. If the RNA sample quality has been adequately controlled, the cycling program has been executed properly, and the thresholds have been defined correctly, then the value of C_t^{PPC} should be 20 ± 2 across all of your arrays or samples. If not, see the Troubleshooting and FAQ section.

iii. Export the resulting threshold cycle values for all wells to a blank Excel spreadsheet for use with the SABiosciences qBiomarker PCR Array Data Analysis Excel Template.

The Data Analysis Template for each qBiomarker PCR Array can be downloaded from SABiosciences at:
http://www.sabiosciences.com/qBiomarkeriPSC.php/DataAnalysis

Data Analysis Templates automatically perform the calculations and interpretation of the control wells, as well as the biological results, after importing the raw threshold cycle values (C_t) from your qPCR instrument. The templates will automatically calculate the fold change values for the genes on your qBiomarker PCR Array, as well as interpret the genomic DNA contamination assay (GDC) the reverse transcription control (RTC) and the pcr control (PPC). In addition, the gene signature will be interpreted as a predictive biomarker signature which is used to assign experimental results to your samples.

Instructions for Data Analysis:

1. Download the appropriate Data Analysis Template based on the catalog number of the qBiomarker PCR Array. Open the template. The templates can be found at: (**http://www.sabiosciences.com/ipscpcrarraydataanalysis.php)**

2. After defining the baseline and setting the threshold, import the raw Ct values into the Instructions Tab of the template. This can be accomplished by simply "cutting and pasting" the values from your qPCR instrument or the exported results file. Enter the corresponding Ct values according to the correct position on the qBiomarker PCR Array starting with well A1. The template will automatically convert empty wells and Ct values greater than 35 to a value of 35.

3. Click on the QC&Results Tab. The Data Quality for the sample is interpreted by analyzing the GDC, RTC and PPC controls. If any of the results indicate "INQUIRY" please see the Troubleshooting and FAQ section on page 27.

The GDC, RTC and PPC Assays are automatically calculated. Below is an interpretation of the results:

a. Genomic DNA Control (GDC):
   i. If the value is greater than 35, then the level of genomic DNA contamination is too low to affect gene expression profiling results. No action is needed.
   ii. If the value is less than 35, then genomic DNA contamination is evident. See the Troubleshooting and FAQ section (page 27).

b. Reverse Transcription Control (RTC):
   Any impurities in your RNA sample that affect the reverse transcription of the RT^2 First Strand Kit's built-in external RNA control also affect the reverse transcription of your messages of interest.
   i. Calculate RTC Efficiency: = $\Delta C_t^{RTC} - \Delta C_t^{PPC}$.
   ii. If this value is less than 5, then no inhibition is apparent.
   iii. If this value is greater than 5, then evidence of impurities that inhibited the reverse transcription phase of the procedure is evident. See the Troubleshooting and FAQ section (page 27).

c. Positive PCR Control (PPC):
   Any impurities in your RNA sample that affect the PCR amplification of the positive control also affect the PCR amplification for your messages of interest.
   i. The average PPC value should be 20 ± 2 on each PCR Array and should not vary by more than two cycles between samples on the same qBiomarker PCR Arrays being compared.
   ii. Larger differences in average C_t^{PPC} values between samples indicate the presence of different amounts of PCR amplification inhibitors in each sample and that all of the RNA samples require further purification. See the Troubleshooting and FAQ section (page 27).

4. Results:
The normalized expression values (ΔCt) are automatically calculated using the ΔCt formula $\Delta Ct = Ct_{GOI} - Ct_{HKG}$. These values are then used against index scores that were experimentally obtained and used to predict the biological outcome of the experiment.

5. Data Presentation Tab
The Data Presentation Tab will create custom graphs for comparing the experimental samples to one control sample. Simply select the control sample using the "Control button" and all of the results will be compared to this reference samples. Two different graphs are provided, one that compares normalized gene expression values (ΔCt) for each sample and one that compares normalized expression of the biomarker genes (ΔCt) from different samples.

6. Additional Graphs Tab
This tab has a series of graphs showing the Fold Change (ΔΔCt) between the selected reference sample and the rest of the samples.

7. Calculations Tab
This tab shows the calculations that were used to generate the graphs and results.

NOTE: Detailed Mathematical Explanation of $\Delta\Delta C_t$ Data Analysis Method

Due to the inverse proportional relationship between the threshold cycle (C_t) and the original gene expression level, and the doubling of the amount of product with 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:

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

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:

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

The complete calculation is as follows:

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

VI. Troubleshooting and FAQs

A. Troubleshooting:

1. Removal of Genomic DNA Contamination:

You must perform the on-column DNase treatment step included in the protocol of QIAGEN's RNeasy Mini Kit (Catalog # 74104). You must also then use the RT^2 First Strand Kit (Catalog # 330401) with its genomic DNA elimination step.

If the genomic DNA contamination proves difficult to remove, fold-changes in gene expression may still be obtained. However, it will then be very important to verify any results for individual genes by a separate more rigorous real-time PCR analysis that includes a "minus RT" control.

Apparent genomic DNA contamination may also indicate evidence of more general DNA contamination of other reagents, tips, and tubes. See the Note about Preparing a Workspace Free of DNA Contamination at the beginning of the protocol in this User Manual. The No Template Control (NTC) in the RT^2 RNA QC PCR Array provides a sense of how well your technique minimizes the introduction of general DNA contamination into your assay system.

2. Improving Poor Reverse Transcription Efficiency:

Double-check the A260:A280 and A260:A230 ratios of your RNA samples and be sure to perform the dilutions for spectrophotometry in RNase-free Tris pH 8.0 buffer. If necessary, re-purify your RNA samples using the QIAGEN RNeasy Kit.

3. Improving Poor PCR Amplification Efficiency:

Different instruments have different levels of sensitivity. If an average C_t^{PPC} value of 20 ± 2 is difficult to obtain for your instrument, the observed average C_t^{PPC} value should be acceptable as long as it does not vary by more than two cycles between PCR Arrays being compared.

Be sure that the initial heat activation step at 95°C had been lengthened to 10 minutes from the shorter time in the default program. Be sure that all other cycle parameters also have been correctly entered according to the recommendations in this User Manual. Also, double check the quality of your RNA as described in "Evidence of Poor Reverse Transcription Efficiency" above.

B. Frequently Asked Questions:

1. Will pipetting error affect the PCR Array results?

Inaccurate pipeting errors can have effects on the RTC Ct value and the ratio between the RTC and PPC assays. Be sure to use calibrated pipets and the most accurate pipet for the volumes you are dispensing.

2. How can I prevent the evaporation of reaction volume from the wells?

Be sure to carefully and completely seal the PCR Array with the optical thin-wall 8-cap strips or the optical adhesive film before placing it into your thermal cycler. Also, be sure to use a compression pad if directed by the manufacturer of your real-time PCR instrument.

3. Can I use cDNA synthesized from a first-strand kit from another manufacturer?

If your cDNA template is not synthesized using an RT^2 First Strand Kit, cDNA from 500 ng to 5 µg total RNA may be used. Adjust the H_2O amount for a final reaction volume of 25.0 µl per reaction. However, the RTC Assay will not be detected, and the efficiency of the reverse transcription reaction can not be evaluated between different samples.

4. Do I need to prepare my reactions or pre-mix on ice?

You can prepare your reactions at room temperature. Since the qBiomarker Mastermixes include a Hot-Start DNA Polymerase that is active only upon heat activation, you can be assured that no non-specific amplification results will be produced.

Please check our website for a more complete listing of Frequently Asked Questions (FAQs) (www.SABiosciences.com), or call our Technical Support Representatives at 1-888-503-3187 or 301-682-9200.

Ordering Information

Product	Contents	Cat. no.
qBiomarker Screening PCR Array	PCR array for screening and validating pluripotency biomarkers	Varies
qBiomarker Validation PCR Array	PCR array for validating lineage-specific differentiation biomarkers	Varies

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Notes

Trademarks: QIAGEN®, RNeasy® (QIAGEN Group); SYBR® (Molecular Probes, Inc.); Roche®, LightCycler® (Roche Group); ROX™, StepOnePlus™, ViiA™ (Applera Corporation or its subsidiaries); Eppendorf®, Mastercycler® (Eppendorf AG); Stratagene®, Mx3005P®, Mx3000P®, Mx4000® (Stratagene); Bio-Rad®, iCycler®, Chromo4™, CFX96™, DNA Engine Opticon®, CFX384™, iQ™5, MyiQ™ (Bio-Rad Laboratories, Inc.); SmartCycler® (Cepheid); LabChip® (Caliper Technologies Corp.); DNA-free™ (Ambion, Inc.).

Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the qBiomarker PCR Array to the following terms:

1. The qBiomarker PCR Array may be used solely in accordance with the *qBiomarker PCR Array Handbook* and for use with components contained in the Kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this Kit with any components not included within this Kit except as described in the *qBiomarker PCR Array Handbook* and additional protocols available at www.qiagen.com.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this Kit and/or its use(s) do not infringe the rights of third-parties.
3. This Kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the Kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the Kit and/or its components.

For updated license terms, see www.qiagen.com.

NOTICE TO PURCHASERS

Use of kit components for reproduction of any primer pair mix, to modify kit components for resale or to use qBiomarker PCR Arrays to manufacture commercial products without written approval of SABiosciences Corporation is expressly prohibited.

PRODUCT WARRANTY

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