



ExProfile™ Gene qPCR Arrays

For high-throughput profiling of coding-gene expression

User Manual

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USER MANUAL

ExProfile™ Gene qPCR Array

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I. Introduction

The ExProfile™ Gene qPCR Arrays are designed for profiling the expressions of pre-defined or customized sets of coding-genes in various tissues or cells. The differential expressions of profiled genes help researchers to identify and/or validate those that are biologically significant and important for their research.

For catalog arrays, each 96-well plate contains up to 84 pairs of qPCR primers (each 384-well plate contains up to 360 pairs of qPCR primers), which have been pre-validated and coated in designated wells. In the same plate, there are 12 wells in 96-well plate (or 24 wells in 384-well plate) that contain different types of controls for monitoring the efficiency of the entire experimental process: from reverse transcription to qPCR reaction.

The All-in-One™ First-Strand cDNA Synthesis Kits (AORT-0020, AORT-0060) and the SYBR® Green-based All-in-One™ qPCR Mix (AOPR-0200, AOPR-1000, AOPR-4000) are the designed reagents for use with the ExProfile gene qPCR arrays. These reagents have been optimized to produce high sensitivity, efficiency, and specificity. Similar reagents from third-party vendors may be compatible with the arrays but not recommended.

Key advantages

- **Validated primers**
Each pair of primers for a specific gene is designed using a proprietary algorithm and has been experimentally validated.
- **Robust performance**
 - Sensitive** – Detects as low as 4 copies of RNA using ExProfile gene qPCR array and recommended reagents/conditions.
 - Broad linearity** – Simultaneously detects mRNAs at different expression levels.
 - Reproducible** – High reproducibility ($R^2 > 0.99$) for inter-array and intra-array replicates.
- **Genome-wide coverage for large selection of catalog and custom arrays**
Catalog arrays for pathway analysis, cancer research and other focused studies
Customized arrays for researcher-selected gene groups
- **Flexible compatibility**
Arrays are available in multiple plate formats to ensure compatibility with most commercial RT-PCR instruments.

Convenient data analysis

Developed specially for ExProfile arrays, a data analysis tool is available for convenient data processing and statistical analysis.

Protocol overview

A. Prepare cDNA from your RNA Samples

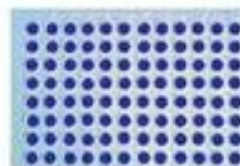
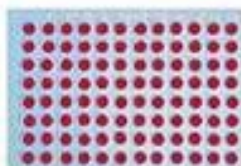


sample cDNA

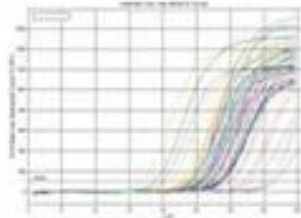
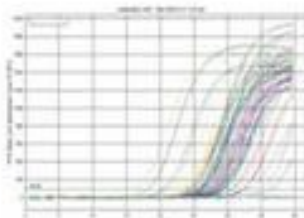


control cDNA

B. Add qPCR Mix and cDNA to the qPCR Array Plate



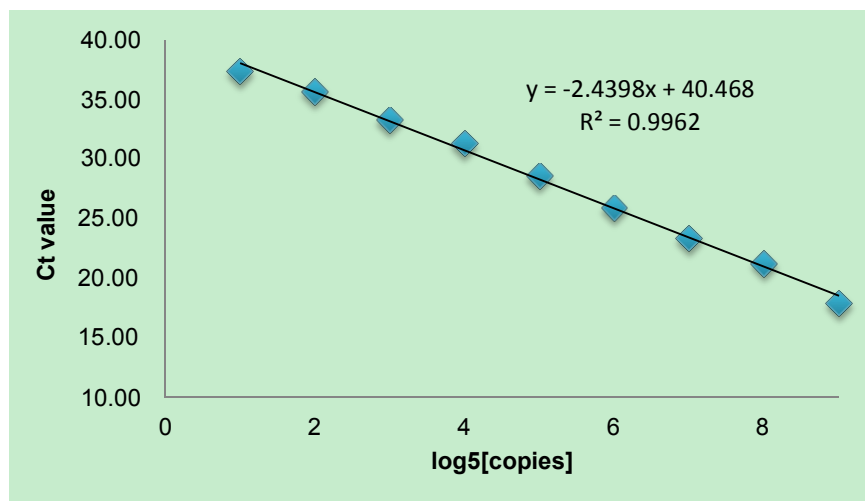
C. Perform real-time PCR



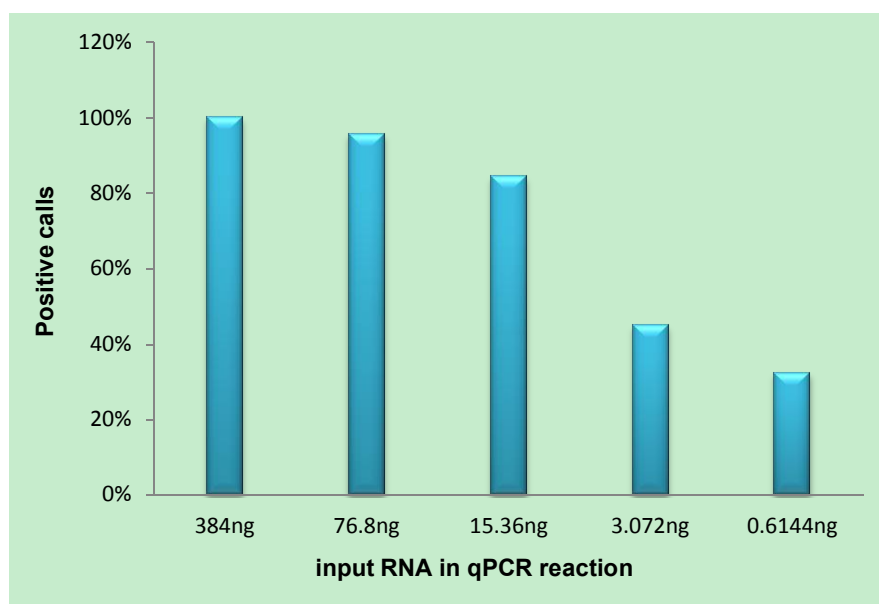
D. Analyze the qPCR Results with GeneCopia's Online Data Analysis System

| qPCR Array Catalog# | | Hm19WG-07 | Homo Brain Array Data (Sample) | | | Homo Liver Array Data (Control) | | | Fold Change | T test |
|---------------------|--------------|----------------|--------------------------------|------------|------------|---------------------------------|------------|------------|--------------------|----------|
| No. | Accession# | miRNA ID | Ct Value-1 | Ct Value-2 | Ct Value-3 | Ct Value-1 | Ct Value-2 | Ct Value-3 | 2 ^{-ΔΔCt} | P Value |
| 1 | MIMAT0004982 | hsa-miR-929 | 26.27 | 26.00 | 26.32 | 25.79 | 26.06 | 26.28 | 112.49 | 1.31E-05 |
| 2 | MIMAT0004993 | hsa-miR-687 | 27.14 | 27.15 | 27.30 | 26.84 | 26.78 | 26.95 | 98.55 | 3.97E-06 |
| 3 | MIMAT0004994 | hsa-miR-425-5p | 32.75 | 31.59 | 31.55 | 33.93 | 34.02 | 33.87 | 22.32 | 5.57E-05 |
| 4 | MIMAT0004636 | hsa-miR-323-5p | 20.12 | 20.05 | 20.82 | 27.49 | 27.81 | 27.59 | 1154.03 | 4.69E-06 |
| 5 | MIMAT0003324 | hsa-miR-481 | 27.08 | 27.25 | 27.12 | 31.49 | 31.69 | 31.05 | 156.93 | 6.99E-05 |
| 6 | MIMAT0004983 | hsa-miR-940 | 18.95 | 19.02 | 18.89 | 19.12 | 18.95 | 19.02 | 8.27 | 1.51E-04 |
| 7 | MIMAT0003304 | hsa-miR-436 | 20.40 | 20.39 | 20.45 | 24.97 | 24.79 | 25.11 | 182.40 | 1.49E-04 |
| 8 | MIMAT0005887 | hsa-miR-648b | 27.41 | 27.39 | 27.42 | 22.80 | 22.48 | 22.59 | 8.28 | 1.81E-03 |
| 9 | MIMAT0002179 | hsa-miR-407a | 22.93 | 22.94 | 24.81 | 31.83 | 31.56 | 31.76 | 1703.40 | 9.72E-07 |
| 10 | MIMAT0003180 | hsa-miR-487b | 21.42 | 22.81 | 21.89 | 26.77 | 26.89 | 26.59 | 987.94 | 1.18E-05 |
| 11 | miR-1 | miR-1 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 1.00 | 0.00 |

Figure 1. Gene qPCR array experiment work flow

Performance data**Linear Range and Sensitivity (spike-in control RNA)****Figure 2. Broad linear range and high sensitivity**

Mouse total RNA with serially diluted Spike-in control RNA were reverse-transcribed using All-in-One first strand cDNA synthesis kit. The reverse-transcribed cDNA samples were detected using All-in-One qPCR mix and spike-in control specific primers deposited in a 96-well plate. The resulting Ct values were plotted against the log₅ of the amounts of spike-in control RNA. The data demonstrated a broad linear dynamic range from 4 to 1.6×10^6 copies of input RNA as well as high sensitivity.

**Figure 3. High positive calls with as little as 15.36 ng of total RNA**

Different amounts of MCF_7 total RNA (1000, 200, 40, 8, 1.6ng) were analyzed with the Human Breast Cancer Gene qPCR Array (PAG-HGBE96-01). The percentage of positive calls (Ct < 35) is plotted against the input amount of total RNA in each qPCR reaction.

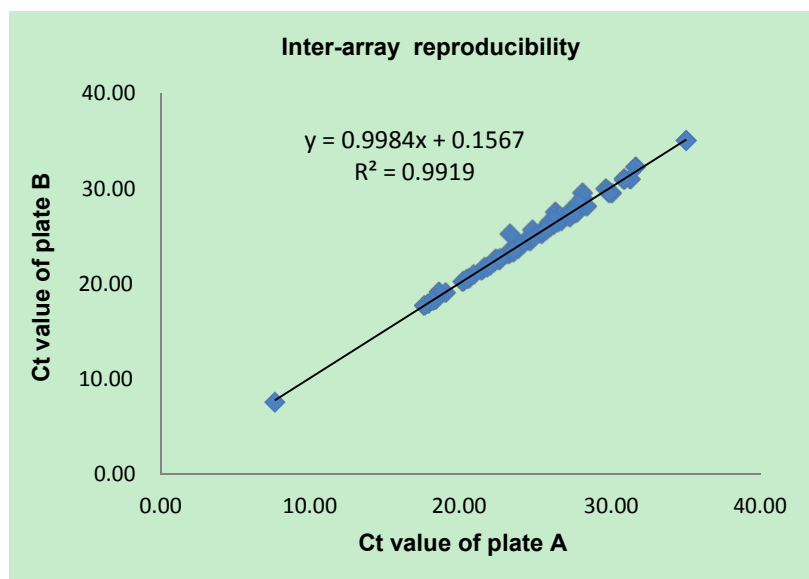


Figure 4. High inter-array reproducibility

Two ExProfile qPCR gene array replicates (plate A and B) were analyzed using human total RNA (10-tissue mix) on the Bio-Rad iQ5. The Ct values of the replicate plates were plotted against each other. $R^2 > 0.99$ was observed for high inter-array reproducibility. $R^2 > 0.99$ was also observed for intra-array reproducibility (data not shown).

II. Array Format and Layout

Array format options

Important note: Upon receiving, please check to make sure that the correct array format was ordered to ensure the compatibility with your qPCR instrument. GeneCopoeia provides five qPCR array formats (A, B, C, D, and E) suitable for use with the following real-time cyclers.

| Plate format | Instrument provider | qPCR instrument model |
|------------------------|-----------------------|--|
| A (96-well) | Applied Biosystems | 5700, 7000, 7300, 7500, 7700, 7900HT (Standard 96-well block), ViiA™7 (Standard 96-well block) |
| B (96-well) | Applied Biosystems | 7500 (Fast block), 7900HT (Fast block), StepOnePlus™, ViiA™7 (Fast block) |
| C (96-well) | Bio-Rad Laboratories | iCycler iQ®, MyiQ™, iQ™5 |
| D (96-well) | Bio-Rad Laboratories | CFX96™, DNA Engine Opticon™, DNA Engine Opticon 2™, Chromo4™ |
| E (96-well) | Roche Applied Science | LightCycler® 480 (96-well block) |
| F (384-well) | Applied Biosystems | 7900HT (384-well block), ViiA 7 (384-well block) |
| G (384-well) | Bio-Rad Laboratories | Bio-Rad CFX384™ 384-well |
| H (384-well) | Roche Applied Science | Roche LightCycler 480 (384-well block) |

Catalog Array layout

| | 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 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
| C | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 |
| D | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 |
| E | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 |
| F | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 |
| G | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 |
| H | GDC | GDC | HK1 | HK2 | HK3 | HK4 | HK5 | HK6 | RT | RT | PCR | PCR |

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 |
|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 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 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 |
| C | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 |
| D | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 |
| E | 97 | 98 | 99 | 100 | 101 | 102 | 103 | 104 | 105 | 106 | 107 | 108 | 109 | 110 | 111 | 112 | 113 | 114 | 115 | 116 | 117 | 118 | 119 | 120 |
| F | 121 | 122 | 123 | 124 | 125 | 126 | 127 | 128 | 129 | 130 | 131 | 132 | 133 | 134 | 135 | 136 | 137 | 138 | 139 | 140 | 141 | 142 | 143 | 144 |
| G | 145 | 146 | 147 | 148 | 149 | 150 | 151 | 152 | 153 | 154 | 155 | 156 | 157 | 158 | 159 | 160 | 161 | 162 | 163 | 164 | 165 | 166 | 167 | 168 |
| H | 169 | 170 | 171 | 172 | 173 | 174 | 175 | 176 | 177 | 178 | 179 | 180 | 181 | 182 | 183 | 184 | 185 | 186 | 187 | 188 | 189 | 190 | 191 | 192 |
| I | 193 | 194 | 195 | 196 | 197 | 198 | 199 | 200 | 201 | 202 | 203 | 204 | 205 | 206 | 207 | 208 | 209 | 210 | 211 | 212 | 213 | 214 | 215 | 216 |
| J | 217 | 218 | 219 | 220 | 221 | 222 | 223 | 224 | 225 | 226 | 227 | 228 | 229 | 230 | 231 | 232 | 233 | 234 | 235 | 236 | 237 | 238 | 239 | 240 |
| K | 241 | 242 | 243 | 244 | 245 | 246 | 247 | 248 | 249 | 250 | 251 | 252 | 253 | 254 | 255 | 256 | 257 | 258 | 259 | 260 | 261 | 262 | 263 | 264 |
| L | 265 | 266 | 267 | 268 | 269 | 270 | 271 | 272 | 273 | 274 | 275 | 276 | 277 | 278 | 279 | 280 | 281 | 282 | 283 | 284 | 285 | 286 | 287 | 288 |
| M | 289 | 290 | 291 | 292 | 293 | 294 | 295 | 296 | 297 | 298 | 299 | 300 | 301 | 302 | 303 | 304 | 305 | 306 | 307 | 308 | 309 | 310 | 311 | 312 |
| N | 313 | 314 | 315 | 316 | 317 | 318 | 319 | 320 | 321 | 322 | 323 | 324 | 325 | 326 | 327 | 328 | 329 | 330 | 331 | 332 | 333 | 334 | 335 | 336 |
| O | 337 | 338 | 339 | 340 | 341 | 342 | 343 | 344 | 345 | 346 | 347 | 348 | 349 | 350 | 351 | 352 | 353 | 354 | 355 | 356 | 357 | 358 | 359 | 360 |
| P | GDC | GDC | GDC | GDC | HK1 | HK1 | HK2 | HK2 | HK3 | HK3 | HK4 | HK4 | HK5 | HK5 | HK6 | HK6 | RT | RT | RT | RT | PCR | PCR | PCR | PCR |

B

Figure 5. Illustration of ExProfile™ gene qPCR array layout (A: 96-well plate, B: 384-well plate)

- **RNA primer pairs:** Wells 1-84 are designated for a real-time PCR assay for genes.
- **HK1-6:** Six pre-deposited housekeeping gene (HK1-6) primer pairs, which can be used as endogenous positive controls as well as for array normalization.
- **GDC:** Genomic DNA Controls, which can be used to specifically detect genomic DNA contamination with a high level of sensitivity.
- **RT:** Spike-in RNA reverse transcription controls, which can be used to monitor the efficiency of the RT reactions. These pre-deposited primer pairs specifically amplify the cDNA template reversed transcribed from the spike-in exogenous RNA in the sample.
- **PCR:** Positive PCR controls, which are used to verify the PCR efficiency by amplifying the pre-deposited DNA template with its specific pre-deposited primer pairs.

III. Arrays and Reagents

Catalog arrays

For a complete list of catalog arrays, please see Appendix II or visit following webpage.

<http://www.genecopoeia.com/product/gene-qpcr-array/>.

RT-PCR and RNA extraction reagents (sold separately)

| Cat. No. | Products | Quantity/set | Shipping and storage condition |
|-------------------------------------|---|---|--|
| AORT-0020 AORT-0060 | All-in-One™ first-strand cDNA synthesis kit | 20 reactions 60 reactions | Shipped with dry ice. Store at –20°C (Stable for at least 12 months).Alternatively, store at –80°C in aliquots. Avoid repeated freezing/ thawing. |
| AOPR-0200 AOPR-1000 AOPR-4000 | All-in-One qPCR mix | 200 reactions 1000 reactions 4000 reactions | Shipped with dry ice. Store at –20°C (Stable for at least 12 months).Alternatively, store at –80°C in aliquots. Avoid repeated freezing/ thawing. |
| E01010A | RNAzol® RT RNA isolation reagent | 50 ml | Shipped at room temperature. Stable for at least two years when stored at room temperature. |

Other materials required but not provided

Total RNA extraction kit (RNAzol® RT RNA extraction reagent is recommended)
DNase/RNase free tips, PCR reaction tubes, 1.5 ml microcentrifuge tubes
5 ml and 10 ml graduated pipettes, beakers, flasks, and cylinders
10 µl to 1,000 µl adjustable single channel micropipettes with disposable tips
5 µl to 20 µl adjustable multichannel micropipette, disposable tips, and reservoir
qPCR instrument, compatible with gene qPCR arrays ordered

IV. Preparation

Important notes

- Before use, remove any condensation that has accumulated on the plate sealing surface and centrifuge plates briefly to collect the contents to the bottom of the plate wells.
- Strictly follow the standard procedures for qPCR to avoid nucleic acid contamination and non-specific amplifications.
- Read the instructions thoroughly before attempting to perform the procedures.

Estimates of RNA and number of RT-PCR reactions required for EACH SAMPLE

| Array format | Number of plates per sample | Total RNA recommended per sample | Number of RT reactions per sample | Number of qPCR reactions per sample |
|----------------|-----------------------------|----------------------------------|-----------------------------------|-------------------------------------|
| 96-well plate | 2 | 0.04-2 µg | 1 | 220 |
| | 5 | 0.12-6 µg | 3 | 550 |
| | 10 | 0.2-10 µg | 5 | 1,100 |
| | 20 | 0.4-20 µg | 10 | 2,200 |
| | 40 | 0.8-40 µg | 20 | 4,400 |
| 384-well plate | 1 | 0.08-4 µg | 2 | 450 |
| | 2 | 0.16-8 µg | 4 | 900 |
| | 5 | 0.4-20 µg | 10 | 2,250 |
| | 10 | 0.8-40 µg | 20 | 4,500 |

RNA quantification and quality control

1. Dilute the RNA sample with the RNase-free water and measure the absorbance at 260 nm and 280 nm. A260/280 should be greater than 1.8.
2. Use the formula $A_{260} \times \text{dilution} \times 40 = \mu\text{g RNA/mL}$ to determine the RNA concentration.
3. Check the RNA integrity by agarose electrophoresis.

Genomic DNA contamination control

The Genomic DNA Control (GDC) in each ExProfile gene qPCR array specifically tests for genomic DNA contamination in each sample during each qPCR performance. A Ct value of genomic DNA control less than 35 indicates the presence of a detectable amount of genomic DNA contamination that should be addressed. So it is necessary to remove genomic DNA and all residual contamination from your RNA samples.

V. Procedure**First-strand cDNA synthesis**

Note: High-quality cDNA is a prerequisite for accurate detection of gene expression. It is important to remove genomic DNA and all residual contamination from your RNA samples before using our All-in-One First Strand cDNA Synthesis Kit.

1. Thaw all reverse transcription reagents from the First Strand cDNA Synthesis Kit. Mix reagents well by gently inverting the tubes. Centrifuge briefly and place on ice.
2. Prepare the RNA–primer mix: Add the following reagents to an RNase-free reaction tube that has been placed on ice. The final volume is 13µl.

| Component | Volume | Final concentration |
|--------------------------------------|----------------|---------------------|
| Total RNA | | 1 µg ^a |
| Spike-in control RNA | 1 µl | 0.08 ng |
| Random primer (250 µM) | 1 µl | 10 µM ^b |
| Oligo (dT) ₁₈ (60 µM) | 1 µl | 2.4 µM |
| ddH ₂ O(RNase/DNase free) | to final 13 µl | |

- a. The final concentration given is recommended. Each reaction can contain 10ng to 5µg total RNA. Low-abundance RNA may not be detected when using less than 10ng total RNA.
- b. Using both the Random Primer and the Oligo(dT)₁₈ primer is recommended for optimal reaction efficiency.

3. Mix thoroughly and centrifuge briefly. Heat the RNA–primer mix (from step 2) at 65°C for 10 minutes to denature the RNA. After incubation, cool immediately on ice then centrifuge briefly.
4. Prepare the reverse-transcription master mix, keeping the tubes (from step 3) on ice while working. The final volume is 25µl.

| Component | Volume | Final concentration |
|---------------------------------------|---------------|---------------------|
| RNA–primer mix | 13µl | |
| 5 × RT Reaction Buffer | 5µl | 1× |
| dNTP (25mM) | 1µl | 1mM |
| RNase Inhibitor(25 U/µl) | 1µl | 1U/µl |
| M-MLV RTase(200U/µl) | 1µl | 8U/µl |
| ddH ₂ O (RNase/DNase free) | to final 25µl | |

5. Prepare the reverse transcription reaction by adding the reverse-transcription master mix to the RNA–primer mixture. Mix thoroughly and centrifuge briefly. Incubate at 37°C for 60 minutes.
6. Terminate the reaction by heating at 85°C for 5 minutes.
7. Add 225µl sterile water to each 25µl of reverse transcription reaction (1:10dilution). Mix thoroughly and centrifuge briefly.

qPCR reaction

Note: Be sure the ExProfile gene qPCR Array plate is compatible with your qPCR instrument before beginning this protocol.

1. Thaw the reagents of All-in-One qPCR Mix Kit. Invert the tubes to mix gently but thoroughly. Briefly centrifuge to bring the contents to the bottom of the tubes and then place them on ice.
2. Remove any condensation that has accumulated on the plate sealing surface and centrifuge briefly to collect the contents to the bottom of the plate wells. Carefully remove sealing film before use 96-Well qPCR.
3. Prepare qPCR solution on ice

| Components | 1 well | N well ^a |
|-------------------------------------|--------|---------------------|
| 2×All-in-One qPCR Mix | 10µl | 11µl × N |
| cDNA (10 times dilution) | 1µl | 1.1µl × N |
| 50 X Rox Reference Dye ^b | 0.4µl | 0.44µl × N |
| ddH ₂ O | | |
| ■ Not using Rox Reference Dye | 9µl | 9.9µl × N |
| ■ Using Rox Reference Dye | 8.6µl | 9.5µl × N |
| Final Volume | 20µl | 22µl × N |

- a. The ExProfile gene qPCR array is used to detect multiple genes simultaneously in the same sample. Ensure sufficient mix is available by preparing enough for the number of reactions to be used with a 10% additional volume for pipetting loss.
- b. 50×Rox Reference Dye is added only for qPCR instruments that require ROX for calibration.

4. Mix the qPCR solution thoroughly and centrifuge briefly. Accurately transfer exactly 20 µl reaction mix to each well. Change tips after each transfer to avoid cross-contamination.
5. Tightly seal the qPCR reaction plate with a new sealing film, Ensure that the film seals smoothly to prevent refraction of light, and tightly to prevent from evaporation loss. Centrifuge briefly in order to remove bubbles.
6. Run qPCR. The following three-step PCR program is recommended for running qPCR.

| Cycles | Steps | Temperature | Duration | Detection |
|--------|----------------------|-------------------|--------------------|-----------|
| 1 | Initial denaturation | 95°C | 10min ^a | No |
| 40 | Denaturation | 95°C | 10sec. | No |
| | Annealing | 60°C ^b | 20 sec. | No |
| | Extension | 72°C ^c | 15 sec. | Yes |

- a. The DNA polymerase used in the 2X All-in-One qPCR Mix is a special chemically modified hot-start enzyme. The indicated initial denaturation is sufficient to activate the enzyme.
- b. The annealing temperature of the cross-linked primer is 60°C when using the optimized All-in-One qPCR Mix.
- c. The extension time indicated above is suitable for Bio-Rad's iQ5 real-time PCR instrument. Adjust the time duration according to the documentation provided with your instrument.

When using SYBR Green dye to monitor the qPCR reaction, a melting curve analysis should be performed immediately after qPCR cycling.

| Temperature range | Heating rate | Constant temperature | Detection |
|-------------------|-----------------|----------------------|-----------|
| 72°C~95°C | 0.5°C/unit time | 10sec./unit time | Yes |
| 25°C | | 30 sec | No |

VI. Data Analysis

1. Define the baseline
The baseline is the noise level in early cycles. Each real-time PCR instrument has algorithms to perform the baseline-setting. This may be a fixed number of cycles for all samples or adaptive for each sample, depending on the type of instrument that is being used. If the lowest Ct is less than the upper limit of the baseline setting, then the baseline should be manually adjusted. Use the "Linear View" of the amplification plot to determine the earliest visible amplification, and then set the baseline from cycle 2 to two cycles before the earliest visible amplification. Normally it is between 2 to 10 cycles. Do not use cycles greater than 15.
Ensure that baseline settings are the same across all PCR runs in the same analysis to allow comparison of results.
2. Set threshold
Correct placement of the threshold is the next crucial step in data analysis. To adjust the threshold properly, set the threshold value within the exponential phase of all amplification plots when viewed using the logarithmic scale for the y axis. Generally, the expression level of each reference gene should be higher than most other genes.
3. Obtain the Ct or Cp values
The Ct is defined as the cycle when sample fluorescence exceeds a chosen threshold above background fluorescence. This is also known as the Cp or crossing point.
4. Export the data. Most qPCR instruments provide a function for exporting Ct or Cp values to Excel.
5. Analyze the qPCR results using the $\Delta\Delta C_t$ method of relative quantification and interpretation of the control wells.
6. All Ct values reported as greater than 35 or as N/A (not detected) are considered as not detectable.

QC analysis

1. Examined amplification and melting status of each gene using the qPCR instrument software. Each reference gene, RT control and PCR control should exhibit only one melting peak per reaction.
2. Examined CT values of the genomic DNA contamination wells (GDC).
A CT values of genomic DNA control should be more than **35**, which indicate a little of genomic DNA contamination can not effect the real-time gene expression profiling result.
3. Examined CT values of the RT control and positive PCR control wells (PCR).
If the RNA sample is of high quality, the cycling program has been correctly run, and the thresholds have been correctly defined, the value of Ct of RT control should be **20±3**, and the value of Ct of PCR should be **20±2** across all arrays or samples.

Data analysis

Analyze the qPCR result with GeneCopia's online Data Analysis System (free), which is available at http://www.genecopia.com/product/gene-qpcr-array/#Analysis_Tool.

This Data Analysis System uses the $\Delta\Delta C_t$ method to perform fold-change analysis or simple statistical analysis of the expression level (C_t or C_p values) for each gene.

1. Download and read the "Primer Array Data Analysis Operation Guide" before performing analysis.

- Import the C_t or C_p values into the corresponding data analysis template form (*Sample Data.xls* and *Control Data.xls*). Upload the template form and choose the correct reference and analysis factors.

Note: The reference factor chosen for qPCR Primer Array for normalization with the $\Delta\Delta C_t$ method must not be influenced by the experimental design. Therefore use one or more factors that have been previously verified experimentally. A single value or an average of the C_t values for the reference factor can be used for normalization.

- Perform the specified analysis. When a test is repeated at least three times, statistical results (p value) are provided. The analysis results allow genes of interest to be simply and rapidly selected for further study.

VII. Appendix I

$\Delta\Delta C_t$ data analysis method

$\Delta\Delta C_t$ data analysis, a relative quantitative analysis technique, is the most simple and direct method for gene expression analyses. The method requires stable expression from a reference gene to normalize the variation introduced by each step, including sample collection, RNA isolation, reverse transcription and amplification. Typically housekeeping genes are used as reference genes.

In qPCR, as in any amplification-based technique, the number of amplification products (N) is calculated as follows:

$$N = N_0 \times (1 + E)^{C_t}$$

N_0 : number of template molecules

C_t : threshold cycle

E: amplification efficiency

When the amplification efficiency E is 100%, the number of template molecules in pre-amplification mix is calculated as follows:

$$N_0 = N \times 2^{-C_t}$$

To analyze the change in expression level for the gene of interest in multiple samples using the $\Delta\Delta C_t$ method, the amount of the amplification template from different samples is normalized by dividing the expression level of the gene of interest (x) with the reference factor (r) as follows:

$$N_{rel} = N_0x/N_0r = N \times 2^{-C_{tx}} / N \times 2^{-C_{tr}} = 2^{-(C_{tx} - C_{tr})} = 2^{-\Delta C_t}$$

The change in normalized expression levels of the gene of interest (x) between experimental sample (sample 1) and the control sample (sample 2) is as follows:

$$N_{rel1}/N_{rel2} = 2^{-\Delta C_{t1}} / 2^{-\Delta C_{t2}} = 2^{-(\Delta C_{t1} - \Delta C_{t2})} = 2^{-\Delta\Delta C_t}$$

The value of $2^{-\Delta\Delta C_t}$ is the change in expression level of the gene of interest between different samples.

VIII. Appendix II

Catalog ExProfile gene qPCR array list

| Cat. No. | qPCR array products | Quantity/set | Shipping and storage condition |
|------------|---|--------------------------------|--|
| PAG-HCAD96 | ExProfile™ Human Adenocarcinoma Gene qPCR Array | 168 genes 2 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |

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| | | | |
|------------|---|--------------------------------|--|
| PAG-HCBA96 | ExProfile™ Human Brain Cancer Gene qPCR Array | 252 genes 3 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HCBE96 | ExProfile™ Human Breast Cancer Gene qPCR Array | 504 genes 6 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HCBL96 | ExProfile™ Human Bladder Cancer Gene qPCR Array | 420 genes 5 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HCCR96 | ExProfile™ Human Colorectal Cancer Gene qPCR Array | 336 genes 4 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HCCV96 | ExProfile™ Human Cervical Cancer Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HCED96 | ExProfile™ Human Endometrial Cancer Gene qPCR Array | 82 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HCNN96 | ExProfile™ Human Head and Neck Cancer Gene qPCR Array | 504 genes 6 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HCKD96 | ExProfile™ Human Kidney Cancer Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HCLK96 | ExProfile™ Human Leukemia Gene qPCR Array | 504 genes 6 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HCLU96 | ExProfile™ Human Lung Cancer Gene qPCR Array | 504 genes 6 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HCLV96 | ExProfile™ Human Liver Cancer Gene qPCR Array | 168 genes 2 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HCLY96 | ExProfile™ Human Lymphoma Cancer Gene qPCR Array | 420 genes 5 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HCML96 | ExProfile™ Human Myeloma Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HCOV96 | ExProfile™ Human Ovarian Cancer Gene qPCR Array | 336 genes 4 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HCPC96 | ExProfile™ Human Pancreatic Cancer Gene qPCR Array | 168 genes 2 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HCPS96 | ExProfile™ Human Prostate Cancer Gene qPCR Array | 412 genes 5 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HCSK96 | ExProfile™ Human Skin Cancer Gene qPCR Array | 252 genes 3 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |

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| PAG-HCSM96 | ExProfile™ Human Stomach Cancer Gene qPCR Array | 168 genes 2 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HCTR96 | ExProfile™ Human Thyroid Cancer Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HCTT96 | ExProfile™ Human Testicular Cancer Gene qPCR Array | 68 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HPCD96 | ExProfile™ Human Cancer Drug Resistance & Metabolism Related Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HPCR96 | ExProfile™ Human Cytokine Receptor Related Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HPEP96 | ExProfile™ Human EGF/PDGF Signaling Related Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HPFT96 | ExProfile™ Human FoxP3 Target Genes qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HPGT96 | ExProfile™ Human Growth and Development Toxicity Related Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HPHI96 | ExProfile™ Human Insulin Signaling Related Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HPIA96 | ExProfile™ Human Innate & Adaptive Immune Response Related Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HPIC96 | ExProfile™ Human Inflammatory Cytokines & Receptors Related Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HPIF96 | ExProfile™ Human Interferon Signaling & Response Related Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HPIL96 | ExProfile™ Human Innate Immune Signaling Related Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HPIN96 | ExProfile™ Human Interferon Related Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HPJS96 | ExProfile™ Human JAK/STAT Signaling Related Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HPKA96 | ExProfile™ Human NFΚΒ Signaling Pathway Related Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HPPK96 | ExProfile™ Human PI3K-AKT Signaling Related Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |

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| PAG-HPRA96 | ExProfile™ Human Inflammatory Response and Autoimmunity Related Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HPST96 | ExProfile™ Human Signal Transduction Related Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HPTB96 | ExProfile™ Human T-cell and B-cell Activation Related Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HPTC96 | ExProfile™ Human Cell Cycle Toxicity and Cancer Related Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HPTG96 | ExProfile™ Human TGF-β Signaling Related Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HPTH96 | ExProfile™ Human Th1-Th2-Th3 Related Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HPTM96 | ExProfile™ Human Tumor Metastasis Related Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-HPTS96 | ExProfile™ Human T helper 17 (Th17) Related Gene qPCR Array | 84 genes 1 x 96-well plate | Shipped at room temperature Stable for at least 6 months when stored at -20°C |
| PAG-CS | ExProfile™ Custom Gene qPCR Arrays | Variable | Shipped at room temperature Stable for at least 6 months when stored at -20°C |

Note: New catalog ExProfile gene qPCR arrays will be continuously added to the product line. Check out <http://www.genecopoeia.com/product/gene-qpcr-array/>

IX. Limited Use License and Warranty

Limited use license

Following terms and conditions apply to use of ExProfile™ Gene qPCR Arrays (the Products). If the terms and conditions are not acceptable, the Product in its entirety must be returned to GeneCopoeia within 5 calendar days. A limited End-User license is granted to the purchaser of the Product. The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged or modified for resale, or used to manufacture commercial products or deliver information obtained in service without prior written consent from GeneCopoeia. This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the Product constitutes acceptance of the above terms.

Limited warranty

GeneCopoeia warrants that the Product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the Product fails to meet these specifications, GeneCopoeia will replace the Product. In the event a replacement cannot be provided, GeneCopoeia will provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to GeneCopoeia within 30 days of receipt of the Product. GeneCopoeia's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. GeneCopoeia's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. GeneCopoeia does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

GeneCopoeia is committed to providing our customers with high-quality products. If you should have any questions or concerns about any GeneCopoeia products, please contact us at 301-762-0888.

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