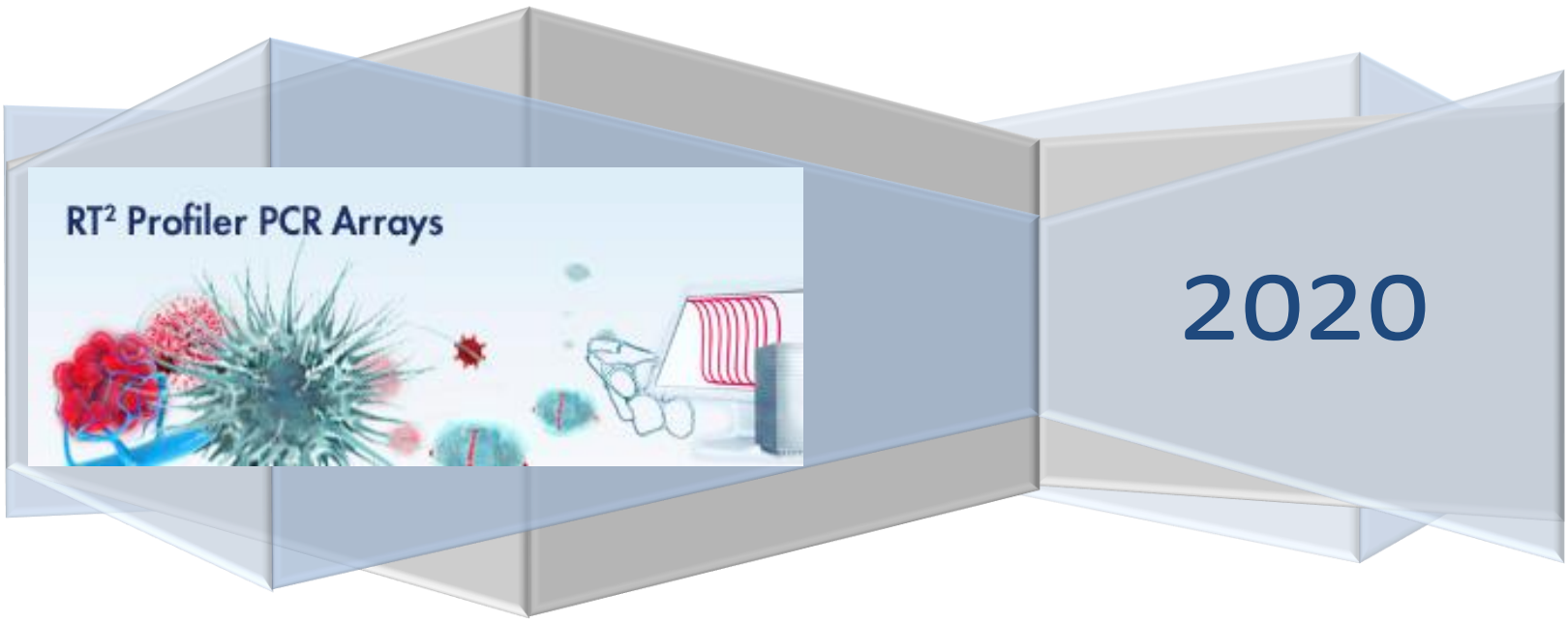
RT-PCR
Array Services

at the Analytical Instrumentation Core

Boston University, AIC, DOM, BMC, BUMC



Analytical Instrumentation Core Mission:

The AIC supports investigators through both cutting-edge analytical instruments and services that facilitate research and education in the Department of Medicine, Boston University School of Medicine, and Boston Medical Center. Our goals are to:

1. Maintain core instruments
2. Train investigators how to use equipment and to help design experiments
3. Share the resources and increase accessibility of instruments for investigators
4. Introduce innovative analytical instrumentation by regularly holding seminars
5. Support investigators in their efforts to win extramural research funding

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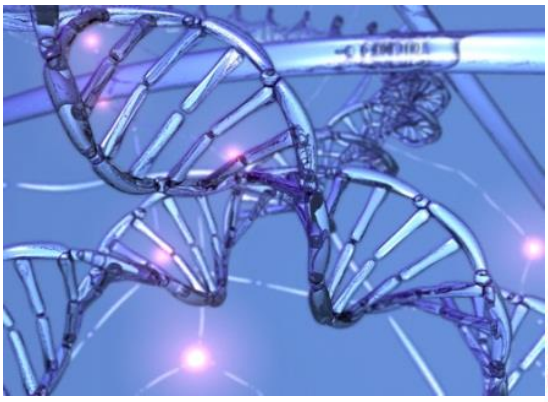
Introduction to Array Services at the AIC:

The Analytical Instrumentation Core offers an Array Service for RT-PCR Arrays to measure gene expression and to identify miRNA. Researchers are able to screen samples against a set of known genes contained on a 384-well PCR microplate (84 genes x 4 replicates + controls). These genes can be related to a disease (such as breast cancer) or a pathway (such as WNT signaling) proposed by literature and bioinformatics studies.

RT-PCR Arrays for gene expression and miRNA identification are useful for:

- Preliminary studies to obtain grants to identify target genes
- Hypothesis driven studies (to verify target genes)
- Verification of high throughput data (to validate microarrays)



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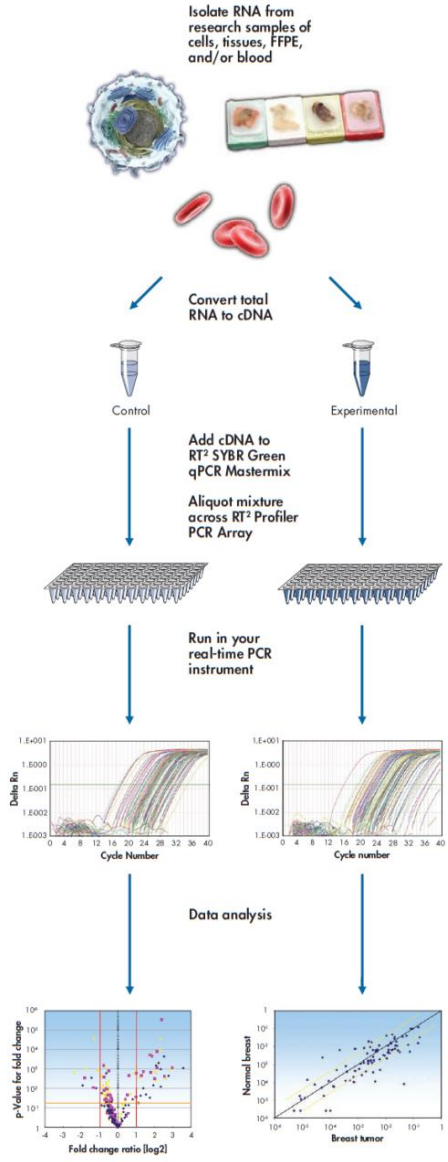
What are PCR Arrays?

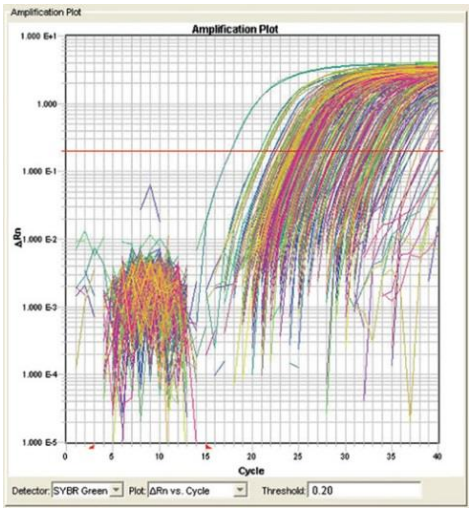
RT-PCR arrays allow for reliable gene expression analysis of a disease or pathway focused set of genes for any sample quantity or type using real-time PCR. Each catalogued PCR array contains 84 genes in a 384-well PCR plate along with 5 housekeeping genes and 7 controls to monitor DNA contamination, successful cDNA synthesis, and RT-PCR efficiency (x 4 replicates).

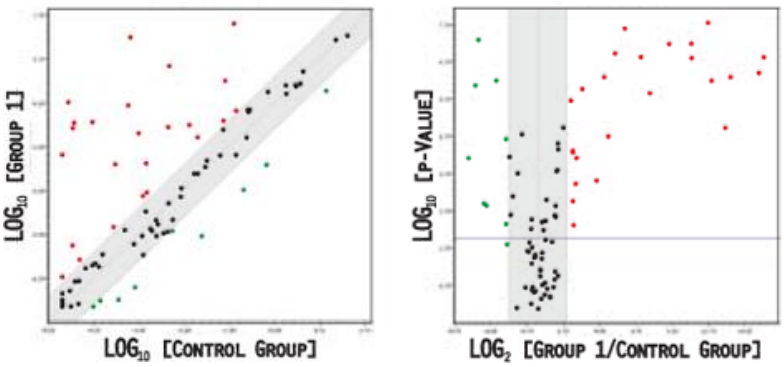
Total RNA first needs to be isolated and purified from samples and converted to cDNA. The cDNA samples are then combined with SYBR Green mastermix and plated into the corresponding wells in the 384-well PCR plate. The plates are run using standard PCR thermal profile settings and raw data is generated in as little as 2 hours. The raw data is then further analyzed and the results are ready to be used for either grant submissions or final publications.



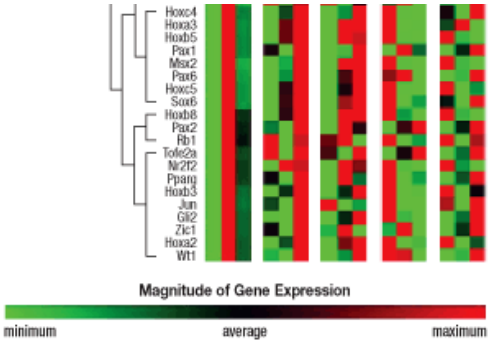
What do the results look like?

Data-analysis software and Excel-based templates allow for quick and easy interpretation of raw RT-PCR. The software identifies up and down-regulated genes and organizes the data into plots and charts which can immediately be integrated into a publication, poster, or presentation.

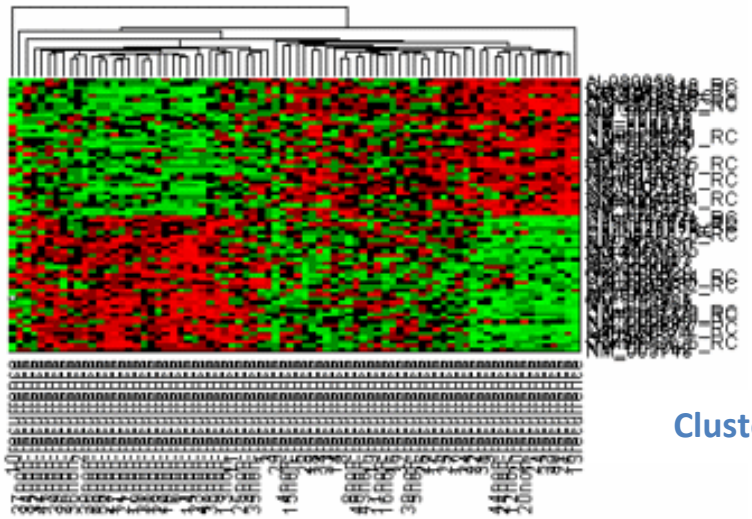




Scatter and Volcano Plots



Heat Maps



Clustergrams

Catalogued Arrays Available

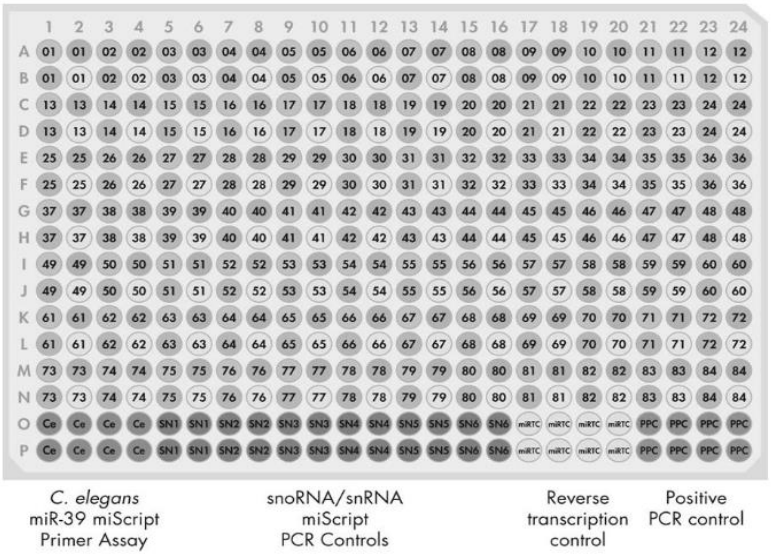
There are over 150 cataloged arrays available for human, rat, and mouse cell lines.

Most Popular Catalogued Arrays:

Inflammatory Cytokines & Receptors
Apoptosis
Extracellular Matrix & Adhesion Molecules
Toll-Like Receptor Signaling Pathway
Innate & Adaptive Immune Response
Oxidative Stress and Antioxidant Defense
Epithelial to Mesenchymal Transition (EMT)
Lipoprotein Signaling & Cholesterol Metabolism

Stress and Toxicity Pathway Finder
WNT Signaling Pathway
Signal Transduction Pathway Finder
Cell Cycle
Angiogenesis
Fibrosis
Mitochondria
Cancer Pathway Finder
Fatty Acid Metabolism

Example of a 384-well RT-PCR Array Plate Format (84 genes x 4 replicates)

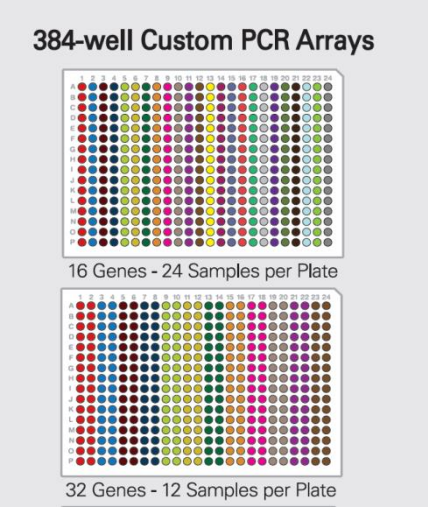


Modified Arrays

Modified Arrays are available in which up to 4 genes can be added or exchanged on any catalogued plate. However, a minimum of 4 plates must be ordered when creating a modified array plate and the cost will be slightly higher.

Custom Arrays

If you have a specific list of targeted genes and want **Custom Arrays**, they are also available, allowing for the analysis of 8 to 384 genes of interest specific to your analysis. Custom Arrays can be created for use with dog, pig, cow, chicken, horse, zebrafish, Chinese hamster, rabbit, rhesus monkey, and drosophilia cell lines. Additionally, once an order has been fulfilled for a custom array, reprints of the same custom array are available at a discounted price. With custom arrays, a minimum of 6 plates must be purchased.



384-well Custom PCR ARRAYS (#330131)	
Format	**Number of Arrays** (minimum)
8 Genes, 48 Samples / Plate 12 Genes, 32 Samples / Plate 16 Genes, 24 Samples / Plate	6
24 Genes, 16 Samples / Plate 32 Genes, 12 Samples / Plate 48 Genes, 8 Samples / Plate 64 Genes, 6 Samples / Plate 96 Genes, 4 Samples / Plate 128 Genes, 3 Samples / Plate	6
192 Genes, 2 Samples / Plate 384 Genes, 1 Sample / Plate	24
All Formats	Per Additional 6 Arrays

Workflow of AIC Array Service

1. Consultation

During the consultation phase, researchers will discuss their research goals and work with the AIC technicians to develop a project overview. The best array options are explored to fit the researcher's needs and sample preparation is discussed. The pricing of the service is determined based on the specific experiment and a research service agreement form is signed by both parties. The AIC then orders the necessary plates and supplies and provides a time estimate of when the project will be completed.

2. Researchers Provide Samples

Samples are typically submitted as cell lines, extracted RNA, or cDNA as prepared by the researcher. However, you may discuss with the AIC the option of submitting other sample type for analysis (such as tissues). All samples must meet biosafety standards for a BSL2 laboratory and any remaining sample not used in the arrays will be returned to the researcher at the end of the experiment.

3. AIC Technicians Prepare Samples

If researchers prefer that the AIC performs the sample preparation, the AIC will extract and purify the RNA from the submitted samples and will synthesize cDNA from the purified RNA. The quality of the RNA and cDNA is determined during the sample prep stage by a NanoQuant Spectrophotometer to ensure the RNA is purified and the cDNA is synthesized successfully.

4. Running of Arrays

The samples are then combined with SYBR Green mastermix and are plated on to the respective wells in the RT-PCR plate. The plates are run on an ABI 7900 HT Fast Real-Time PCR instrument under normal cycling conditions. A melting curve analysis is also performed at the end of each PCR run.

5. Data Analysis

The raw data from the PCR run is then analyzed by the AIC Array Service technician using online software specifically designed for the RT-PCR array plates. A list of up and down-regulated genes including fold changes is provided to the researcher along with a project summary.

Cost of Array Service

The **Basic Service** applies to researchers who wish to do their own sample preparation and will be providing purified cDNA to the AIC.

Basic Service (Internal Rate): Purified cDNA supplied to AIC

Labor, Instrument Usage, and Consumables* = $**139 per plate**

*Does not include cost of MasterMix and/or Assay Plates which will be determined separately

Basic Service (External Rate): Purified cDNA supplied to AIC

Labor, Instrument Usage, and Consumables* = $**235.72 per plate****

*Does not include cost of MasterMix and/or Assay Plates which will be determined separately

**Total Costs are also subject to a 15% Administrative Fee

The **Full Service** applies to researchers who want the AIC to perform the sample preparation for them. They will provide samples in the form of cell lines, tissues, or extracted RNA to the core for further processing. The charges for the sample preparation are separate from the charges for running the array and will vary depending on the number of samples being analyzed.

Full Service (Internal Rate): RNA extraction, purification, and cDNA synthesis

RNA Extraction/Purification = $35 per sample
cDNA Synthesis = $30 per sample
Labor, Instrument Usage, and Consumables to Run Array* = $139 per plate

*Does not include cost of MasterMix and/or Assay Plates which will be determined separately

Full Service (External Rate): RNA extraction, purification, and cDNA synthesis

RNA Extraction/Purification = $50.60 per sample
cDNA Synthesis = $41.70 per sample
Labor, Instrument Usage, and Consumables to Run Array* = $235.72 per plate**

*Does not include cost of MasterMix and/or Assay Plates which will be determined separately

**Total Costs are also subject to a 15% Administrative Fee

Advantages of the Array Service

The Array Service provides many and is great for researchers!

- Having this service facilitates on-going research in the field of gene expression analysis and miRNA analysis, especially for those who have limited starting funds in array services, to jump start research

- Obtain the most out of the data by using the intellectual products and software created by Bio-tech companies

- Researchers do not have to train bench scientists to learn array practices and run the risk of wasting expensive plates and reagents

- The service is performed by AIC professionals with consistency and accuracy

- AIC acts as liaison between array companies and BU researchers, allowing for the lowest prices on plates, supplies, and reagents.

- Researchers are able to get results in as little as one week, depending on the scope of the project and the number of plates being analyzed.