

RT-PCR

Array Services

at the Analytical Instrumentation Core

Boston University, AIC, DOM, BMC, BUMC



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Analytical Instrumentation Core Mission:

The AIC supports investigators through cutting-edge analytical instruments and services to 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 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 regular seminars
5. Support investigators in their efforts to win extramural research funding

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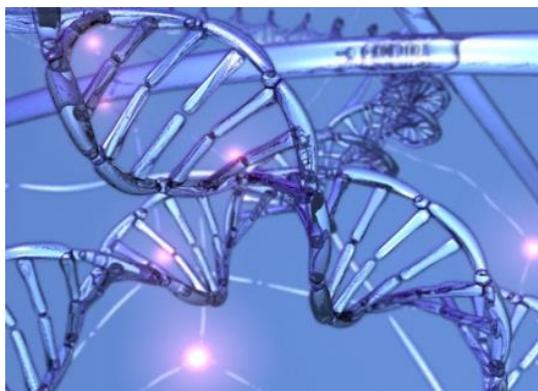
Introduction to Array Services at the AIC:

Our new Plate Array Service Facility has formally opened for business!

The Analytical Instrumentation Core now offers Array Services for RT-PCR Arrays. Researchers are able to screen samples against a set of known genes contained in 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 micro arrays)**

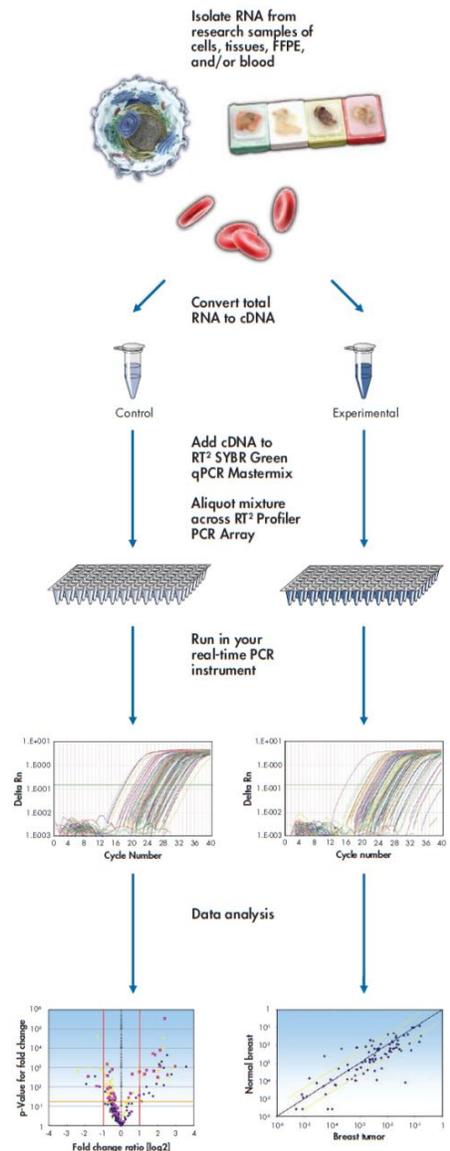


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What are PCR Arrays?

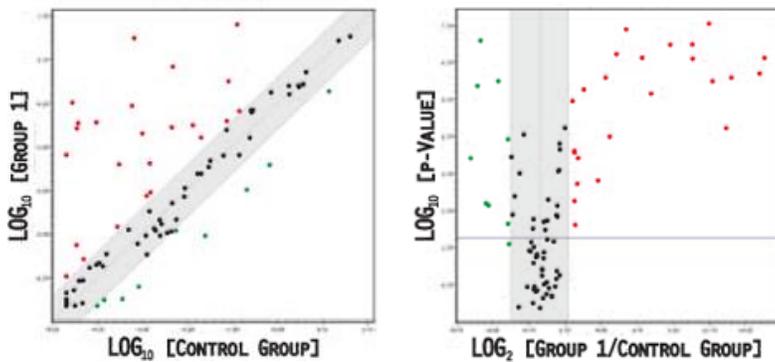
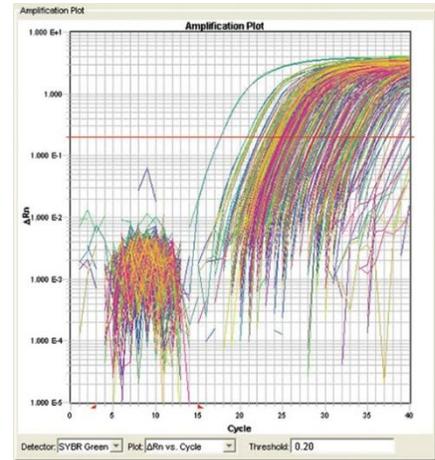
RT-PCR arrays allow for reliable gene expression analysis of a disease or pathway focused set of genes for any sample quantity or type using real-time PCR. Each catalogued PCR array contains 84 genes in a 384-well PCR plate along with 5 housekeeping genes and 7 controls to monitor DNA contamination, 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 analyzed and the results can be used for grant submissions or final publications.

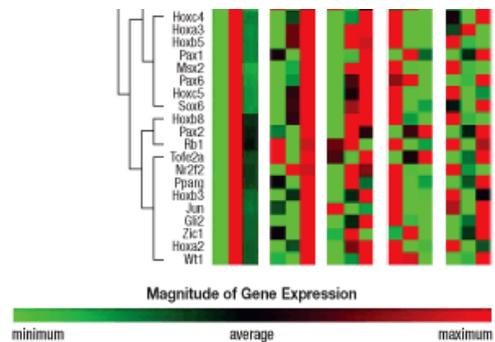


What do the results look like?

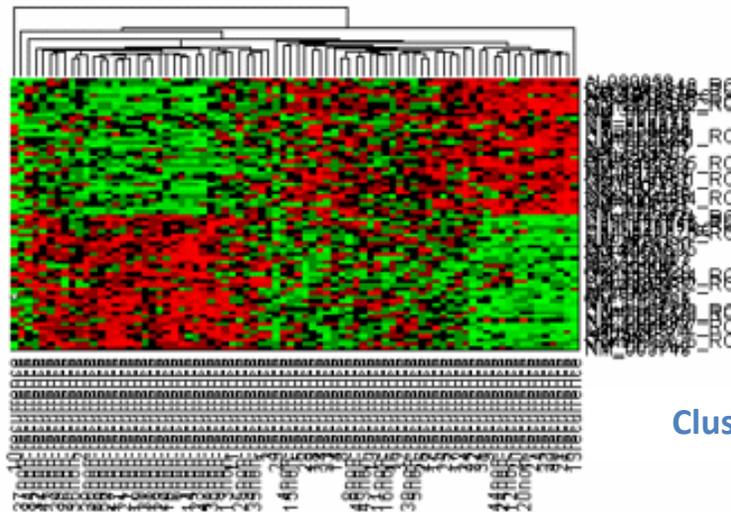
Free online data-analysis software allows for quick and easy interpretation of raw RT-PCR results using excel-based templates. The software identifies up and down-regulated genes and organizes the data into plots and charts which can immediately be integrated into a publication, poster, or presentation.



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

	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	01	01	02	02	03	03	04	04	05	05	06	06	07	07	08	08	09	09	10	10	11	11	12	12
B	01	01	02	02	03	03	04	04	05	05	06	06	07	07	08	08	09	09	10	10	11	11	12	12
C	13	13	14	14	15	15	16	16	17	17	18	18	19	19	20	20	21	21	22	22	23	23	24	24
D	13	13	14	14	15	15	16	16	17	17	18	18	19	19	20	20	21	21	22	22	23	23	24	24
E	25	25	26	26	27	27	28	28	29	29	30	30	31	31	32	32	33	33	34	34	35	35	36	36
F	25	25	26	26	27	27	28	28	29	29	30	30	31	31	32	32	33	33	34	34	35	35	36	36
G	37	37	38	38	39	39	40	40	41	41	42	42	43	43	44	44	45	45	46	46	47	47	48	48
H	37	37	38	38	39	39	40	40	41	41	42	42	43	43	44	44	45	45	46	46	47	47	48	48
I	49	49	50	50	51	51	52	52	53	53	54	54	55	55	56	56	57	57	58	58	59	59	60	60
J	49	49	50	50	51	51	52	52	53	53	54	54	55	55	56	56	57	57	58	58	59	59	60	60
K	61	61	62	62	63	63	64	64	65	65	66	66	67	67	68	68	69	69	70	70	71	71	72	72
L	61	61	62	62	63	63	64	64	65	65	66	66	67	67	68	68	69	69	70	70	71	71	72	72
M	73	73	74	74	75	75	76	76	77	77	78	78	79	79	80	80	81	81	82	82	83	83	84	84
N	73	73	74	74	75	75	76	76	77	77	78	78	79	79	80	80	81	81	82	82	83	83	84	84
O	Ce	Ce	Ce	Ce	SN1	SN1	SN2	SN2	SN3	SN3	SN4	SN4	SN5	SN5	SN6	SN6	mRTC	mRTC	mRTC	mRTC	PPC	PPC	PPC	PPC
P	Ce	Ce	Ce	Ce	SN1	SN1	SN2	SN2	SN3	SN3	SN4	SN4	SN5	SN5	SN6	SN6	mRTC	mRTC	mRTC	mRTC	PPC	PPC	PPC	PPC

C. elegans
 miR-39 miScript
 Primer Assay

snoRNA/snRNA
 miScript
 PCR Controls

Reverse
 transcription
 control

Positive
 PCR control

Modified Arrays

Modified Arrays are available in which up to 4 genes can be added or exchanged on any catalogued plate. A minimum of 4 plates must be ordered when creating a modified array plate.

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 drosophila 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



16 Genes - 24 Samples per Plate



32 Genes - 12 Samples per Plate

384-well Custom PCR ARRAYS (#330131)

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

Workflow of AIC Array Service



1. Consultation

During the consultation, researchers 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 schedules a time slot for the experiment on the core scheduler.

2. Researchers Provide Samples

Samples are typically submitted as cell lines, extracted RNA, or cDNA as prepared by the researcher, although you may discuss with the AIC the option of submitting other samples for analysis (such as tissues). All samples must meet biosafety standards for a BSL2 laboratory. 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 the AIC to do 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 into 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. Preliminary 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: Purified cDNA supplied to AIC*

Plate, instrument usage, and time= approximately \$370 per plate

Reagents and supplies= approximately \$200 per plate

Total= \$570 per plate

The **Full Service** applies to researchers who wish the AIC to do the sample preparation for them. The 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 the array running and will vary depending on the number of samples being analyzed.

Full Service: RNA extraction, purification, and cDNA synthesis*

Sample Preparation= approximately \$50 per sample (4 samples per plate) =

approximately \$200 per plate

Plate, instrument usage, and time= approximately \$384 per plate

Reagents and supplies= approximately \$200 per plate

Total = \$770 per plate

* Costs of plates will be higher for modified or custom arrays

Advantages of the Array Service

The advantages of the Array Service 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 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 discounts 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.

