PCR Arrays

An Advanced qPCR Technology to Enhance Your Analysis of Pathways

RT^2 Profiler PCR Arrays

Microarray Profiling Capabilities with Real-Time PCR Performance

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Product Manager, Gene Expression Analysis

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Topics to be Covered

- **Introduction to the PCR Array**
  - What <u>IS</u> the PCR Array?
  - PCR Array System Brief Protocol Overview
- **Biologically Relevant PCR Array Performance**
  - Sensitivity, Specificity, Reproducibility, & Reliability
- **Applications for the PCR Array**
  - **Immunology Application Example:**
    - Monitor the Induction of Cytokines
  - **Cancer Application Example:**
    - Explore the oncogenic route taken by two breast cancer tumors
  - **Toxicology Application Example:**
    - Distinguish liver toxicity of structurally similar drug candidates

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What <u>is</u> the RT^2 Profiler PCR Array?

- **Expression analysis tool for a focused panel of genes**
  - Pre-designed cataloged arrays for over 100 pathways
  - Customized panels ranging from 4 to 384 genes
  - Compatible with (*almost*) all real-time PCR instruments

- **96- or 384-well plate of SYBR Green-optimized qPCR assays**
  - 84 relevant, pathway- or disease-focused genes
  - 5 housekeeping genes and three RNA quality controls

- **Very simple two-hour protocol that generates:**
  - Microarray-like multi-gene content & results
  - qPCR sensitivity and reproducibility

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RT²*Profiler*™ PCR Array Layout

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

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

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Pathway Design Requirements

- NOT biochemical pathways or kinase cascades
  - Biological Processes, Disease States
- NOT simply every gene list from the entire pathway
  - Use genes regulated at mRNA level

- Publicly Accessible Databases
- Text Mining Relevant Literature
- Reviews by Experts in the Field
- Feedback from Previous & Existing Customers

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Available Cataloged PCR Arrays

Human, Mouse, and Rat

PATHWAY	SPECIES	PATHWAY	SPECIES
Angiogenic Growth Factors & Angiogenesis Inhibitors	HM	Interferons & Receptors	HM
Angiogenesis	HMR	JAK / STAT Signaling	HM
Apoptosis	**HMR**	MAP Kinase Signaling	HMR
Atherosclerosis	HM	Neuroscience Ion Channels & Transporters	HM
Breast Cancer and Estrogen Receptor Signaling	HMR	**Neurotransmitter Receptors & Regulators**	**HMR**
cAMP / Calcium Signaling PathwayFinder	HM	Neurotrophins & Receptors	HMR
Cancer Drug Resistance & Metabolism	HM	NFκB Signaling	HMR
Cancer PathwayFinder™	**HMR**	Nitric Oxide Signaling	HM
Cell Cycle	HMR	Notch Signaling	HM
Chemokines & Receptors	HMR	Obesity	MR
Common Cytokines	**HMR**	Osteogenesis	HM
Diabetes	HMR	Oxidative Stress & Antioxidant Defense	HM
DNA Damage Signaling	**HM**	p53 Signaling	HM
Drug Metabolism	**HMR**	Signal Transduction PathwayFinder™	HMR
Drug Metabolism: Phase I Enzymes	H	Stem Cells	HM
Drug Transporters	H	**Stress & Toxicity PathwayFinder™**	**HMR**
Endothelial Cell Biology	HMR	**TGFβ / BMP Signaling**	**HMR**
Extracellular Matrix and Adhesion Molecules	**HMR**	Th1 Th2 Th3	HMR
Growth Factors	HM	TH17 for Autoimmunity & Inflammation	M
Housekeeping Genes	HMR	TNF Ligands and Receptors	HM
Hypoxia Signaling	HM	Toll-Like Receptor Signaling	HMR
Inflammatory Cytokines & Receptors	**HMR**	**Tumor Metastasis**	**HM**
Insulin Signaling	HMR	WNT Signaling	HMR

Other Pathways? Other Species?

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Human RT^2 miRNA qPCR Arrays & Assays

ALSO NOW AVAILABLE

- Individual Assays for over 420 miRNA Sequences
- Used to Design Pathway-Focused PCR Arrays
  - Cancer
  - Cell Differentiation & Development
  - Genome
  - miFinder™
- Similar System, Packaging, Pricing, and Procedure as RT^2 Profiler PCR Arrays
- Same rigorous assay design & experimental validation

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4 Components of the PCR Array System

- **RT² First Strand Kit**
  - Built-in genomic DNA elimination & external RNA control

- **RT² SYBR Green qPCR Master Mixes**
  - Instrument-specific reference dyes included

- **RT² Profiler™ PCR Array**
  - Instrument-specific plate formats

- **FREE Data Analysis Web Portal or Excel Template**
  - Automatic results from uploaded raw C_t data

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Ease-of-Use: How the PCR Array Works

Control & Experimental Cells or Tissue

1. Isolate Total RNA

2. Reverse Transcription: RT^2 First Strand Kit
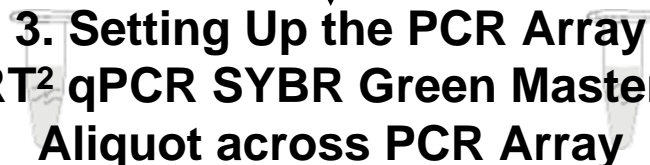
3. Setting Up the PCR Array
Instrument-Specific RT^2 qPCR SYBR Green Master Mix & cDNA Cocktail
Aliquot across PCR Array

4. Real-Time PCR
(40 cycles, 100-120 min)

5. Data Analysis: Determine Fold Changes ($\Delta\Delta$ C$_t$ Method)

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Compatible Instrumentation

- **Matched with the instrument in your lab**
- **Applied Biosystems (ABI)**
  - Standard 96-Well Blocks: 7000, 7300, **7500**, 7700
  - FAST 96-Well Blocks: 7500**, 7900HT**
  - FAST 384-Well Block: **7900HT**
- **Bio-Rad**
  - **iCycler**, MyiQ, iQ5
  - MJ Research: Opticon, Opticon 2, Chromo 4
- **Stratagene**
  - Mx3000p, **Mx3005p**, **Mx4000p**
- **Roche**

  LightCycler 480
- **Eppendorf** masterplex ep

Underlined Instruments run PCR Arrays 24/7/365 in SuperArray R&D and Production Departments

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Performance: SensitivitY

See More Genes with Less RNA
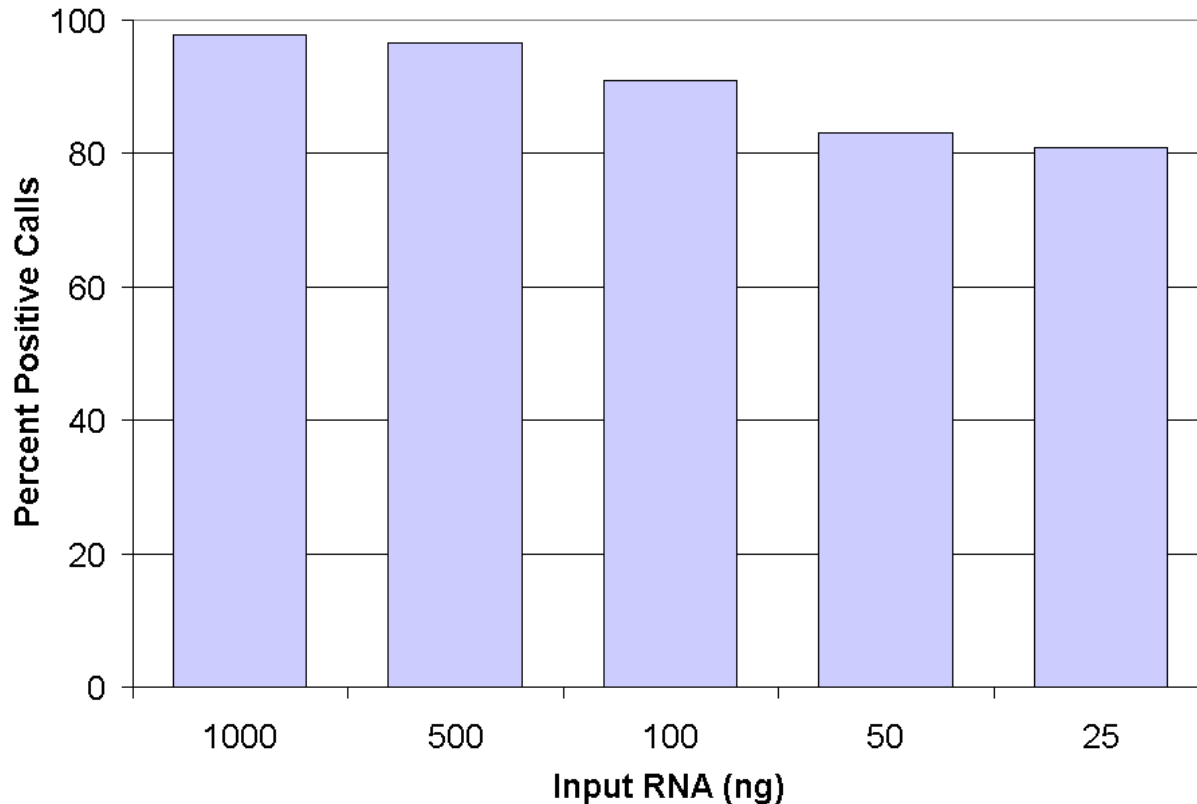
CONCLUSION: See gene expression as rare as <u>UN</u>-induced cytokines.

Human Inflammatory Cytokines & Receptors in Universal Reference RNA

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Performance: Specificity

Dissociation Curves

Agarose Gel

BMP1 BMP2 BMP3 BMP4 BMP5 BMP6 BMP7 BMP15
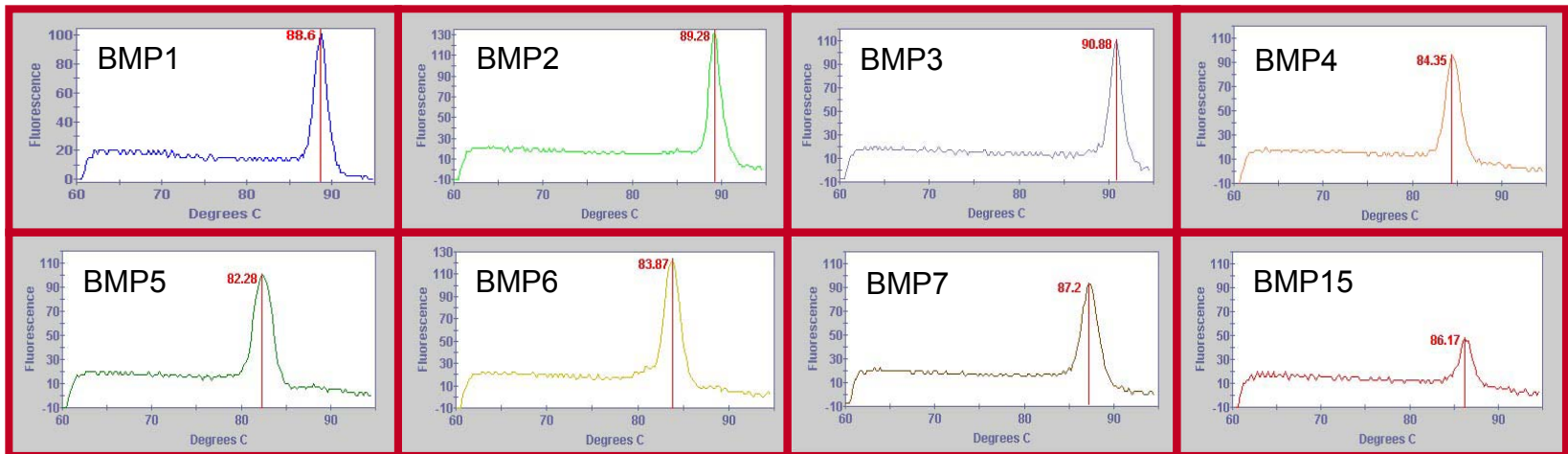
CONCLUSION: Each well detects a single gene-specific product.

Human TGFβ & BMP Signaling Pathway in Universal Reference RNA

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Specificity: Single Gene-Specific Product

Dissociation Curves
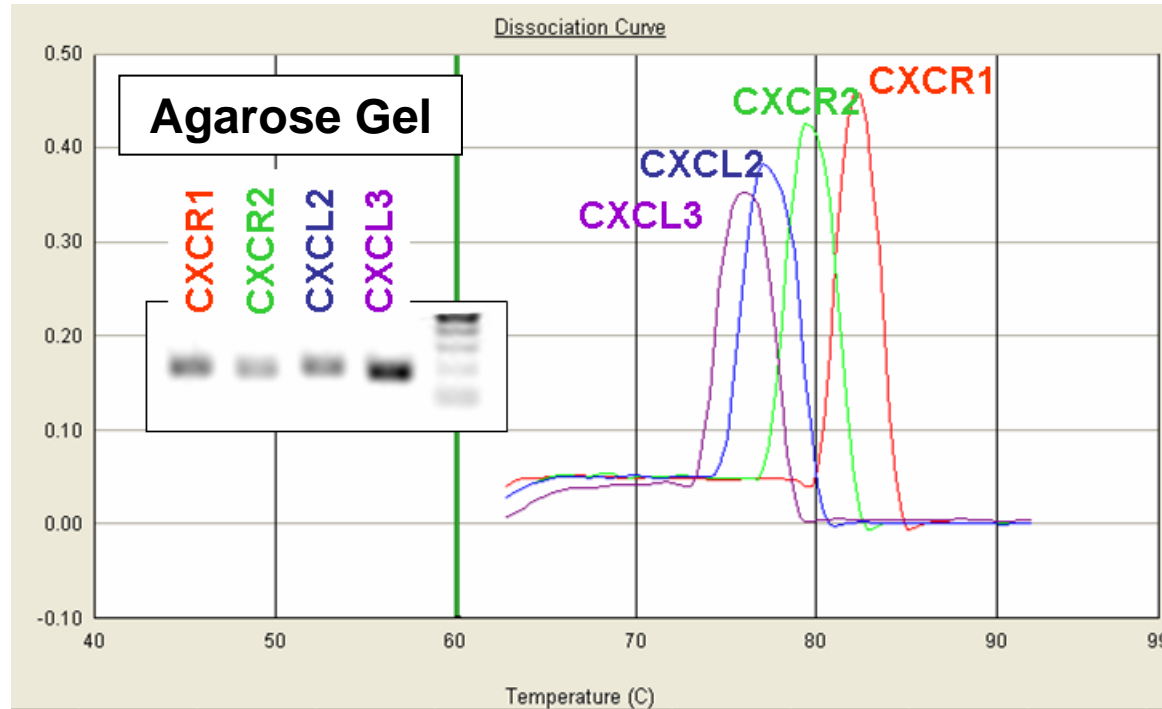
CONCLUSION: PCR Array specifically detects the expression of individual genes despite the presence of members of the same gene family in the same RNA sample.

Human Chemokines & Receptors in Universal Reference RNA

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Reproducibility: Same C_t Values
Different Lots, End-Users, Times

End User A Replicates

1   2   3   4

End User B Replicates

1

2

3

4
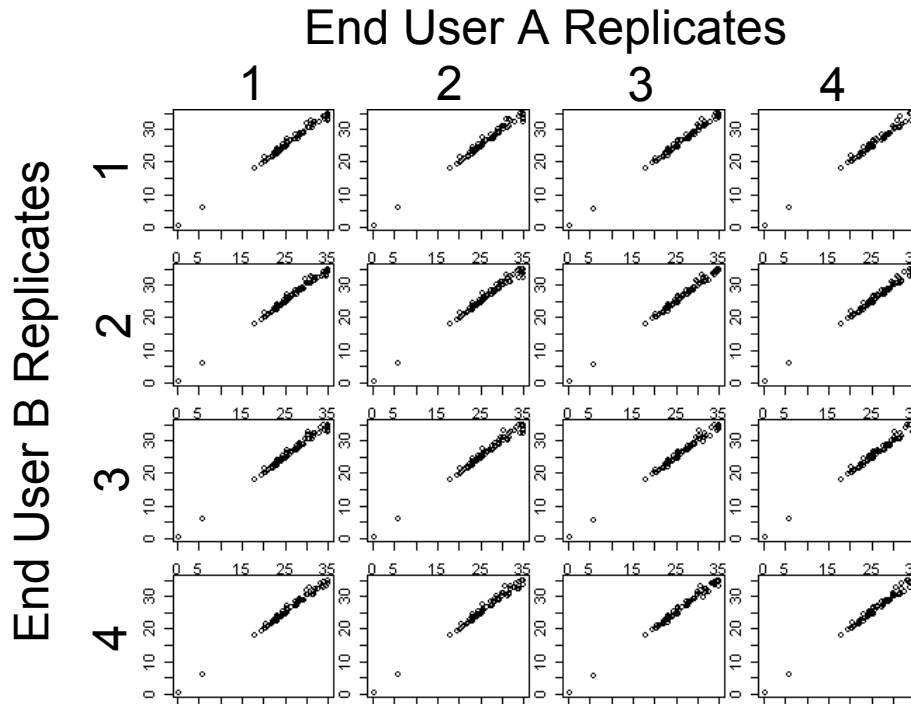
Correlation Coefficients

R^2	A1	A2	A3	A4
B1	0.993	0.989	0.995	0.992
B2	0.994	0.990	0.995	0.992
B3	0.992	0.990	0.993	0.992
B4	0.993	0.992	0.994	0.992

CONCLUSION:

The PCR Arrays are **SO** reproducible that the same raw threshold cycle data can be obtained for the same samples even from different end users at different times.

Human Drug Metabolism in Universal Reference RNA

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Reproducibility: Same Fold-Change Results

Different Instruments
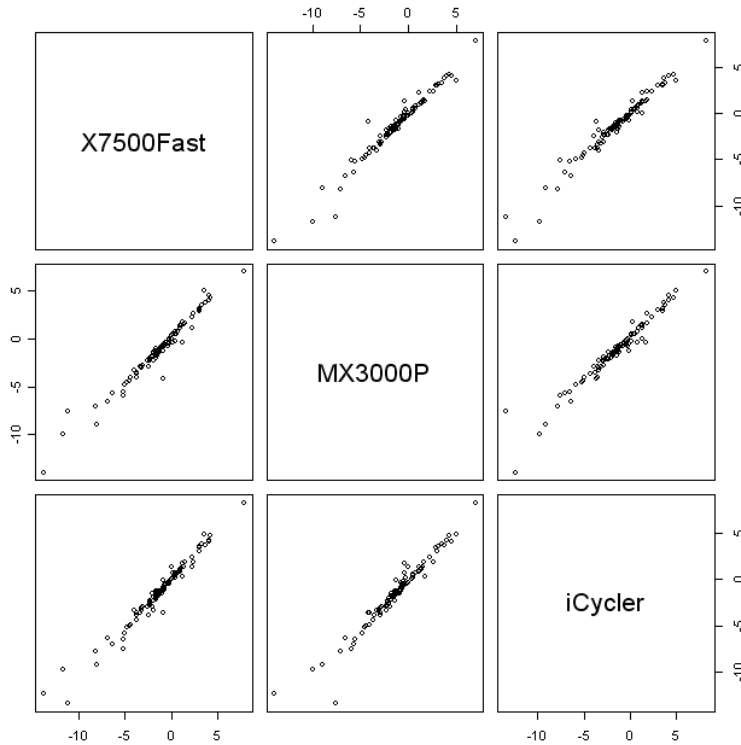
R^2	7500 FAST	Mx3000p	iCycler
7500 FAST	1		
Mx3000p	0.980	1	
iCycler	0.981	0.973	1

CONCLUSION:

The PCR Arrays are **SO** reproducible that the same fold-change results can be obtained for the same samples even from different instruments.

CONCLUSION: PCR Arrays demonstrate a highly reproducible results.

Be confident that observed gene expression changes due to biology.

Human Drug Metabolism in MAQC Standard RNA Samples A & B

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Performance: Reproducibility

- Can I compare results between plates and samples? **YES!**
- Will future lab members get the same results as I did? **YES!**
  - Correlation factor (R) = **0.989 – 0.995**
    For raw C_t value data between different lots, end-users, and times

- Will another lab be able to reproduce my results? **YES!**
  - Correlation factor (R) = **0.973 – 0.981**
    For fold-change results across instrument platforms

- **CONCLUSION: Be confident that gene expression changes are due to <u>biology</u> and <u>not</u> the technology itself**

- Biological Triplicates Recommended

Performance: Reliability

Consistently High Amplification Efficiencies

Average = 99% with a
95% Confidence Interval (CI) from 90 to 110%
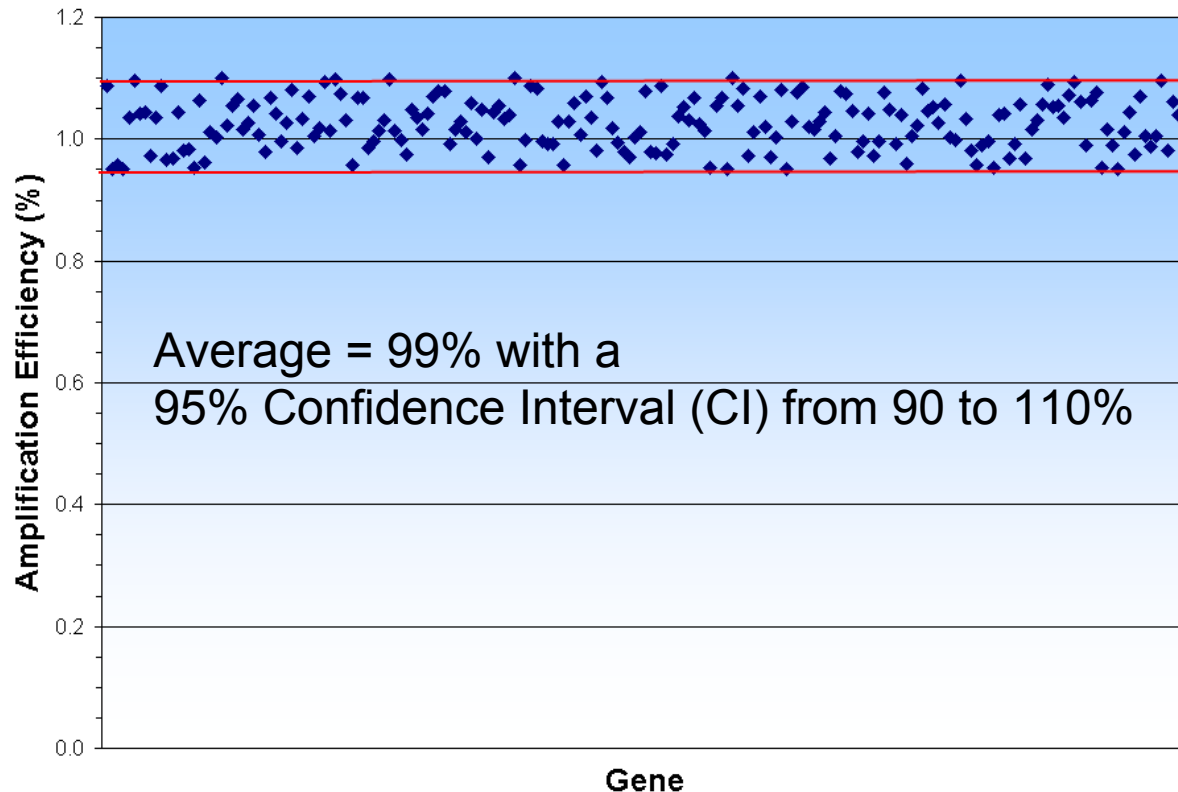
Representative set of 500 out of > 4,000 assays used in PCR Arrays

CONCLUSION: Get accurate and reliable $\Delta\Delta C_t$ results.

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Performance: Accuracy & Reliability

Same Fold-Change Results as TaqMan®

$$y = 0.9941x - 0.5701, R = 0.97$$
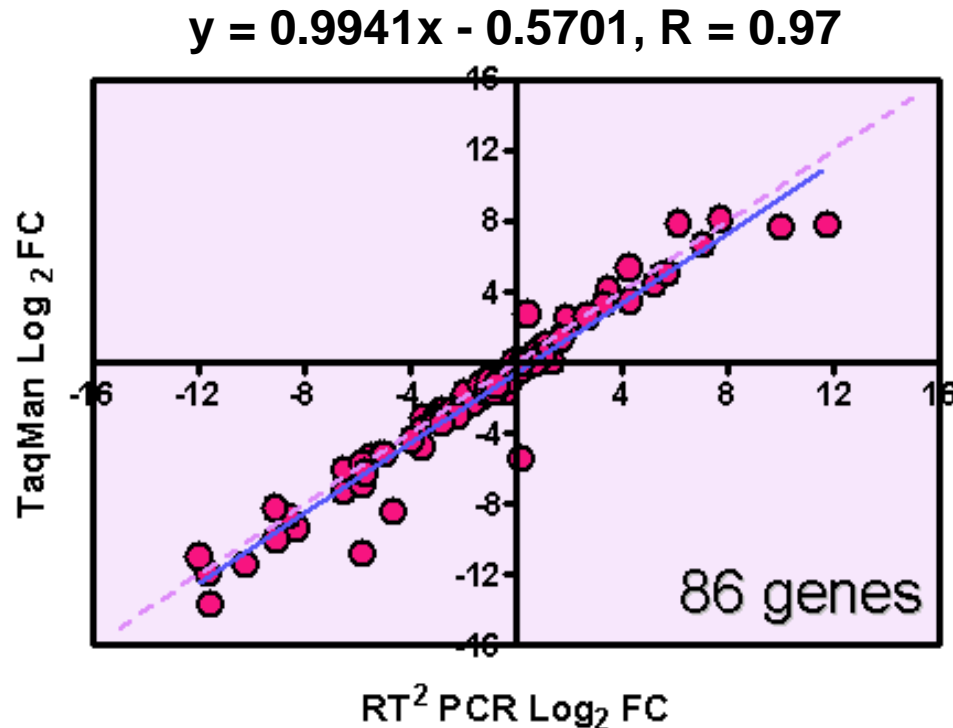
CONCLUSION: Pathway-focused PCR Arrays give the same reliable answers as individual TaqMan Gene Expression Assays.
Profile pathways all at once rather than one gene at a time.

Human Custom PCR Array in MAQC Standard RNA Samples A & B

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Immunology Research Application Example

- Quantification of cytokine expression levels is important for the study of inflammatory and immune responses.

- Correlating changes in secreted protein levels with changes in mRNA levels helps delineate the mechanisms regulating cytokine production.

- Stimulate human peripheral blood mononuclear cells (PBMC) with 50 ng/ml PMA plus 1 μg/ml ionomycin over a time course (0, 6, 12, 24 h).

- Monitor the gene expression levels of 84 different cytokines in technical triplicates using the Human Common Cytokines PCR Array. Validate results with ELISA.

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PBMC Cytokine Induction by PMA-Ionomycin
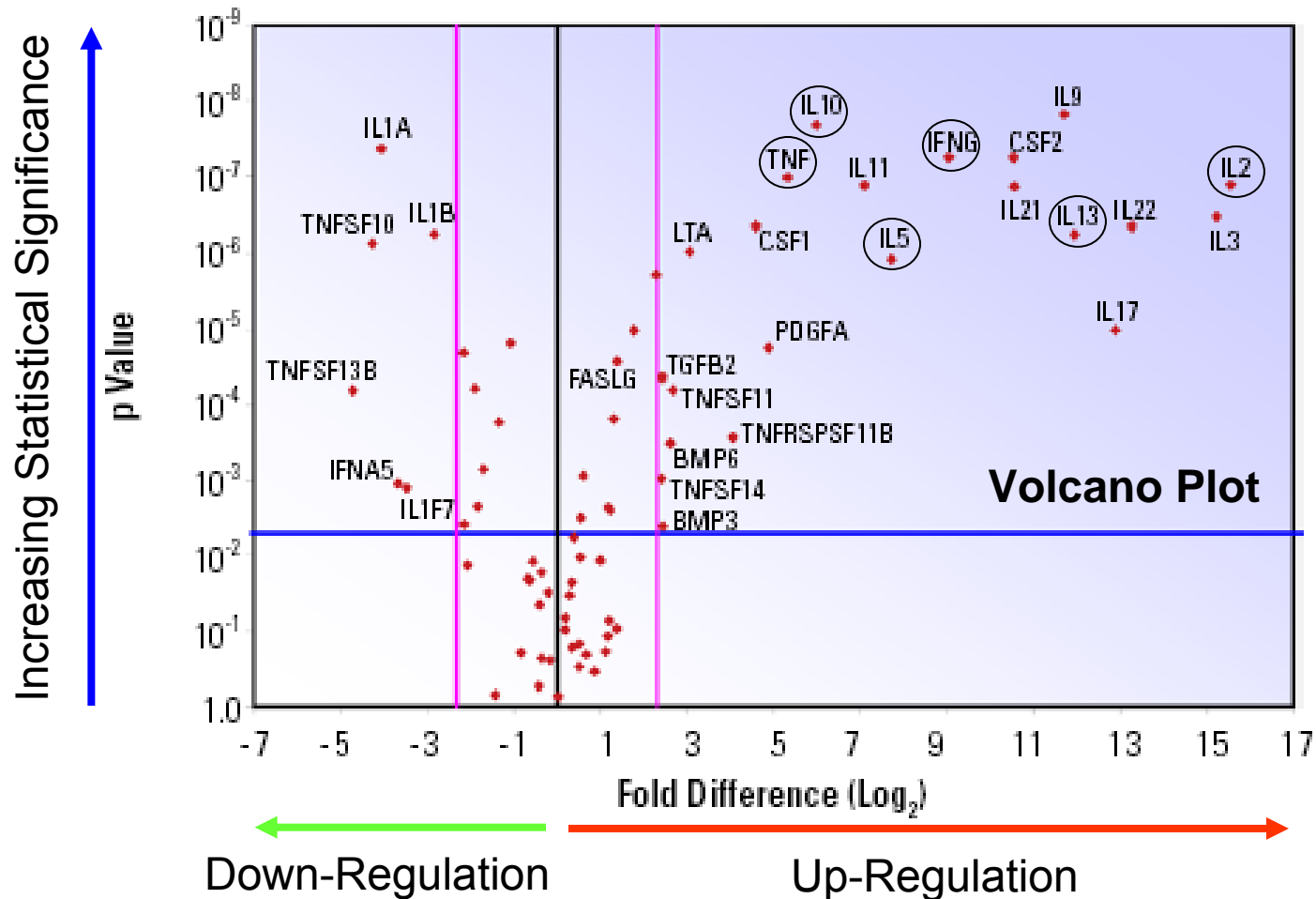
23 Genes Up- & 6 Genes Down-Regulated by > 5-fold (p < 0.0005) with 6-h stimulation

Cytokine Induction in PBMC by PMA-Ionomycin
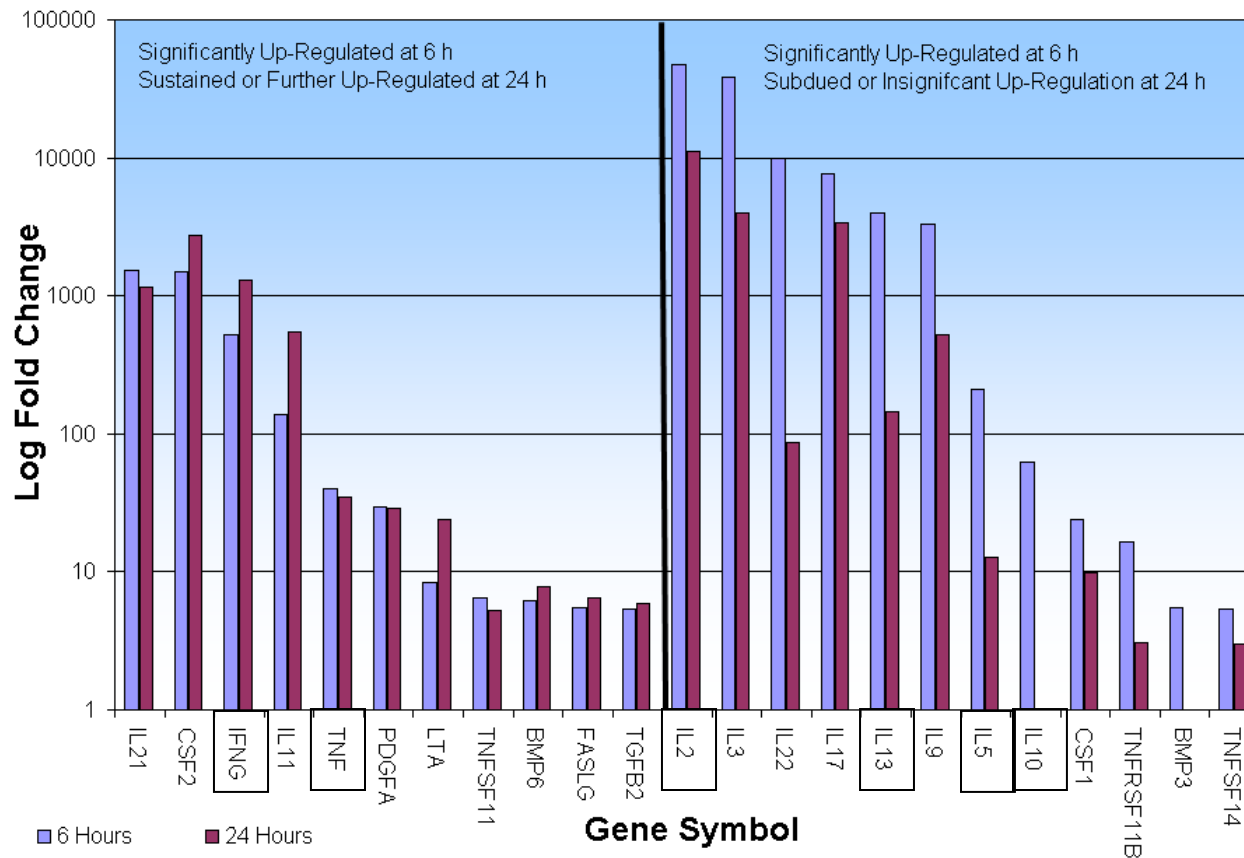
After 24-h stimulation, one half of the up-regulated genes maintains or increases, while the other half decreases their levels of expression.

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Cytokine Secretion & mRNA Expression Correlate
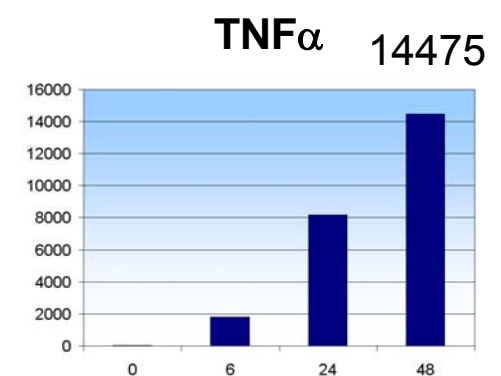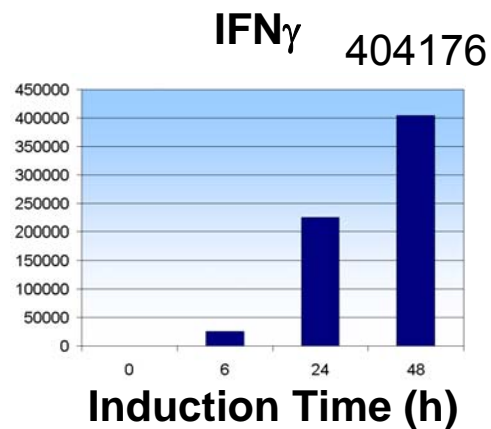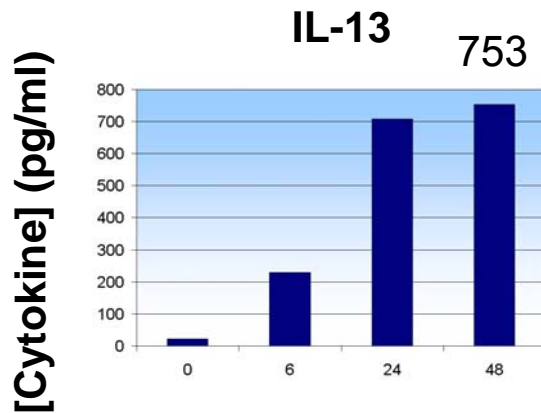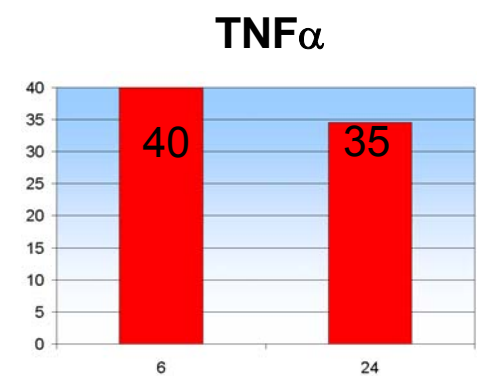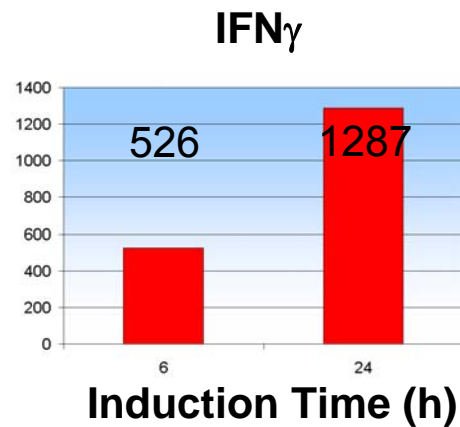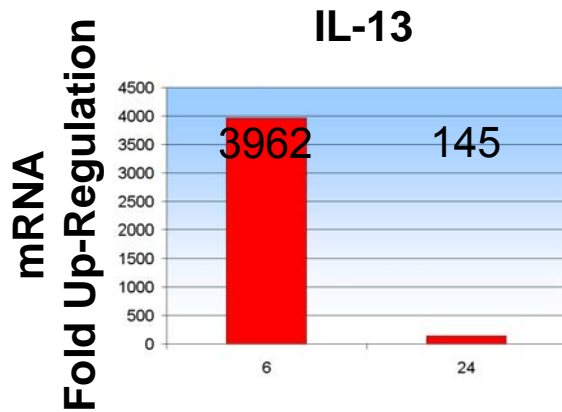
CONCLUSION: Cytokine gene expression correlates with cytokine protein secretion in a complicated time-dependent fashion.

Immunology Application Example

Conclusions

- PCR Arrays have the sensitivity and dynamic range to detect both stimulated and un-stimulated cytokines at the same time.

- Induction in cytokine secretion by PMA-ionomycin was sustained up to 48 hours of stimulation, despite subdued mRNA expression levels for IL-13 and TNF-α (and others) after 24 hours of stimulation.

- Cytokine gene expression correlates with cytokine protein secretion in a complicated time-dependent fashion.

- Profiling cytokine gene expression levels examines more cytokines than ELISA does and can help you probe more deeply into the mechanism of regulating cytokine secretion.

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Cancer Research Application Example

- Ascertain the oncogenic route taken by human breast tumors

- Characterize total RNA from normal human breast and two human breast tumors.

- Compare expression of 84 cancer-related genes
  - Human Cancer PathwayFinder™ PCR Array
  - Human Extracellular Matrix & Adhesion Molecule PCR Array

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Cancer PathwayFinder™ PCR Array

Functional Gene Groupings

- **Adhesion**
- **Angiogenesis**
- **Apoptosis**
- **Cell Cycle Control**
- **Cell Senescence**
- **DNA Damage Repair**
- **Invasion**
- **Metastasis**
- **Signal Transduction Molecules**
- **Transcription Factors**
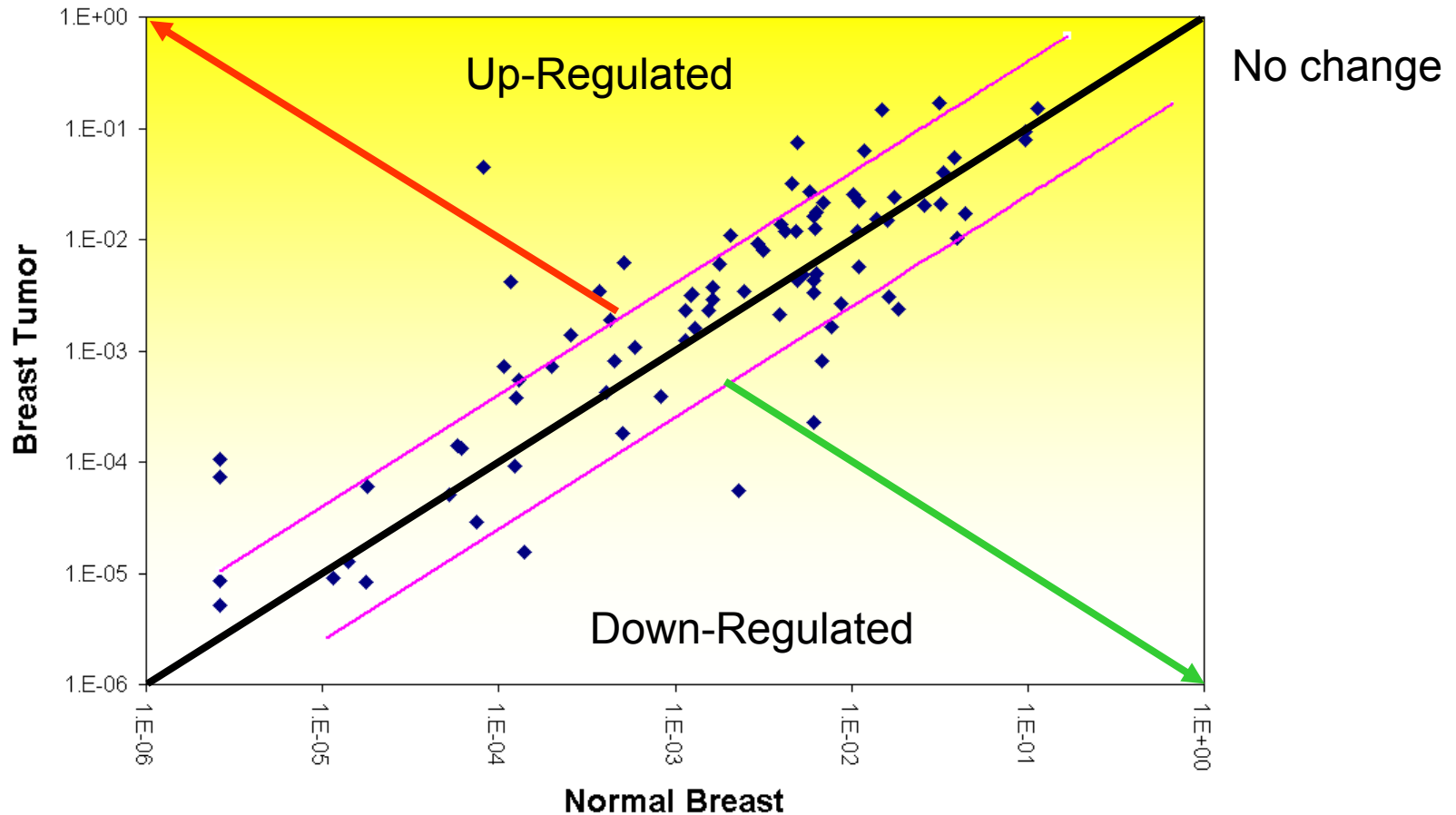
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Cancer PathwayFinder™ Results

Scatter Plot

Normalized tumor gene expression levels ($\log_{10}$) versus normal tissue

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Cancer PathwayFinder™ Results

- 24 genes with significant change; 17 up- and 7 down-regulated in tumor

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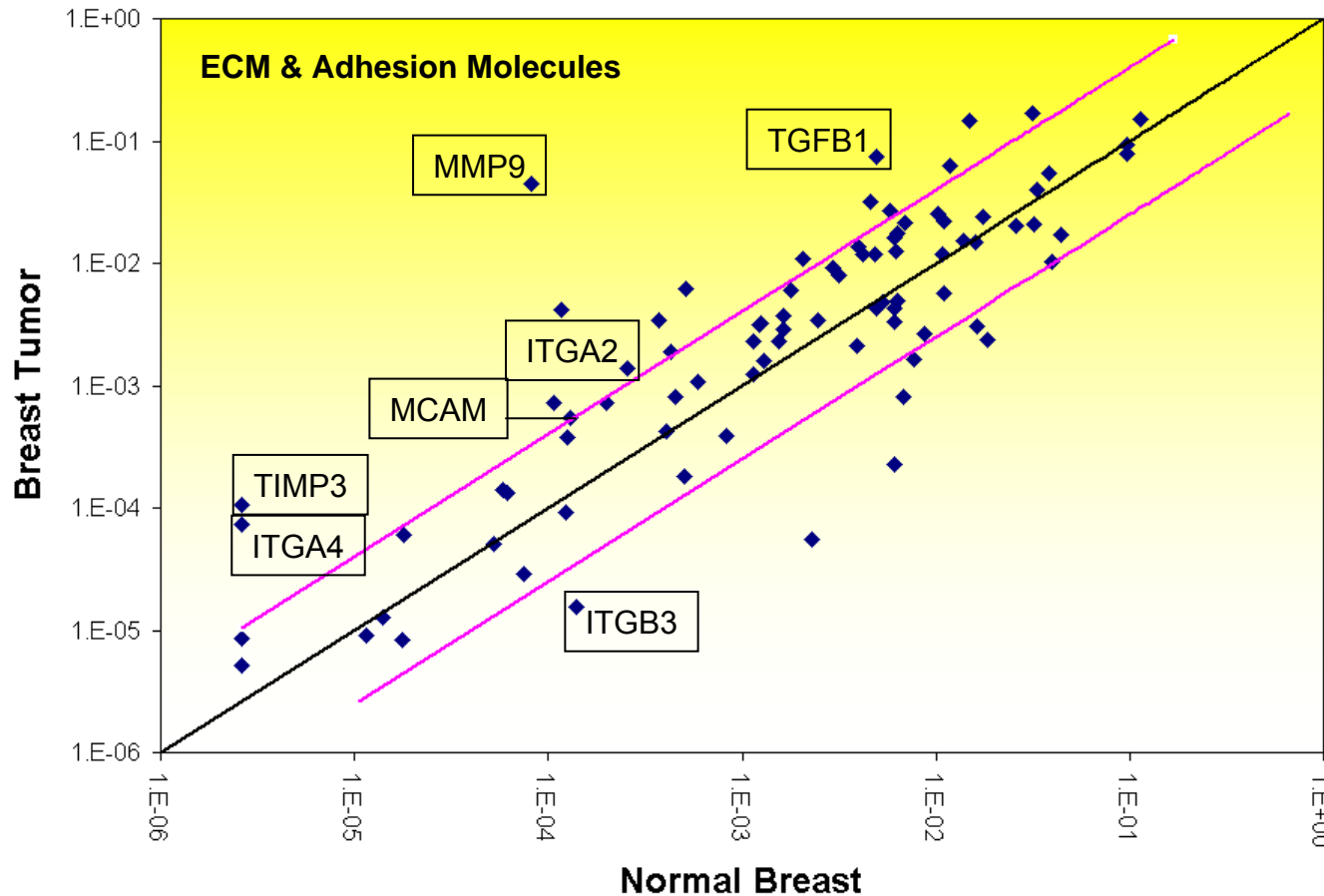Bioscience Corporation

Cancer PathwayFinder™ Results

- 24 genes with significant change; 17 up- and 7 down-regulated in tumor

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Cancer PathwayFinder™ Results

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Cancer PathwayFinder™ Results

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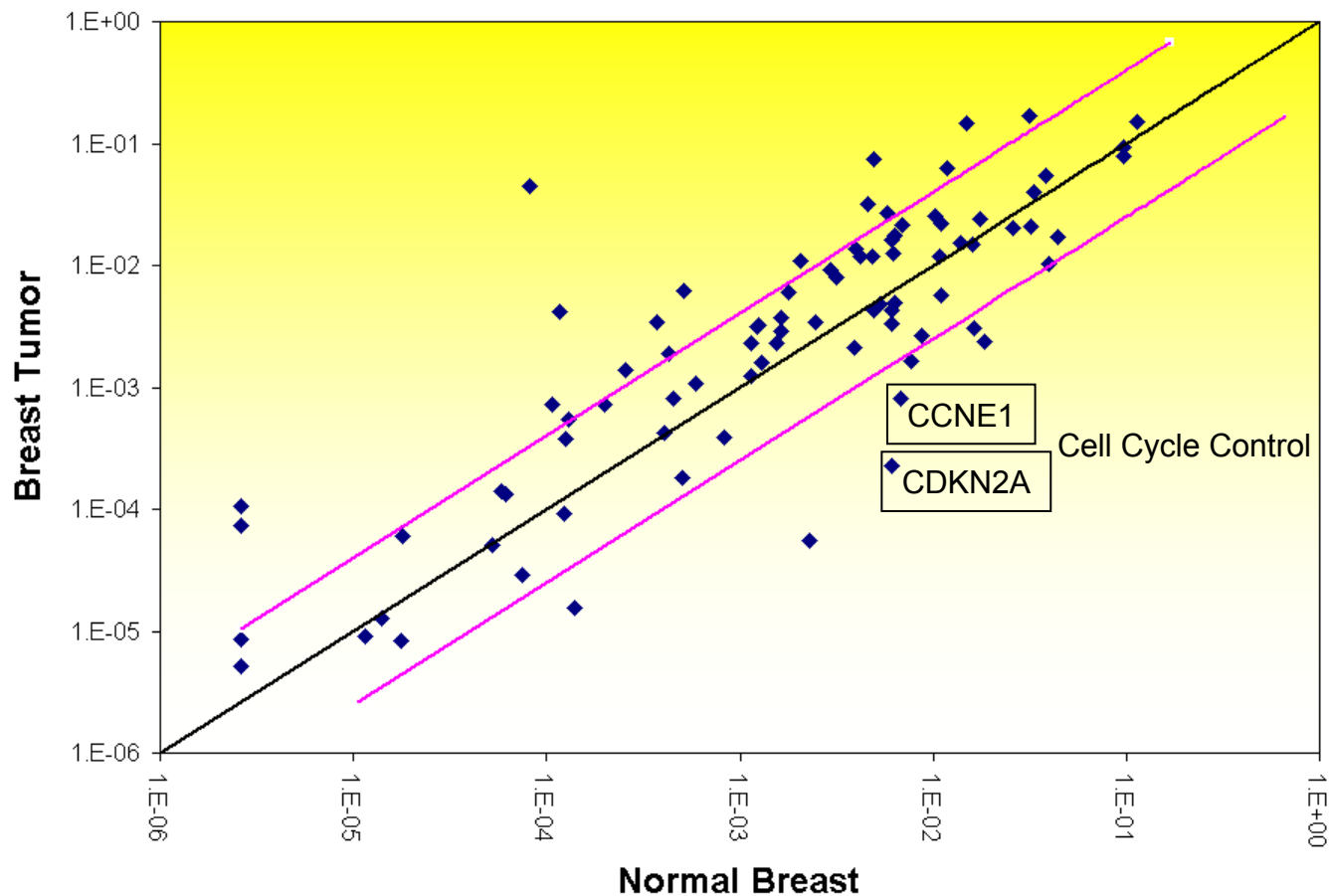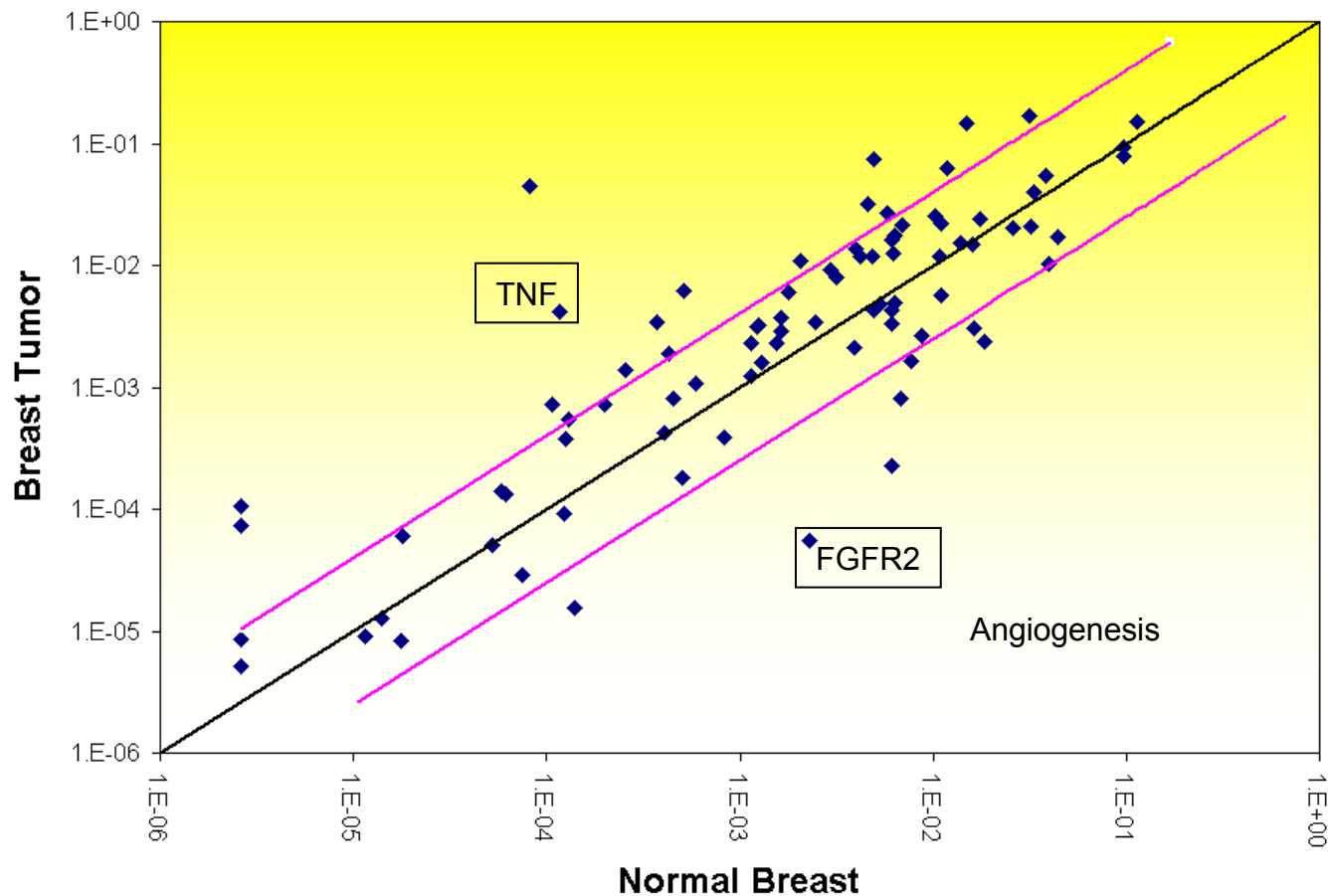
Cancer PathwayFinder™ Results
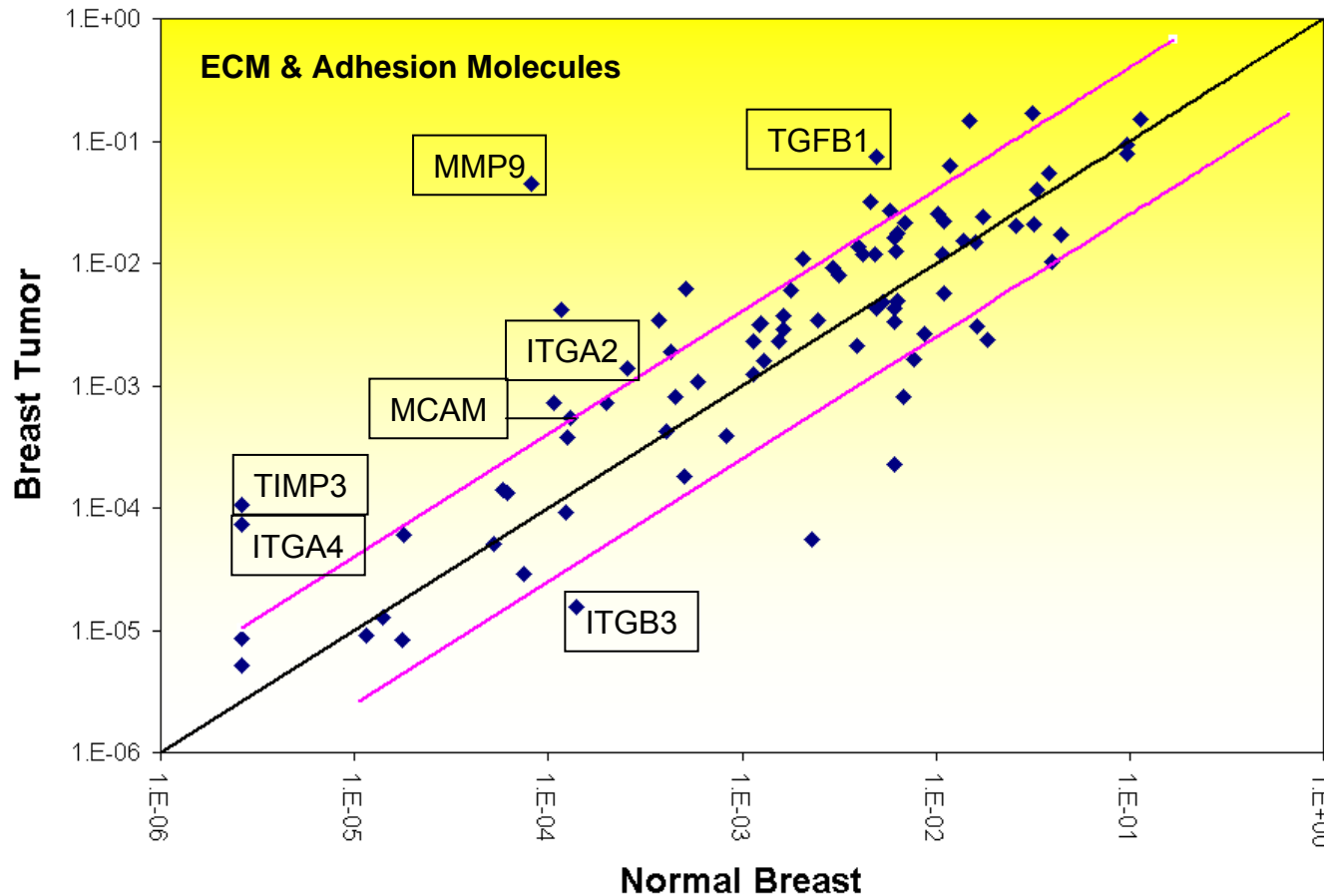
- 24 genes with significant change; 17 up- and 7 down-regulated in tumor
- **CONCLUSION:** Follow Up with ECM & Adhesion Molecule PCR Array

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ECM & Adhesion Molecules PCR Array

Functional Gene Groupings

- **Cadherins**
- **Catenins**
- **Cell Adhesion Molecules (ICAM, VCAM)**
- **Collagens**
- **Integrins**
- **Laminins**
- **Matrix & ADAM Metallopeptidases (MMP, ADAMTS)**
- **Selectins (SELE, SELL, SELP)**
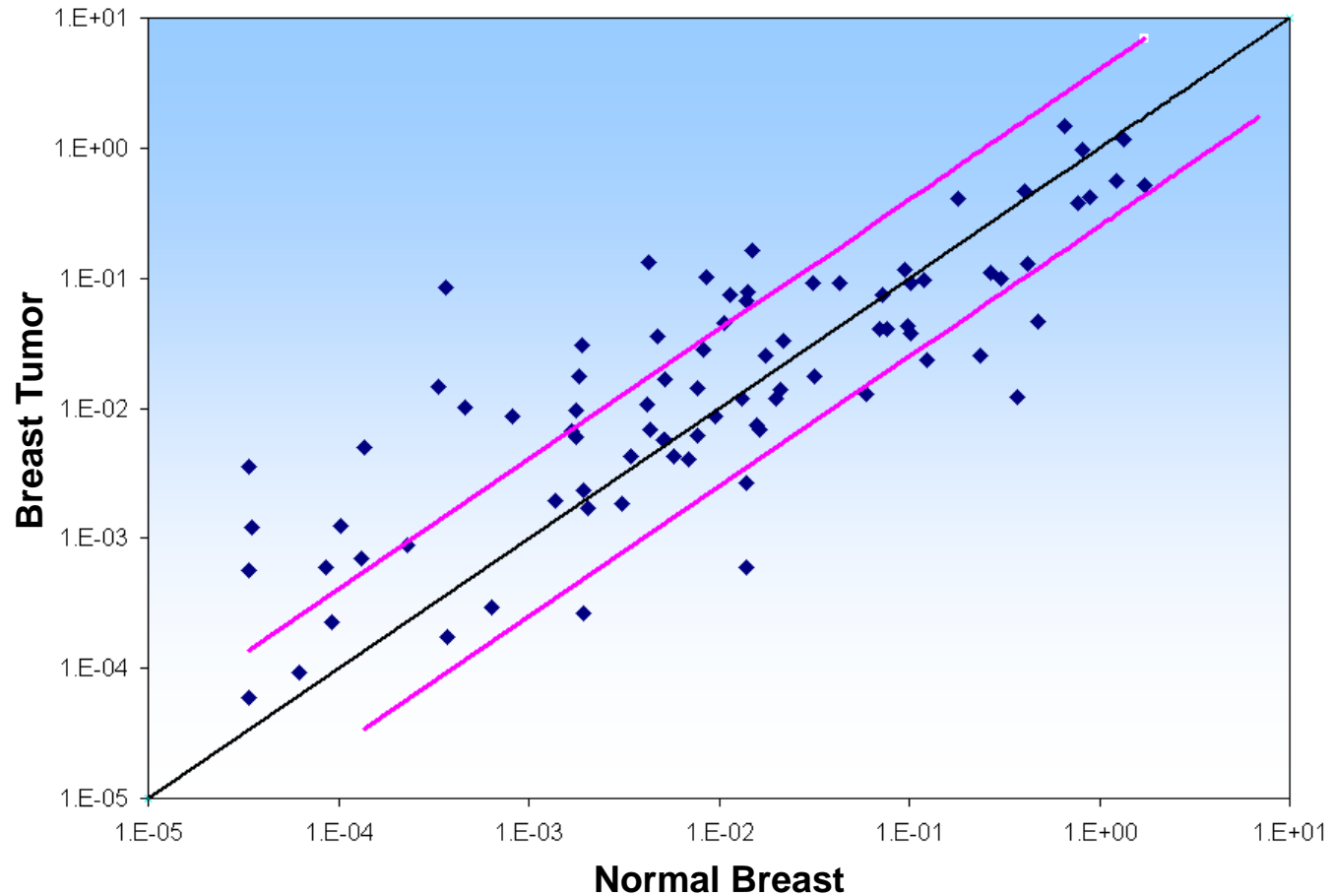- **Tissue Inhibitors of Metallo-Peptidases (TIMP)**
- **Thrombospondins**
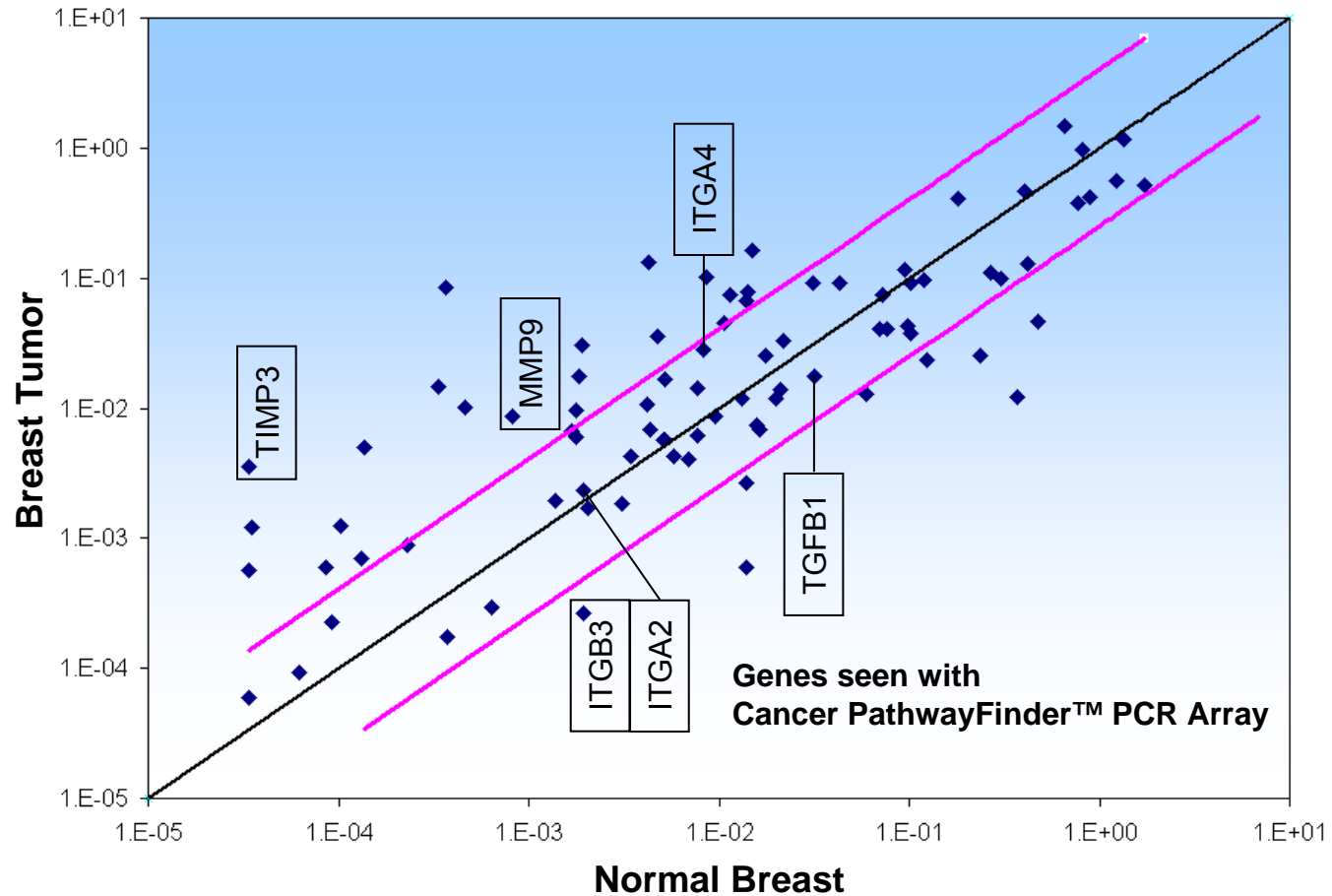
ECM & Adhesion Results
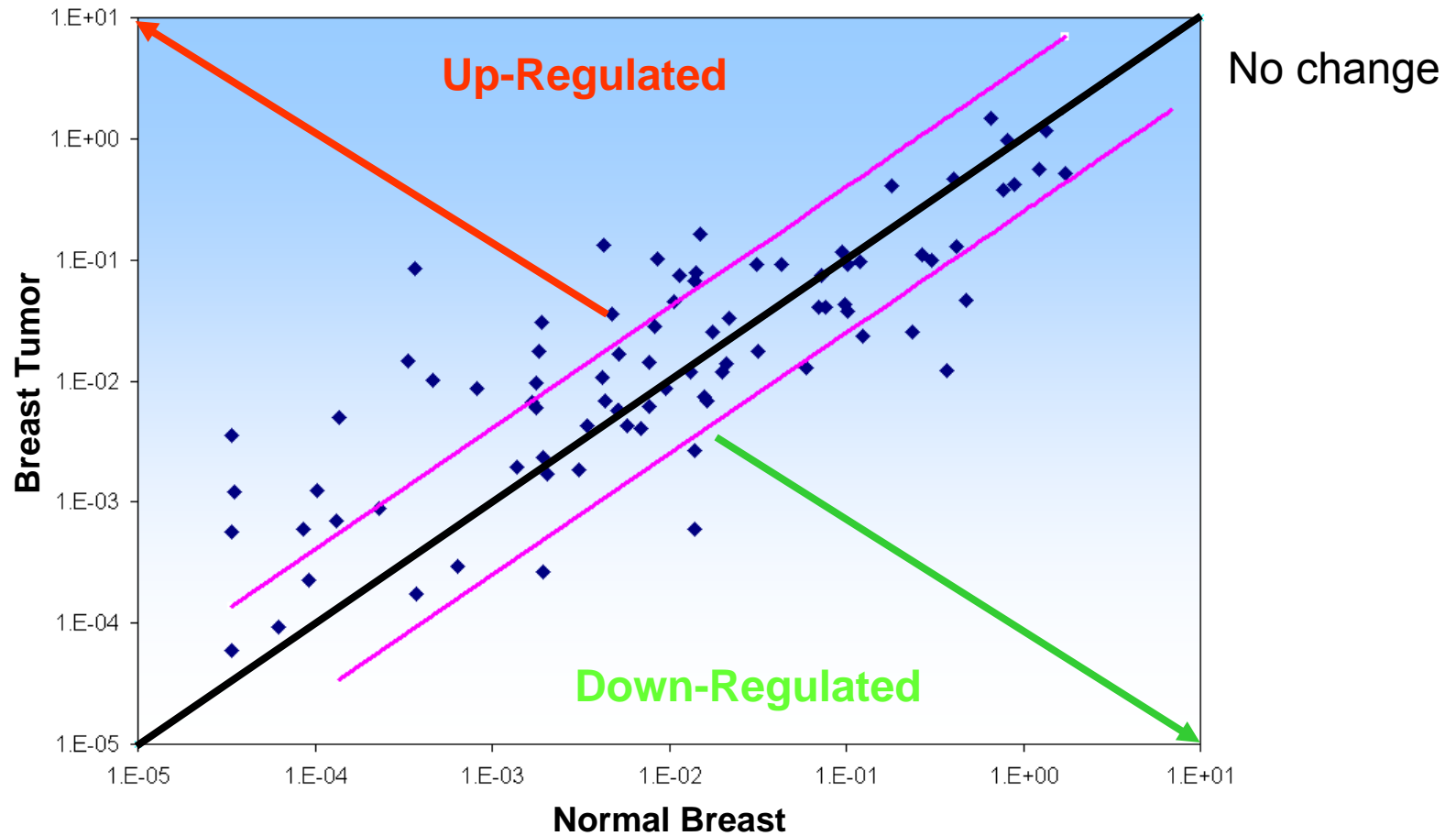
Scatter Plot

Normalized tumor gene expression levels ($\log_{10}$) versus normal tissue
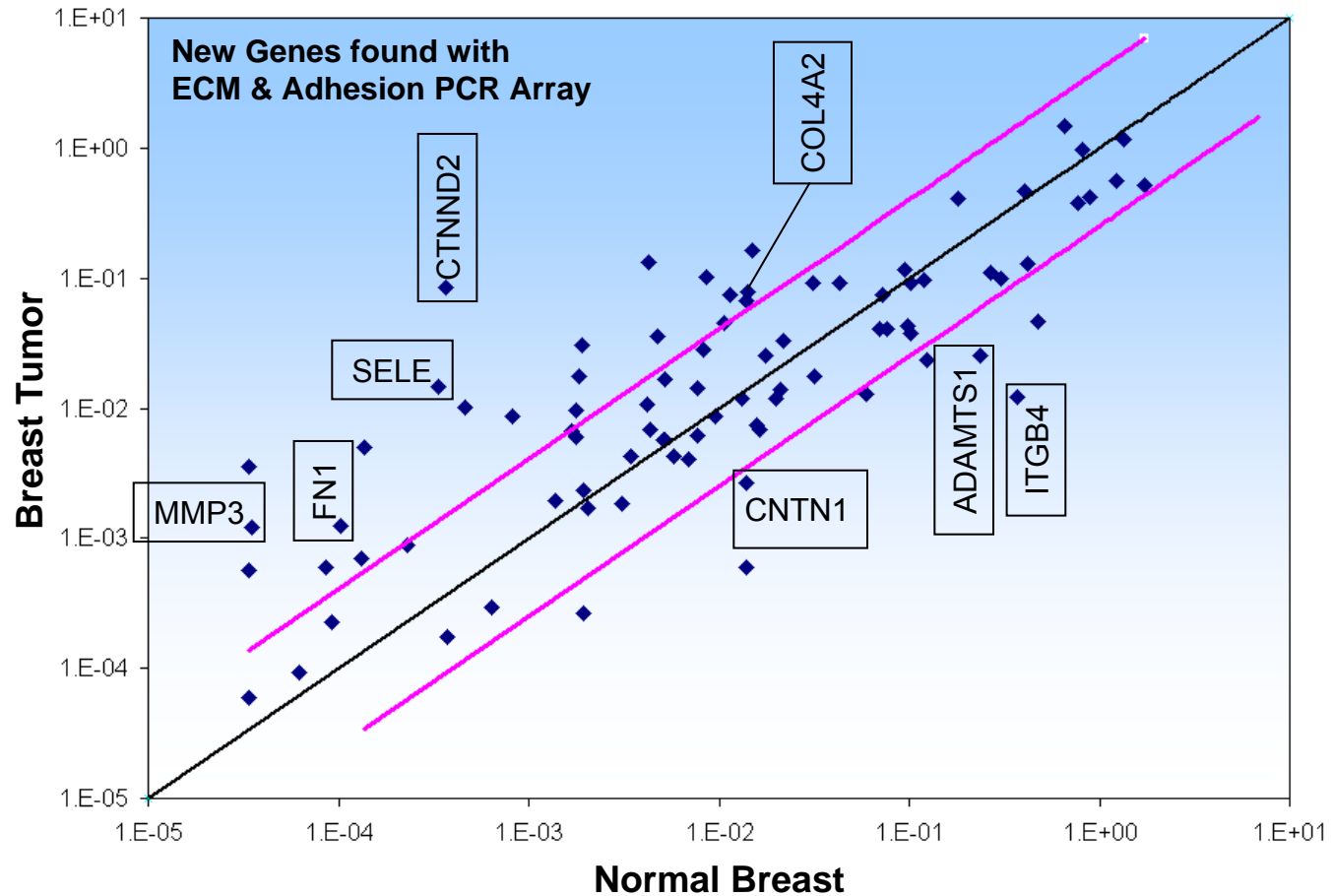
ECM & Adhesion Results

- 38 genes with significant change; 27 up- & 11 down-regulated in tumor

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ECM & Adhesion Results

- 38 genes with significant change; 27 up- & 11 down-regulated in tumor

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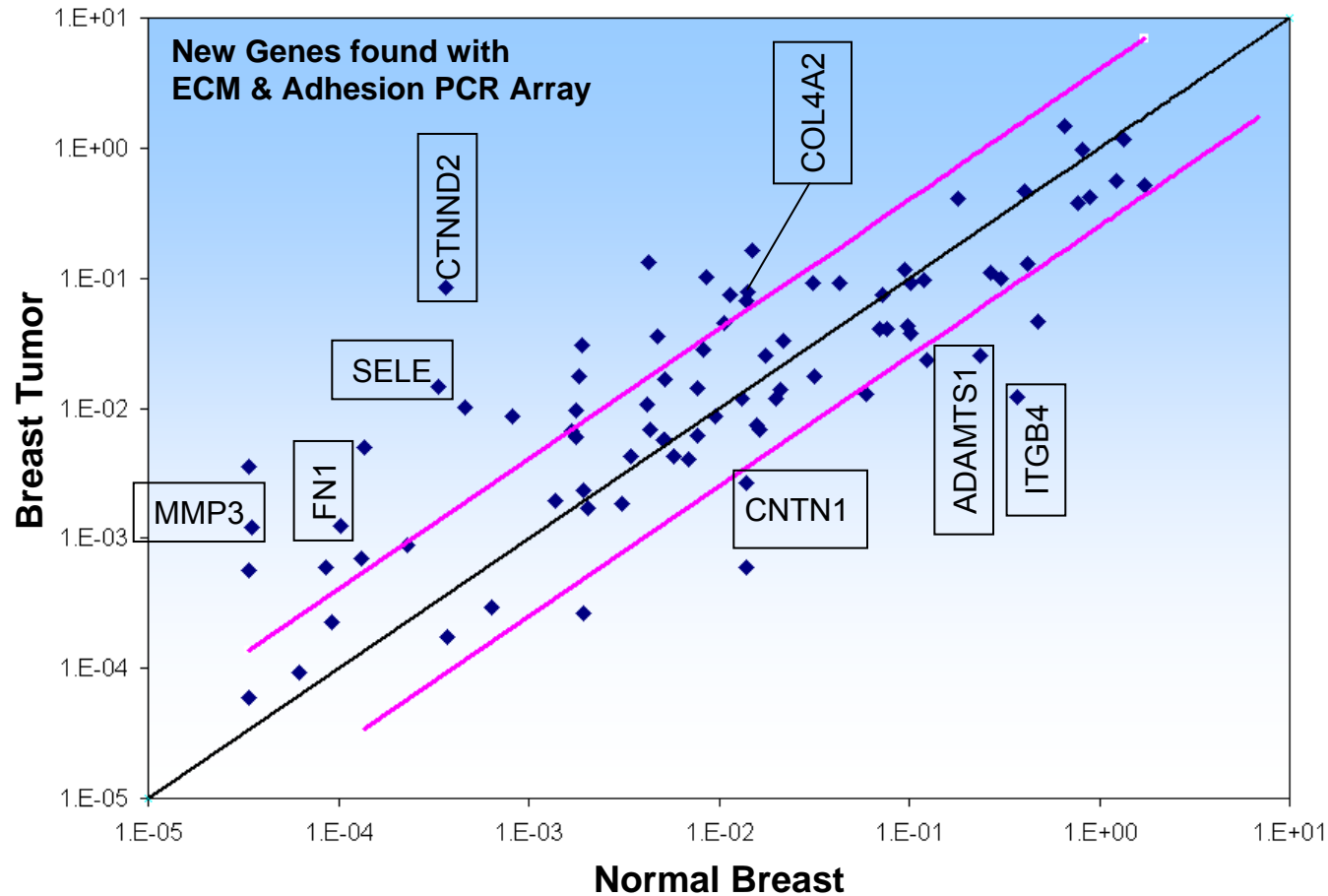# ECM & Adhesion Results

- 38 genes with significant change; 27 up- & 11 down-regulated in tumor

ECM & Adhesion Results

- 38 genes with significant change; 27 up- & 11 down-regulated in tumor
- **CONCLUSION:** Verifiable changes in cell adhesion-related gene expression upon oncogenesis of these and other breast tumors

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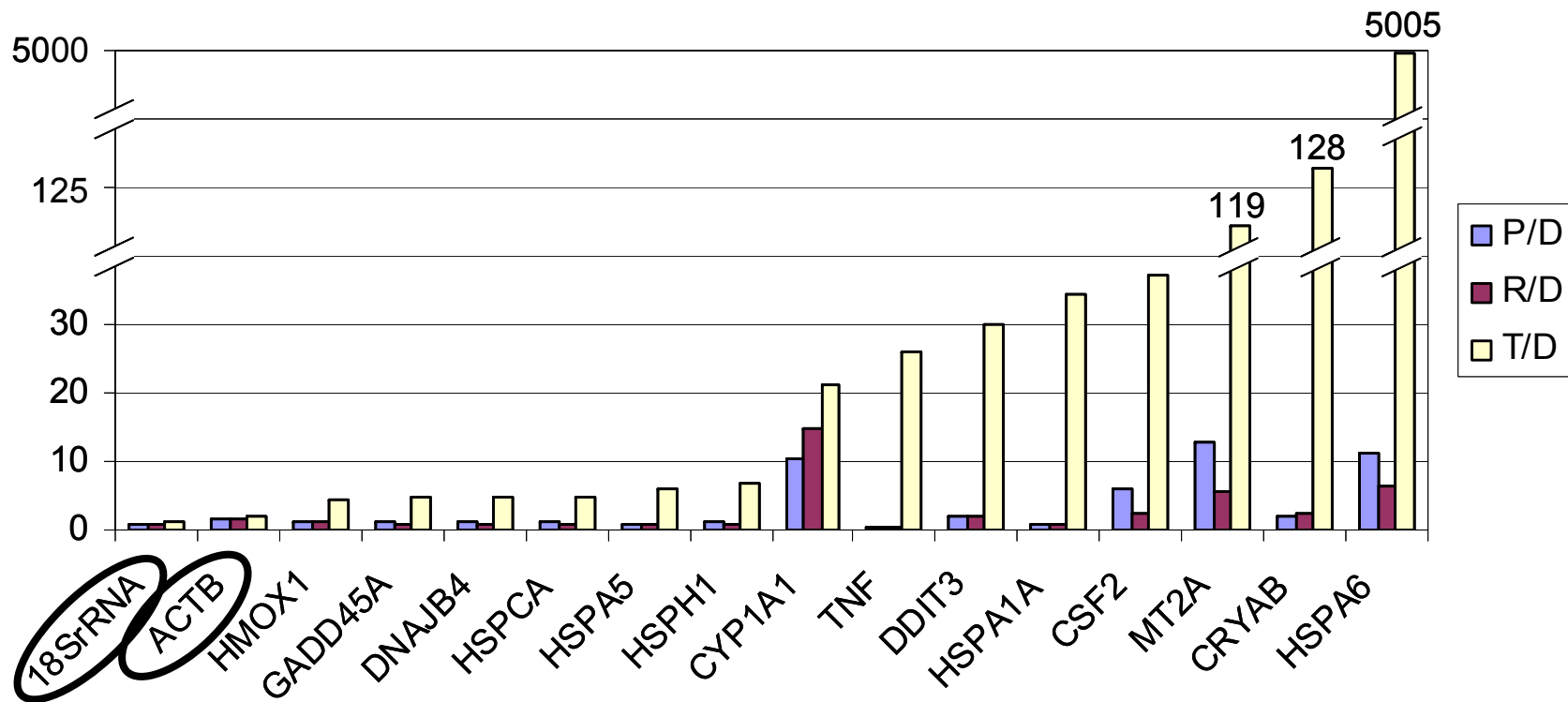   - Ascertain Liver Toxicity of Similar Drug Candidates

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Toxicology Research Application Example

- Rezulin (Troglitazone or "Tro" or "T"), a glitazone PPARγ agonist, was approved for treatment of type 2 diabetes mellitus, but was withdrawn from the market due to idiosyncratic liver toxicity.

- Two similar drugs, Avandia (Rosiglitazone or "Rosi" or "R") and Actos (Pioglitazone or "Pio" or "P"), are considered to be safe treatments for the same condition.

- The expression profile of key drug metabolism genes should be different in cells treated with Rezulin versus those treated with Avandia and Actos.

- Treat HepG2 cells with these three drugs, and characterize gene expression with the Human Drug Metabolism and Stress & Toxicity PathwayFinder™ RT²Profiler™ PCR Arrays.

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Summary Drug Metabolism Results

Symbol	Pioglitazone Fold Change	p-value	Rosiglitazone Fold Change	p-value	Troglitazone Fold Change	p-value
CYP17A1					- 4.4	3.9E-03
CYP1A1	17.8	2.8E-06	21.4	4.3E-06	42.7	1.2E-06
CYP2B6	5.4	7.7E-05	3.5	1.5E-03	4.2	4.5E-04
GPX2					- 9.6	2.8E-03
GSTP1					7.1	2.8E-03
CYP3A5	3.0	2.6E-04	3.3	4.3E-04		
GCKR	3.1	2.2E-06	2.9	3.0E-05		
MPO			3.0	1.7E-03		
MT2A	18.6	1.2E-05	8.1	1.4E-03	297.5	5.5E-07
NAT2					-4.9	5.3E-04
NOS3	6.1	9.7E-07	5.3	3.4E-06	4.2	6.7E-05

Stress & Toxicity PathwayFinder

Housekeeping Genes

FOCUS ON YOUR PATHWAY™

SuperArray
Bioscience Corporation
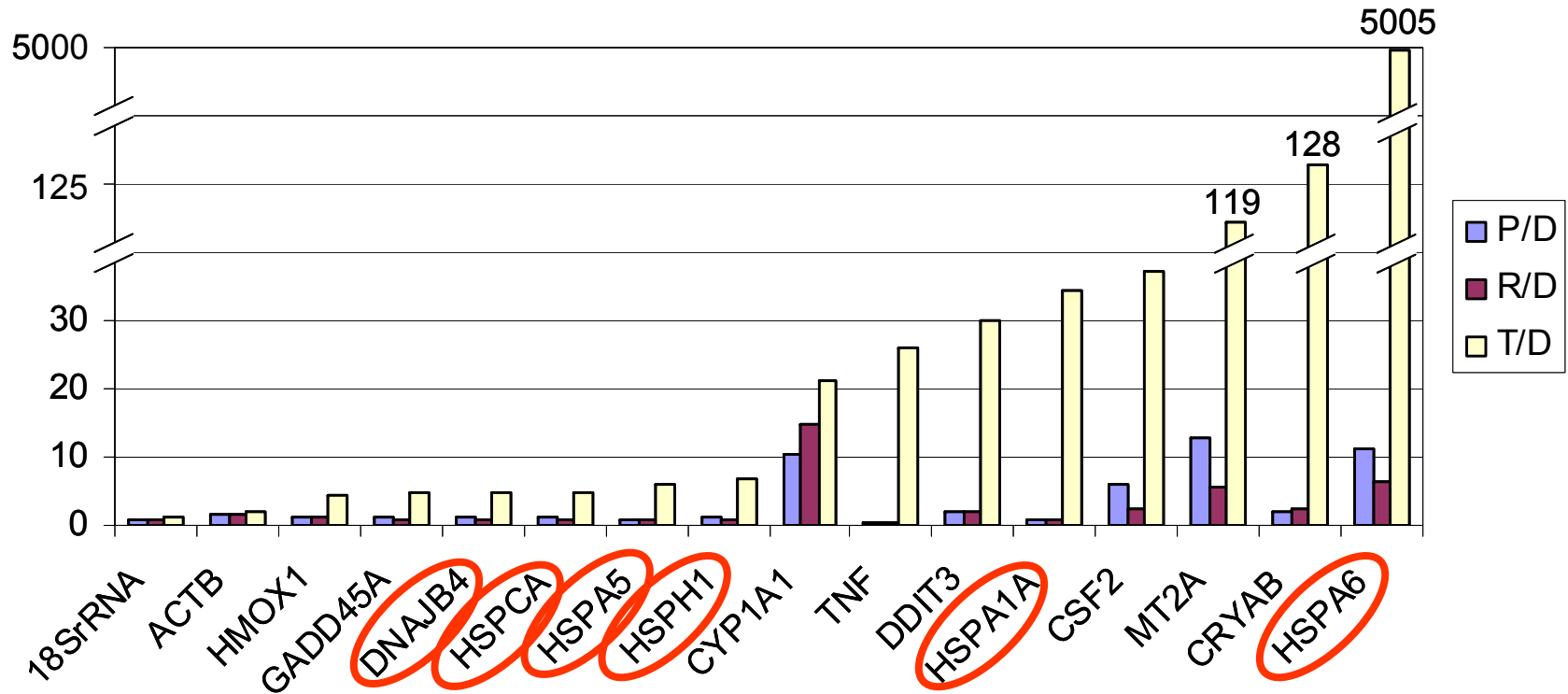
Stress & Toxicity PathwayFinder
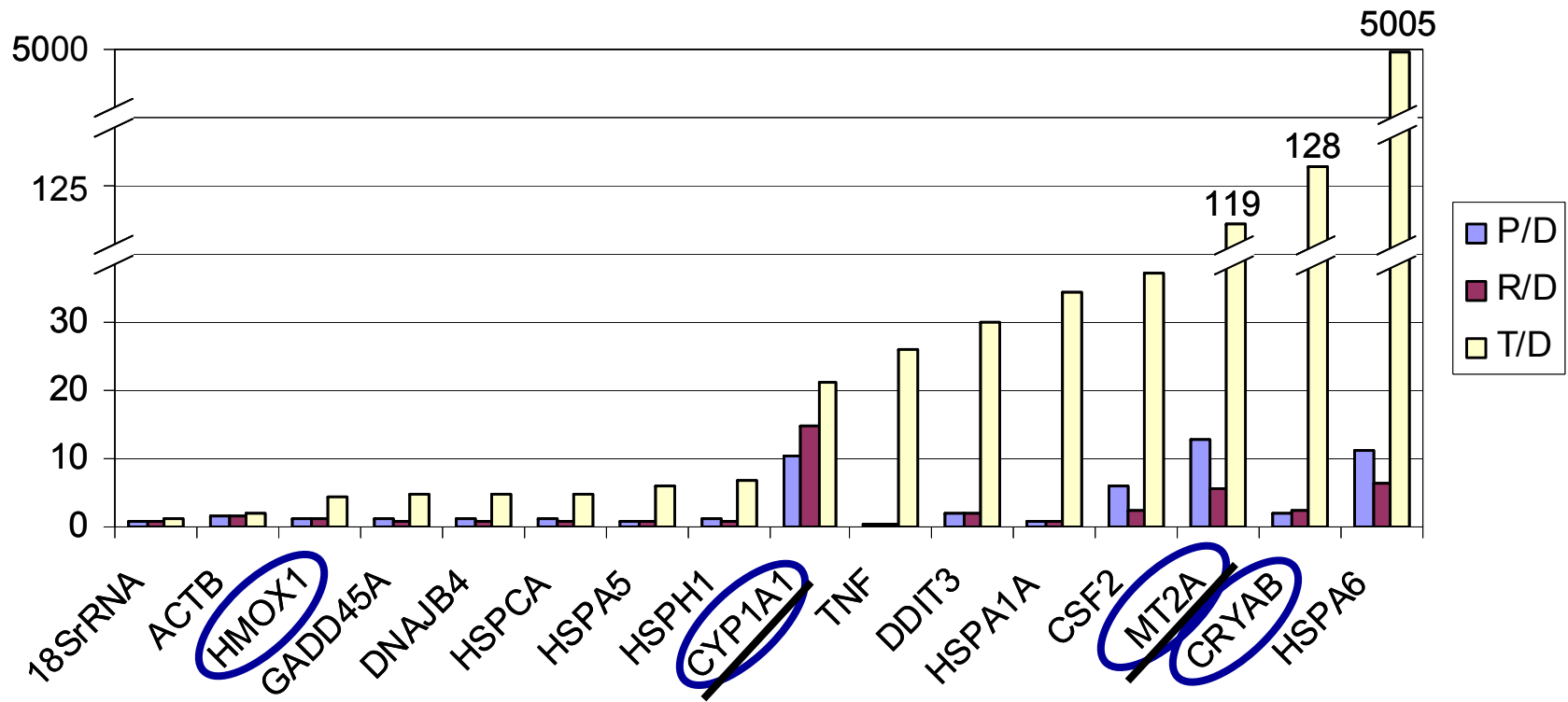
Oxidative / Metabolic Stress

Stress & Toxicity PathwayFinder

Heat Shock

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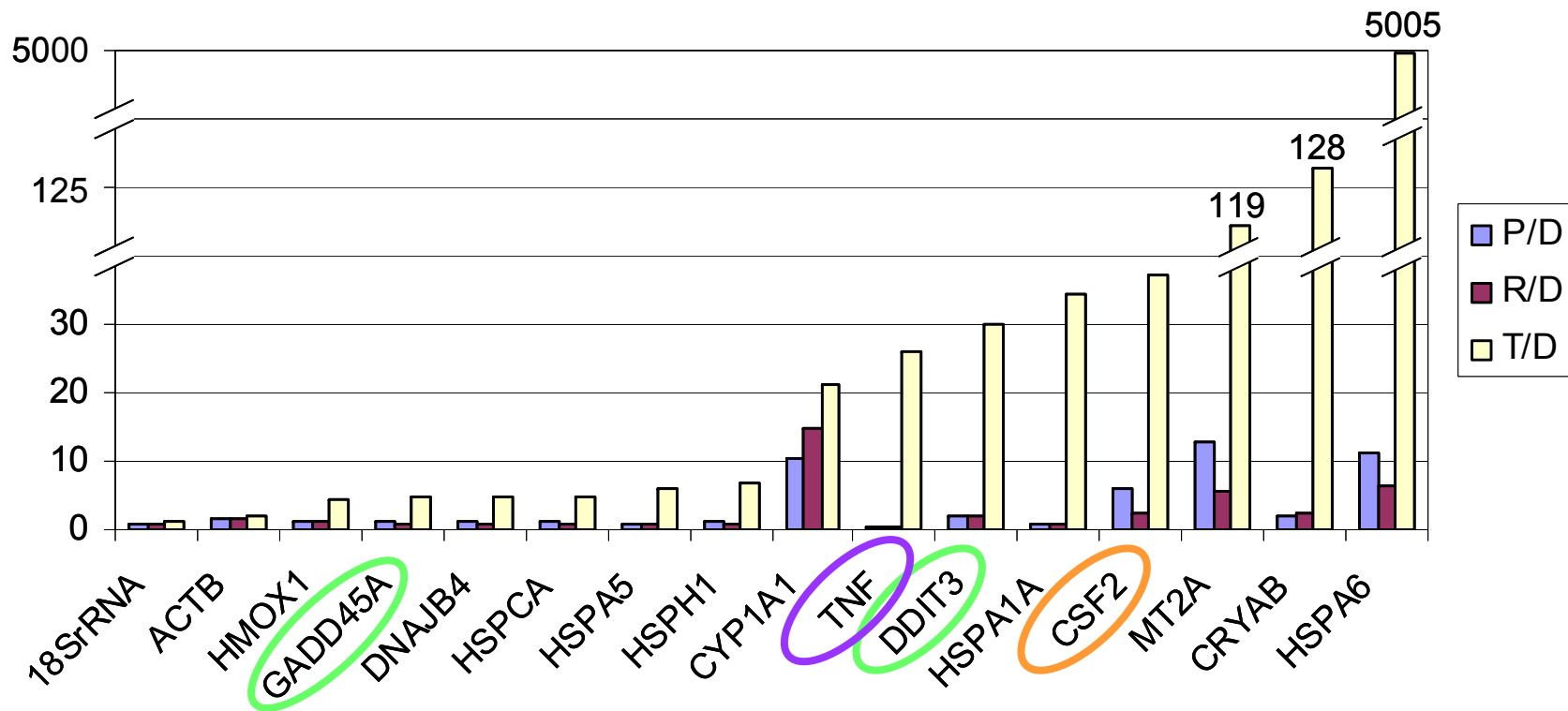
Stress & Toxicity PathwayFinder

Stress & Toxicity PathwayFinder
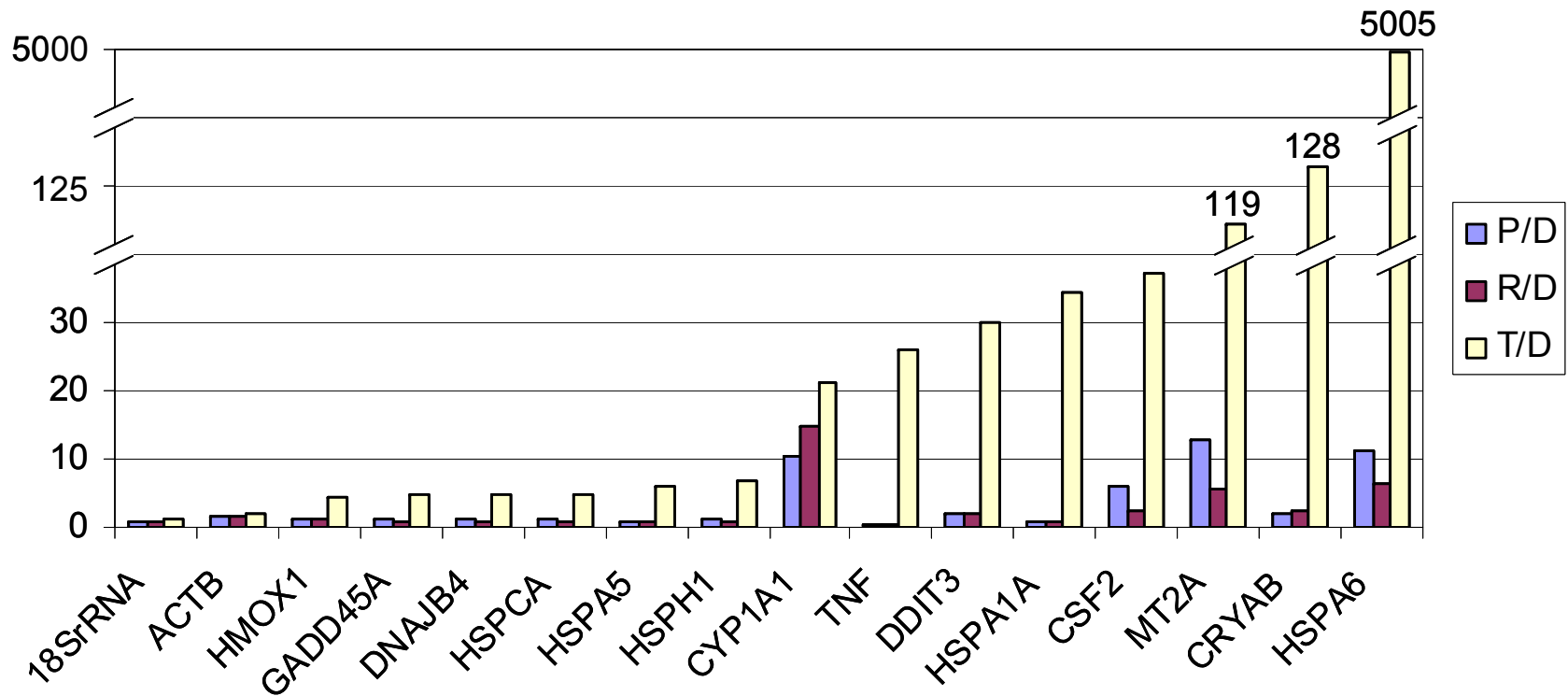
CONCLUSION: Withdrawn drug with idiosyncratic liver toxicity induces very different gene expression profile changes in HepG2 cells than safer drugs still on the market.

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Market Acceptance

- **Launched May 2005**
  - V2.0 November 2006, V3.0 September 2007
- **Current Publication List: 30+**
  - Most added in last 3 months
  - Expected to grow very rapidly
- **Core Facility Adoption**
  - 5 to 10 more in next 3 months

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SUMMARY

- **Simplicity**
  - Pathway-focused gene expression to your lab or core facility
  - Three-component system: arrays, master mix, first strand kit
  - Two-hour protocol from RNA to data
- **Breadth of Pathway Content**
  - Profile the genes and pathways that you really care about
  - More than 45 pathways for human, mouse, and rat
  - Customizable by gene, set, or pathway
- **Real-Time PCR Performance**
  - Reliability, Reproducibility, Sensitivity, Specificity
- **Application Examples**
  - Cancer and Toxicology Research
- **How can <u>YOU</u> use PCR Arrays in <u>YOUR</u> research?**

FREE PCR Array Trial Offer
For First-Time Customers

- First time users may request two free PCR Arrays:
- Package includes Technical Resources and a special quote (50% OFF) for the rest of the PCR Array System!
- Contact your Account Manager:

  Call Toll-Free: 888-503-3187 or 301-682-9200

  Email: support@superarray.net

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PCR Arrays

An Advanced qPCR Technology to Enhance Your Analysis of Pathways

Questions?

Contact Technical Support M-F 9AM-6PM Eastern
Telephone: 888-503-3187; Email: support@superarray.net

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