



***GENpathway***

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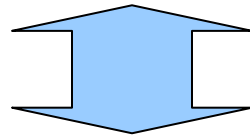
The ChIP Assay Experts™

[www.genpathway.com](http://www.genpathway.com)

# Genpathway - The ChIP Assay Experts

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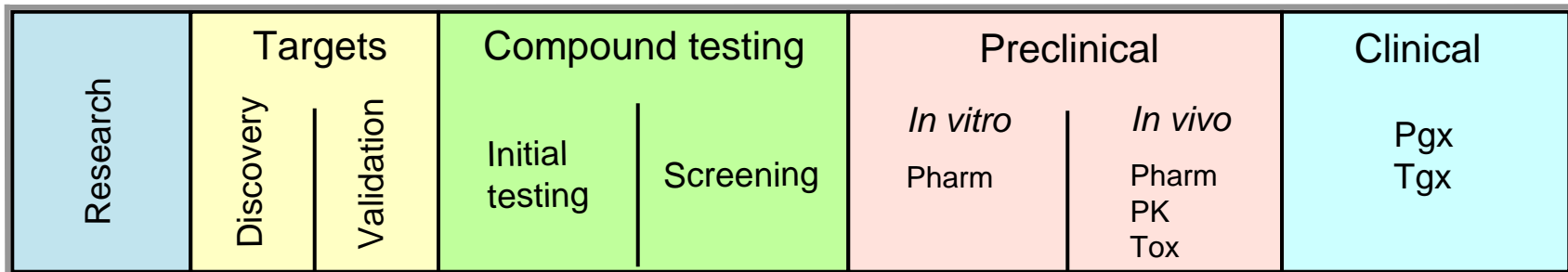
Genpathway: The leading provider of ChIP and DNA methylation assays, expertise & services



Combining Genpathway's assays with Affymetrix tiling arrays provides researchers a complete ChIP-on-Chip and Methylated DNA IP-on-Chip solution

# Where Can You Apply Genpathway's Technology?

- Target Identification
- Target Validation
- Compound Efficacy
- Mechanism of Action
- Pharmacogenomics
- Clinical Diagnostics

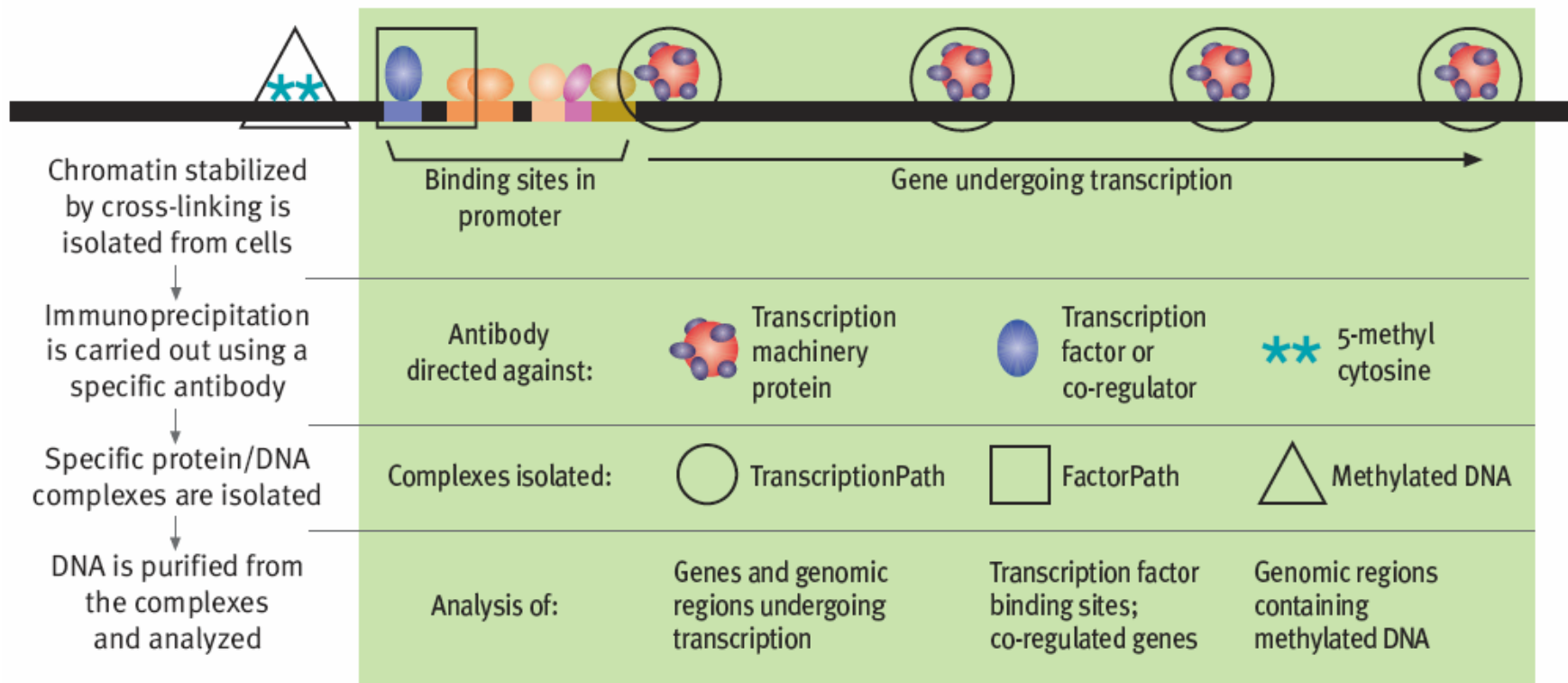


# How can Genpathway's novel assays move your programs forward?

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- **Discover transcription factor and co-regulator binding sites**, plus the genes they regulate, across the entire genome
- **Profile the transcriptome** in your cells, but at the DNA level, which gives highly reproducible results
- **Quantify changes** in gene transcription and regulation at any timeframe, even within minutes
- **Discover and quantify epigenetic changes** including DNA methylation and chromatin modifications
- **Identify new genes** associated with drug treatment or disease mechanisms

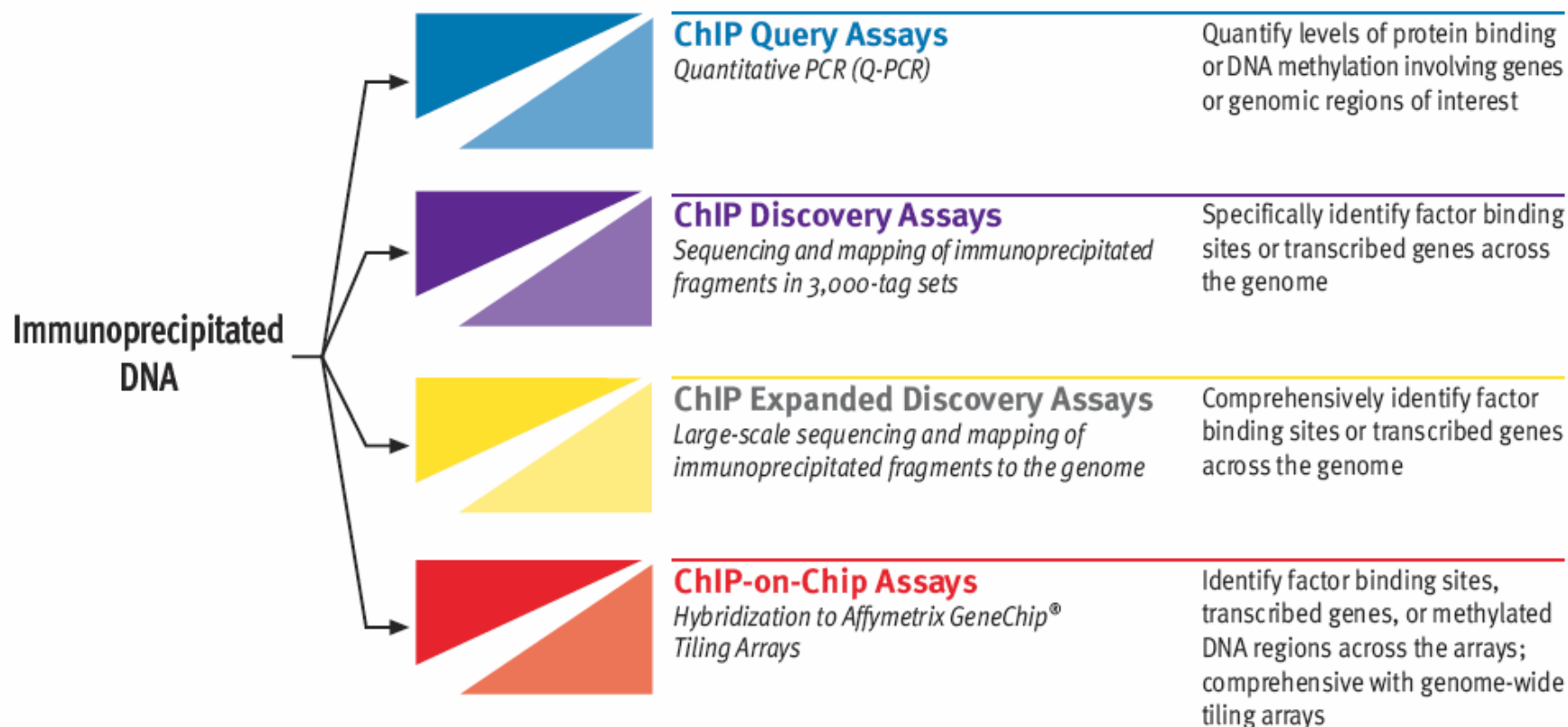
# Genpathway's ChIP Assays: FactorPath, TranscriptionPath & Methylated DNA



\*\* Immunoprecipitations are carried out directly on DNA

*Genpathway's optimized procedures give maximum sensitivity and minimal background binding.*

# Genpathway's analysis modes: Highly informative and reproducible



# Genpathway's ChIP-on-Chip analysis: a high quality combination

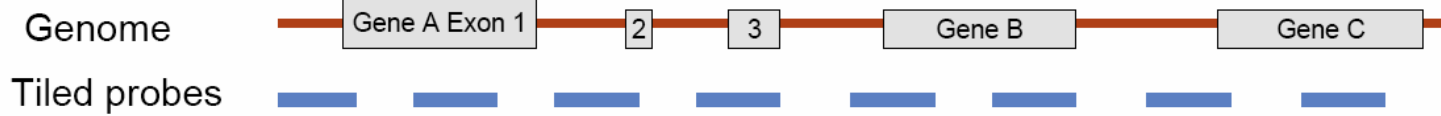


## ChIP-on-Chip Assays

Genome-wide assays combining chromatin immunoprecipitation and Affymetrix GeneChip® Tiling Arrays

<b>FactorPath ChIP-on-Chip/Tiling</b>	Comprehensive discovery of factor binding sites across whole-genome tiling arrays	Analyze hundreds to thousands of binding sites
<b>FactorPath ChIP-on-Chip/Promoter</b>	Discovery of factor binding sites in promoter regions	Analyze hundreds of binding sites
<b>TranscriptionPath ChIP-on-Chip/Tiling</b>	Comprehensive discovery of transcribed genes across whole-genome tiling arrays	Analyze thousands of transcribed genes
<b>Methylated DNA IP-on-Chip/Tiling</b>	Comprehensive discovery of methylated DNA regions across whole-genome tiling arrays	Analyze thousands of methylated DNA regions
<b>Methylated DNA IP-on-Chip/Promoter</b>	Discovery of methylated DNA regions in promoter regions	Analyze thousands of methylated DNA regions

*Affymetrix' Tiling Arrays contain 25-mer probes separated by 10 bp spaces across the genome.*



# How investigators are using the Genpathway-Affymetrix Total Solution

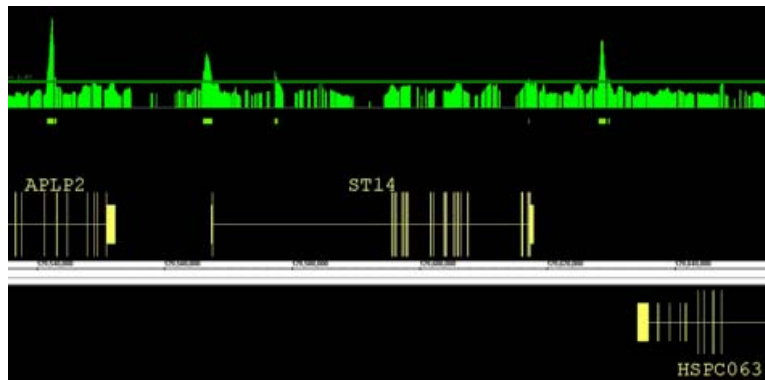
Examples of Therapeutic Areas	<b><i>FactorPath, TranscriptionPath and Methylated DNA assays provide new, valuable information</i></b>
Oncology	Determine drug mechanism of action
Metabolic disorders	Discover and validate drug targets
Toxicology	Identify downstream effects of compounds
Inflammation	Discovery biomarkers for clinical & discovery programs
Endocrinology	Determine disease mechanisms for future targets
Genetic disorders	Identify side effects of compounds
Cardiovascular	Determine efficacy of compounds
Diabetes	Define genetic pathways for important programs
Virology	Validate results obtained by other assays

# Genpathway *Total Solution* for Biomarkers

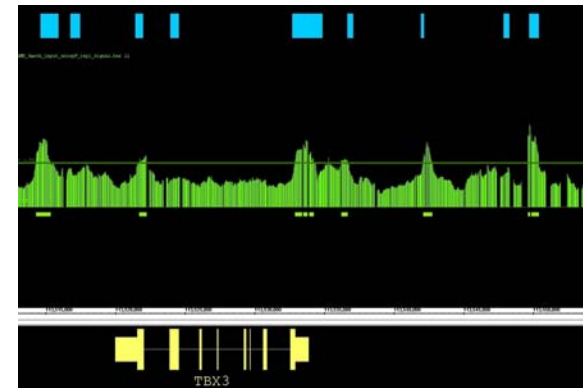
<b>Program</b>	Cancer (or other Therapeutic Areas)
<b>Customer need</b>	Identify <b>biomarkers</b> in disease-relevant samples, e.g., tumors, other tissues or blood
<b>Genpathway solution</b>	1. Screen <b>whole genome for MeDNA regions</b> in sets of affected and unaffected tissues
	2. Select <b>MeDNA panel</b> for custom array(s)
	3. Use <b>custom MeDNA arrays</b> to test large numbers of samples; may develop subpanels
	4. Use to classify patients, predict and correlate response to treatment, predict metastases, screen compounds, predict unwanted side effects

# Detect Me-DNA genome-wide by Methylated DNA IP-on-Chip

- **Methylated IP-on-Chip/Tiling** for detection of methylated DNA regions in MDA-MB-231 cells.



***Methylated DNA regions in promoters, within genes, and between genes***



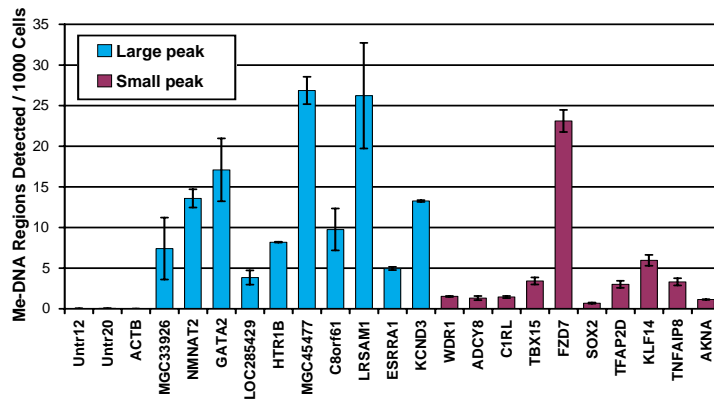
***Very good correlation with CpG islands (blue), but also differences***

Locations of peaks representing Methylated DNA		
Peaks on Array F (chromosomes 8, 11, 12)	Number	Percent
Total number on Array F	997	
Extrapolated to entire genome	6979	
In CpG Islands +/- 200 bp	233	23.4%
In promoters (-1 to -10,000 from TSS*)	123	12.3%
In genes	584	58.6%
In exons +/- 100 bp	137	13.7%
Unannotated (no gene within 10 kb)	216	22.0%

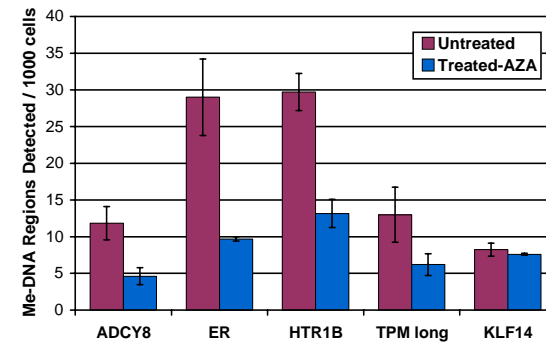
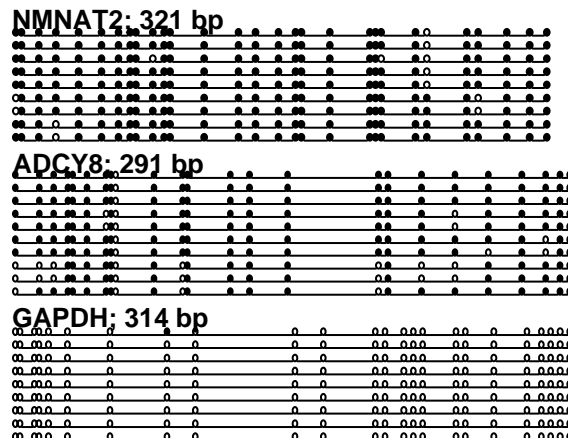
\*TSS = Transcription Start Site

# Validate peaks on the arrays as true methylated DNA regions

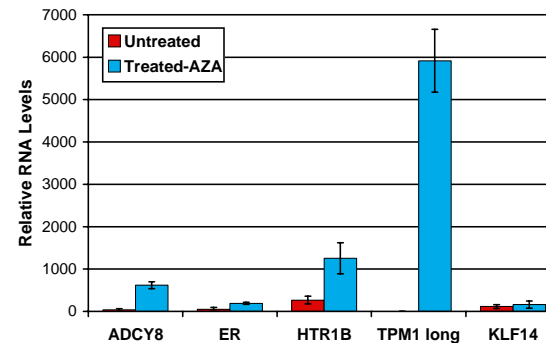
**Methylated DNA Query: Peaks on arrays represent Me-DNA regions**



**Bisulfite sequencing: Peaks on arrays represent Me-DNA regions**








**5-Azacytidine treatment: Me-DNA regions exhibit decreased levels**



**5-Azacytidine treatment: RNAs for corresponding genes increase**

# Genpathway: The Only Whole-Genome Methylated DNA Total Solution

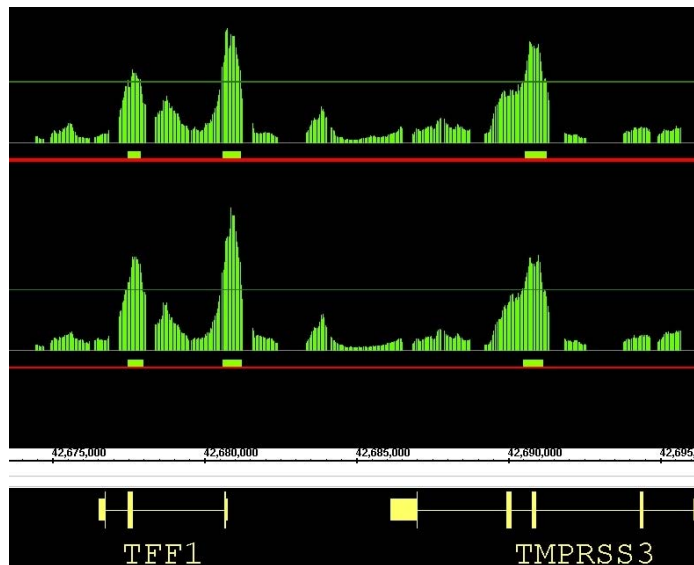
	<b>High Sensitivity</b>	No discrimination against any sequences (vs. enzyme digestion)
	<b>Comprehensive</b>	Whole-genome tiling arrays (vs. CpG island or promoter analysis only)
	<b>High Throughput</b>	Whole-genome tiling arrays (vs. bisulfite-sequencing)
	<b>Robust and Reproducible</b>	Consistent results (vs. variability of bisulfite PCR)
	<b>High Specificity</b>	Genpathway's optimized protocols result in low background binding

# Genpathway *Total Solution* for Targets Based on Pathways

<b>Programs</b>	Metabolic Diseases (or other Therapeutic Areas)
<b>Customer need</b>	<b>Identify and validate targets</b> based on nuclear hormone pathways (or other factors or pathways)
<b>Genpathway solution</b>	1. <b>Identify nuclear hormone receptors</b> and co-regulators important to program (by Customer)
	2. Carry out <b>whole genome FactorPath ChIP-on-Chip</b> for each factor in cells or tissues
	3. Identify factor binding sites (and their regulated genes) across the genome
	4. Compare binding results for factors and co-regulators with <b>genes</b> and cell systems
	5. Use <b>subset of binding sites and genes</b> as biomarkers for testing compounds

# Identify factor binding sites across the genome

- FactorPath ChIP-on-Chip/Tiling to Affymetrix GeneChip whole-genome tiling arrays.



**SRC-3 binding sites in MCF7 cells**

*Hybridization to duplicate arrays shows high reproducibility.*

Examples of array peaks on chromosome 19					
Interval start	Interval end	Gene	Distance to gene start	Distance to gene end	Location
1132744	1133240	KIAA0963	-7981	-74599	Upstream
2486540	2486829	GNG7	110449	-24611	In gene
14815918	14816464	OR7A10	-2775	-3704	Upstream
15460283	15460958	PGLYRP2	-9646	-20495	Upstream
15947884	15948282	LOC126537	22555	3333	Downstream
16434947	16436450	CALR3	31553	14438	Downstream
34854025	34854662	PLEKHF1	6222	-4179	In gene
35865774	35865953	None*			
36463389	36464254	ZNF537	-2239	-6561	Upstream
37545573	37546208	ZNF507	111726	-20256	In gene

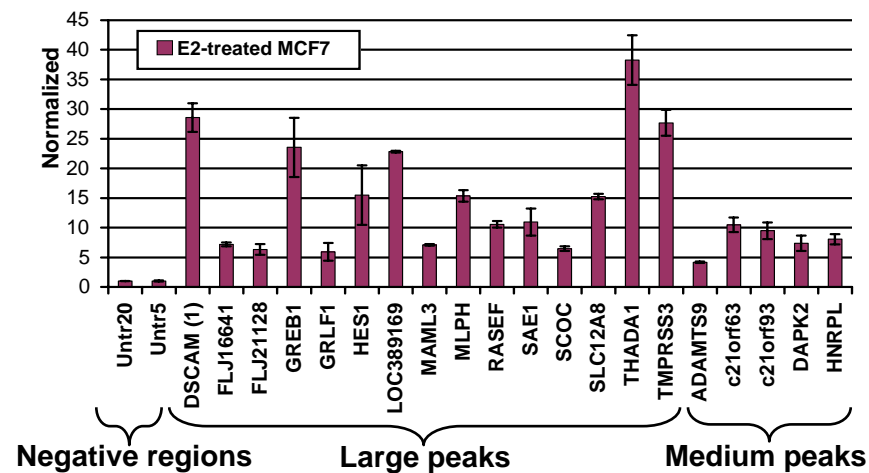
\*No known gene within 20 kb

*Excerpt of list of binding sites provided by Genpathway's software*

# Validate peaks on arrays as true factor binding sites

- FactorPath ChIP-on-Chip/Tiling for detection of SRC-3 binding sites in MCF7 cells.

**FactorPath Query:**  
peaks on arrays  
represent SRC-3  
binding sites



Number of SRC-3 array peaks	
Tiling Array Type	No. of peaks
B (chromosomes 2, 9, 19)	371
C (chromosomes 3, 21, 22, X)	307
D (chromosomes 4, 15, 18, 20)	474
Extrapolated to whole human genome	2688

Peak locations relative to genes		
Peaks on Array B	Number	Percent
Upstream (-5 kb)	16	4.3%
Upstream (-5 to -20 kb)	49	13.2%
In gene	174	46.9%
Downstream (+20 kb)	26	7.0%
No gene within 20 kb	106	28.6%
<b>TOTAL</b>	<b>371</b>	<b>100.0%</b>

*A minority of SRC-3 sites are upstream of genes*

# Genpathway *Total Solution* for Toxicology or Discovery

<b>Program</b>	Toxicology
<b>Customer need</b>	Identify <b>mechanisms</b> of toxicity/action for compounds
<b>Genpathway solution</b>	<b>1. Screen whole genome for differentially transcribed genes</b> in cells or tissues treated with prototype compounds
	2. Identify genes differentially transcribed in treated vs. untreated cells or tissues
	<b>3. Screen additional compounds</b> and identify useful biomarkers
	4. Develop <b>custom panels of genes</b> for screening large numbers of compounds in variety of cells and tissues, including clinical samples



# Evaluate the transcriptome at the DNA level

**TranscriptionPath ChIP-on-Chip/ Tiling identified approx. 10,000 transcribed genes in MCF7 cells.**

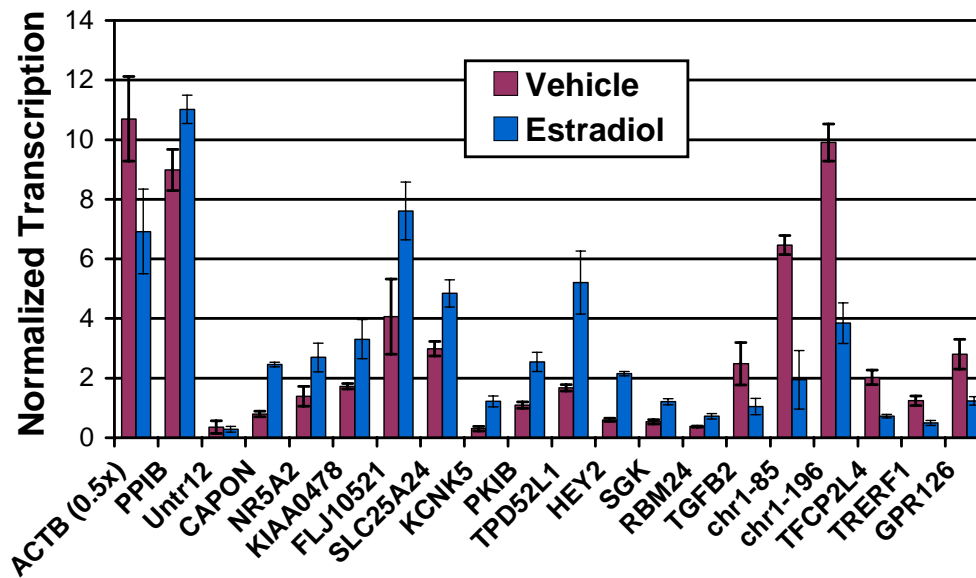
**Approx. 10% were novel genes.**

**Approx. 10% were differentially transcribed.**

**TranscriptionPath Query validated both transcription and differential transcription.**

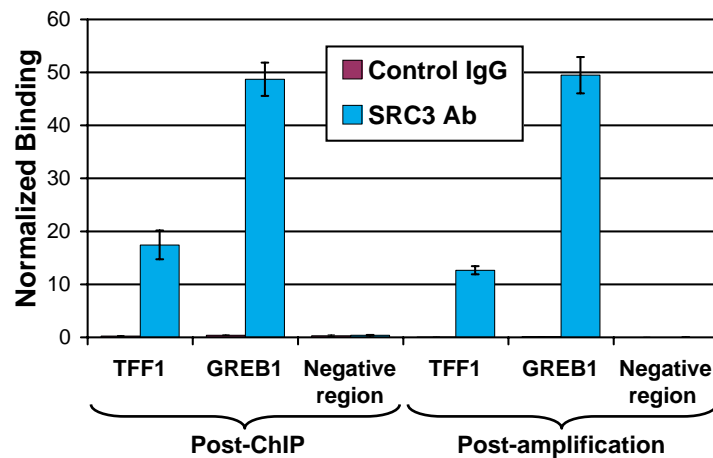
**Chr1-85 and chr1-196 represent novel genes.**

Characteristics of regions undergoing transcription			
	Vehicle-treated cells	Estradiol-treated cells	Differential
Number of regions	1360	1301	136
On chromosome 1	890	863	76
On chromosome 6	470	438	60
Annotated genes	1239	1160	98
Unannotated regions	121	141	38

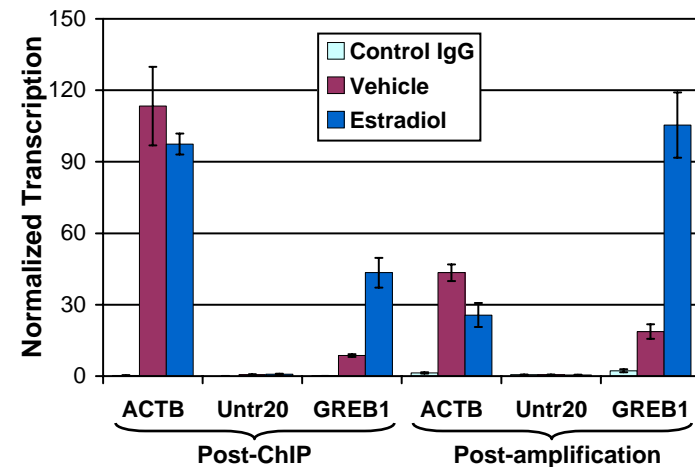


# Ensure ChIP/DNA IP quality with validated antibodies and chromatin

- **FactorPath Query** of ChIP DNA demonstrating specific selection of desired protein/DNA complexes, and differential binding or transcription if possible.



**FactorPath** assay for *SRC-3* binding sites



**TranscriptionPath** assay for transcribed genes

*Validation prior to array hybridization, including antibody testing as needed, ensures high quality array results.*

# Genpathway's ChIP and DNA IP Assay Services

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CHIP Query Assays

FactorPath Query  
TranscriptionPath Query  
Methylated DNA Query



CHIP Discovery Assays

FactorPath Discovery



CHIP Expanded Discovery

FactorPath Expanded Discovery  
TranscriptionPath Expanded Discovery



CHIP-on-Chip Assays

FactorPath CHIP-on-Chip/Tiling  
FactorPath CHIP-on-Chip/Promoter  
TranscriptionPath CHIP-on-Chip/Tiling  
Methylated DNA IP-on-Chip/Tiling  
Methylated DNA IP-on-Chip/Promoter

# Genpathway + Affymetrix - The Total Solution

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- **Genpathway - *The ChIP Assay Experts***
- Affymetrix' Tiling Arrays + Genpathway's Assays & Services provide customers with an immediate total ChIP solution
- Optimized procedures for ChIP/IP give superior results - Validation of Customer's chromatin and Ab's fully qualified
- ChiP Analysis Software (ChAS) provides customers with easily understandable and compiled results
- Superior Technical and Customer Support regarding samples, genomic regions, antibodies, and more
- Quality Control at various steps in the assay - *we take the variability out of the assay*
- **Affymetrix + Genpathway is the only whole-genome ChIP-on-Chip and Methylated DNA IP-on-Chip Assay Total Solution**



***GENpathway***

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The ChIP Assay Experts™

[www.genpathway.com](http://www.genpathway.com)