



Data Sheet

❏ ToxFX™ Analysis Suite

The ToxFX™ Analysis Suite provides a complete toxicogenomics solution. It combines Affymetrix GeneChip® brand arrays with an automated analytic report developed by Iconix Biosciences. The analytical approach used is based upon information contained in the Iconix DrugMatrix® reference database and utilizes Iconix' proprietary Drug Signatures® and pathways libraries. The analysis solution can be used with the whole genome GeneChip® Rat Genome 230 2.0 Array or the focused GeneChip® Rat ToxFX 1.0 Array. The GeneChip Rat ToxFX 1.0 Array contains customized content carefully selected by Iconix scientists and has been designed for use with the ToxFX Analysis Suite.

Applications

As pioneers in the fields of microarray technology and toxicogenomics, Affymetrix and Iconix have worked together to co-develop the ToxFX Analysis Suite, offering an easy-to-use and rapid assay for assessing compound safety. Key applications for the ToxFX Analysis Suite include:

- Compound prioritization during drug discovery
- Understanding mechanisms of toxicity underlying drug candidate safety issues
- Confirmation of drug candidate safety
- Identification of potential safety issues associated with toxicants and/or chemicals in living animals as part of environmental safety studies

Toxicogenomics is rapidly becoming a standard tool during the discovery phase in the pharmaceutical industry. It promises earlier assessment of compound toxic liabilities in a single streamlined assay. This enables the most promising compounds from both a therapeutic and safety perspective to be advanced to the next stages of development, thereby increasing the overall efficiency of the development process.

Benefits of the ToxFX Analysis Suite include:

- **Automated analysis for dramatic time savings in data interpretation**
The ToxFX analysis pipeline offers rapid turnaround, providing a fully annotated toxicology report from the expression data in minutes

- **Faster compound priority decisions**
Following tissue collection from animals, expression data can be generated in as little as three days and a fully documented toxicity report can be produced from these data in minutes
- **Contextual interpretation of results**
Rapid interpretation of gene expression data based on an understanding of how changes in gene expression relate to specific biological endpoints in the DrugMatrix database
- **Single assay assessment of a wide variety of toxic outcomes**
Understanding a compound's safety profile traditionally requires a variety of different assays, including histopathology, hematology and blood chemistry. A single gene expression study can capture the same information as a collection of these traditional approaches
- **Greater sensitivity than either traditional blood chemistry or histopathology**
In many cases evidence of toxic outcomes is detected earlier, reducing the need for more costly, longer-term animal studies
- **Earlier prediction of toxicity reducing animal study length**
Overall reduction in animal study costs due to reduction in animal husbandry costs and compound requirements
- **Economical assay for large-scale compound screening**
The GeneChip® Rat ToxFX 1.0 Array provides substantial savings to both the array and analysis costs, enabling safety assessment of more compounds earlier in the drug development process



DrugMatrix® Reference Database

Developed by Iconix, DrugMatrix® is the world's largest toxicogenomics reference database. DrugMatrix contains results from thousands of experiments in which rats were systematically treated with drugs and toxicants. From each treated rat, total RNA from liver, heart and kidney tissues was run on Affymetrix GeneChip® Rat Genome 230 2.0 Arrays (Rat 230 2.0 Arrays) in conjunction with the collection of standard histopathology, hematology and clinical chemistry data.

The resulting matrix of expression and common toxicology assay data provides Iconix with the unique ability to extensively cross-reference between the two types of data. Additionally, the database provides an interpretive reference set for understanding the key molecular mechanisms causative to a given toxic response. This has enabled Iconix scientists to develop a unique understanding of gene expression patterns that correlate to key endpoints of toxicological interest.

Iconix informatics scientists have mined the DrugMatrix database to define a Drug Signatures® library. Drug Signatures are patterns of gene expression changes that can be used as biomarkers, linking the expression pattern of groups of genes to key

biological endpoints. Signatures represent, in essence, groups of genes whose expression is strongly correlated to a specific biological outcome (e.g., liver necrosis or ALP increase). As such, Drug Signatures can identify specific off-target effects and toxicological reactions in different organs and tissues as well as specific mechanisms of action. A specific and focused set of these Drug Signatures have been selected by Iconix scientists for inclusion in the ToxFX Analysis Suite. The ToxFX analysis, therefore, provides many of the benefits of the DrugMatrix database without the requirement of a subscription or license.

The ToxFX Automated Analysis

A drug treatment will typically alter the expression of several hundred or even thousands of genes in the tissues of a treated animal. It is a complex and time-consuming task to understand which of those changes are meaningful and indicative of a potential toxicity.

A typical ToxFX study is composed of data generated on multiple arrays and represents multiple time points and compound doses. The ToxFX Analysis Suite makes it possible to submit the data, using the ToxFX Study Builder software, and in minutes return an analysis report that provides a clear picture of potential safety problems, the genes that are likely

to be most important in relation to those problems and the biological pathways that are most likely to play a role in any predicted toxicity. These results enable decision-making far sooner than the weeks or months that it takes to produce a typical pathology report. The ToxFX analysis accomplishes this task by using several tools, including Iconix' DrugMatrix reference database, Drug Signatures and Pathway Impact analysis.

Supported Arrays

The ToxFX Analysis Suite has been designed to support the analysis of either the GeneChip Rat ToxFX 1.0 Array (Rat ToxFX 1.0 Array) containing focused content or the whole genome Rat 230 2.0 Array. The choice of GeneChip® array will depend upon the requirements of the study.

The probe sets chosen for the focused Rat ToxFX 1.0 Array are based on the knowledge gained from the thousands of experiments in DrugMatrix and the associated Drug Signatures and pathway library. The probe sets represent a subset from the well-proven content found on the Rat 230 2.0 Array and include the genes that make up a total of 55 toxicological and pharmacological Drug Signatures in rat liver, heart and kidney. Also included are genes involved in 22 key toxicology pathways, as well as a set

Figure 1. The ToxFX study workflow

The ToxFX analysis starts with a typical dose and time response study in the rat. Tissue samples are collected and total RNA is extracted and labeled using standard procedures. The labeled cRNA is then run on an Affymetrix GeneChip® Rat Genome 230 2.0 or GeneChip® Rat ToxFX 1.0 Array. Following data collection, CHP files are generated in Expression Console™ software and transferred to the ToxFX Study Builder for final analysis. The analysis results are summarized in an easy-to-read and comprehensive report delivered as a PDF file directly to the user's computer.

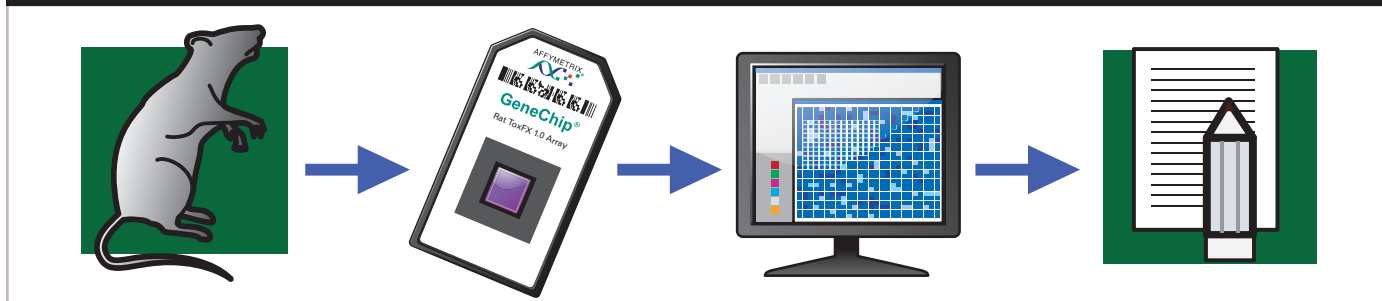


Figure 2. Distribution and use of probe sets included in the GeneChip® Rat ToxFX 1.0 Array focused content.

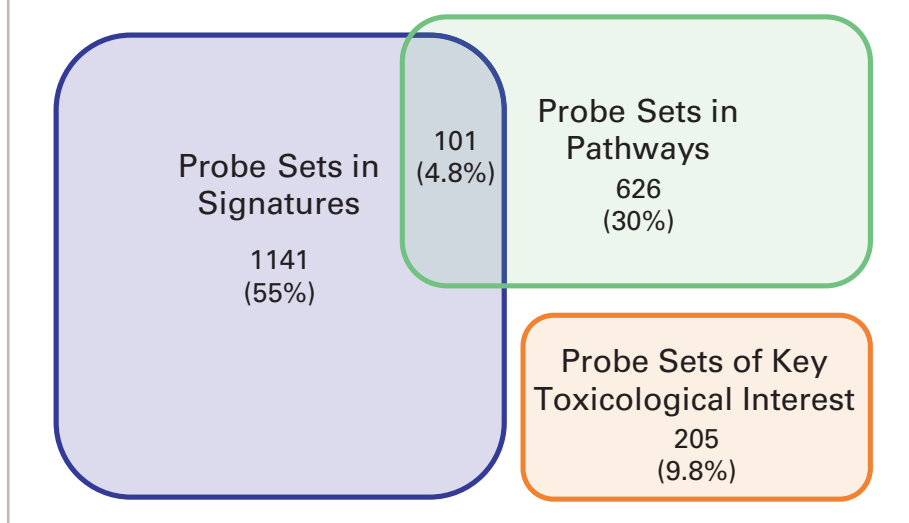
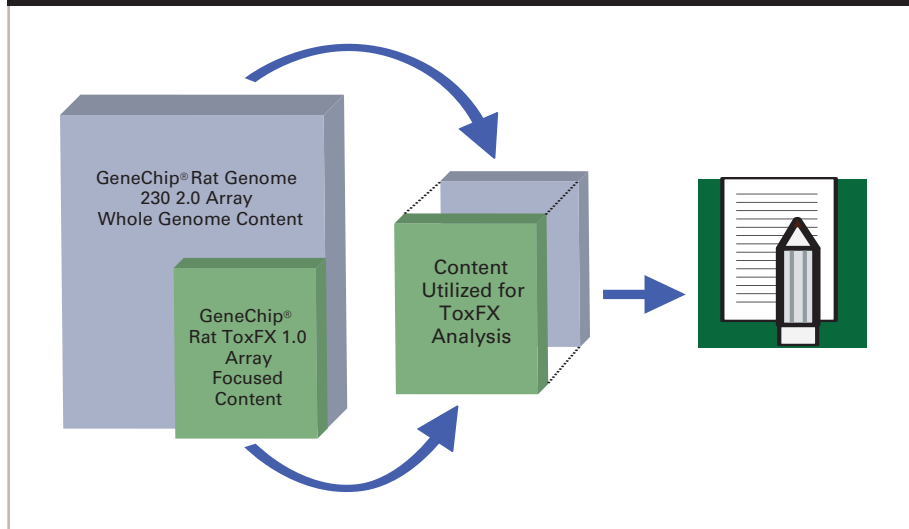


Figure 3. Content relationship between the GeneChip® Rat Genome 230 2.0 and GeneChip® Rat ToxFX 1.0 Arrays.

The identical content from each array is utilized by the ToxFX analysis (Note: 100 percent of the gene content from the whole-genome array is returned to the user already calculated as treatment group ratios and ready for further analysis by the user).



of genes that toxicologists widely agree are vital to the understanding of toxic response mechanisms.

Considerations for each array are:

- **GeneChip® Rat ToxFX 1.0 Array:** Iconix and Affymetrix have combined their experience to design an array

exclusively for the ToxFX automated analysis. Based on approximately 600 *in vivo* compound experiments, the array content focuses specifically on unique probe sets that Iconix' analysis experience indicates are most informative from a toxicology perspective. For compound screening purposes, the

more focused array provides the economical solution for running large numbers of samples.

- **GeneChip® Rat Genome 230 2.0 Array:** In addition to the ToxFX automated analysis, the whole-genome coverage provided by the Rat 230 2.0 Array enables a more in-depth investigation of mechanism of toxicity through Iconix Consulting Services or the bioinformatics analysis capabilities of the user. The report generated by the ToxFX automated analysis using Rat 230 2.0 Arrays focuses on the same toxicologically important genes that are found on the Rat ToxFX 1.0 Array. Gene expression changes for the complete genome are recorded in the supplementary data files returned with the ToxFX Report. These files provide a useful starting point for the user to perform more in-depth analysis of whole-genome effects.

The ToxFX Report

Each ToxFX study is fully documented in a comprehensive and easy-to-read report. The ToxFX Report is automatically generated as a PDF document and provides an in-depth summary of all aspects of the study and its results (Table 2). Data are summarized in the report at a variety of levels allowing the user to first get an overall impression of the study outcome and then delve deeper into specific results of interest. Each ToxFX Report is written using standard toxicology terminology and is designed to be standalone documentation of the study.

Accessing the ToxFX Automated Analysis

Analyzing a ToxFX study does not require a subscription to or licensing of the DrugMatrix reference database or ToxFX Study Builder software. Instead, data submissions are tracked through the use of "Analysis Certificates," providing the researcher with the flexibility and conven-

Table 1. Key attributes for ToxFX-compatible arrays

	GeneChip® Rat ToxFX 1.0 Array	GeneChip® Rat Genome 230 2.0 Array
Number of probe sets	2,073	31,042
Feature size	11µm	11µm
Oligo probe length	25mer	25mer
Probe pairs/sequence	11	11
Control sequences included:		
Hybridization controls	bioB, bioC, bioD and cre	bioB, bioC, bioD and cre
Poly-A controls	dap, lys, phe, thr	dap, lys, phe, thr
Normalization controls	N/A	100 probes sets
Housekeeping controls	GAPDH, beta-Actin, Hexokinase 1	GAPDH, beta-Actin, Hexokinase 1
Detection sensitivity	1:100,000*	1:100,000*

*As measured by detection of pre-labeled transcripts from mouse cDNA clones in a complex rat background.

Table 2. ToxFX Report organization

Section	Description
Executive Signature	High-level single-page summary highlighting the key Drug Summary and Pathway “hits.”
Study Description	Detailed record of the study design as defined by the user during the data submission process.
Drug Signatures	Detailed information documenting Drug Signature matches. Each match is associated with a probability score providing an indication of the strength of the match. Drug Signature matches correlate with specific biological end points (e.g., cholestasis, hepatic duct hyperplasia) and are predictive of potential safety liabilities.
Pathways	Detailed molecular pathway diagrams are directly linked to significance scores indicating treatment impact on 22 molecular pathways of key toxicological interest (e.g., bile acid and cholesterol metabolism or oxidative stress). The pathway information has been assembled exclusively by Iconix toxicologists and is uniquely focused on pathways with roles in toxic responses.
Toxicologically Relevant Gene Groupings	Certain noteworthy genes are of exceptional interest to the toxicologist, such as the cytochrome P450s. To allow the reader rapid access to all data available for these specific gene groups, the expression data for each group member are compiled in a single table. This kind of preassembly allows the scientist to spend time on interpretation and less time on data assembly.
Methodology	Full documentation of the analytical approaches and terms required to interpret study results are provided with every report. Interpretive guidance is provided throughout the report with convenient hyperlinks on each page.

Table 3. Information content provided through the ToxFX automated analysis

Overall Content		Content by Tissue	Tissue		
Total Number of Drug Signatures	55		Liver	Heart	Kidney
Total Number of Reference Compounds	316	Reference Compounds by Tissue	200	71	139
Total Number of Toxicologic Pathways	22	Signatures by Tissue	29	11	15

ience of deciding when and how they perform their ToxFX study.

An Analysis Certificate enables a single array to be submitted as part of a study and is purchased and tracked electronically. To submit a new study, sufficient certificates equal to the number of arrays in the study must be present in the user's account. The number of Analysis Certificates in the user's account and the number required for submission of a study is conveniently displayed within the ToxFX Study Builder software and automatically debited from the user's account when a study is submitted.

For users of the Rat 230 2.0 Array, Analysis Certificates may be purchased directly from Iconix. For more detailed information on purchasing Analysis Certificates, please visit www.toxfx.com.

The purchase of the Rat ToxFX 1.0 Array from Affymetrix includes the ToxFX analysis. For added convenience, the ToxFX Study Builder software automatically recognizes and registers new Rat ToxFX 1.0 Array data at the time the data are submitted, eliminating the need for certificate tracking.

Summary

The ToxFX Analysis Suite offers a rapid and streamlined toxicogenomic solution for understanding the safety issues associated with new drug candidates, compounds or environmental toxicants. Whether the array of choice is the Affymetrix whole genome Affymetrix GeneChip® Rat Genome 230 2.0 Array or the custom-designed GeneChip® Rat ToxFX 1.0 Array, the ToxFX Analysis Suite provides a complete analysis solution.

Table 4. ToxFX Drug Signatures

Signature	Liver	Kidney	Heart
Adrenergic agonist			✓
Bile Duct Hyperplasia	✓		
Cholesterol biosynthesis inhibitor	✓	✓	
Cardiac cellular infiltration			✓
Cardiac myocyte degeneration			✓
DNA damage		✓	
DNA intercalator, anthracycline-like		✓	
Erythrocyte count increase		✓	
Estrogen receptor agonist	✓		
Estrogen receptor alpha binding		✓	
Glucocorticoid and mineralocorticoid receptor agonist	✓	✓	✓
Heart weight increase			✓
Hepatic eosinophilia, centrilobular	✓		
Hepatic eosinophilia, early gene expression	✓		
Hepatic fibrosis	✓		
Hepatic hypertrophy, centrilobular	✓		
Hepatic inflammatory infiltrate, centrilobular	✓		
Hepatic inflammatory infiltrate, early gene expression	✓		
Hepatic lipid accumulation, centrilobular	✓		
Hepatic lipid accumulation, macrovesicular	✓		
Hepatic lipid accumulation, microvesicular, centrilobular	✓		
Hepatic lipid accumulation, periportal	✓		
Hepatic necrosis	✓		
Hepatocellular hypertrophy, diffuse	✓		
Hepatomegaly	✓		
Hypoalbuminemia	✓		✓
Leukocytosis, early gene expression	✓		
Leukopenia	✓		
Lymphocytosis	✓		
Lymphopenia	✓	✓	✓
Nephromegaly		✓	
Neutrophilia	✓		✓
Non-DNA reactive antiproliferative agent			✓
Peroxisome proliferator	✓	✓	✓
Pregnane X receptor activation	✓		
Renal tubular necrosis		✓	
Renal tubular nephrosis		✓	
Renal tubular proteinaceous cast		✓	
Renal tubular regeneration		✓	
Renin-angiotensin-aldosterone inhibitor		✓	
Serum alanine aminotransferase increase	✓		
Serum bilirubin and alkaline phosphatase increase	✓		
Thyropoxidase inhibitor	✓		
Toxicant, DNA alkylator	✓		✓
Toxicant, heavy metal-like		✓	

Table 5. Pathways by tissue

Pathway	Liver	Heart	Kidney
Xenobiotic Metabolism	✓	✓	✓
Aryl Hydrocarbon Receptor Signaling	✓	✓	✓
Apoptosis	✓	✓	✓
Hepatic Stellate Cell Activation and Fibrosis	✓		
Angiotensin II and Cardiac Hypertrophy		✓	
Hepatic Steatosis	✓		
Hepatic Cholestasis	✓		
Cholesterol Biosynthesis	✓		
Beta-Oxidation of Fatty Acid	✓	✓	✓
Fatty Acid Biosynthesis and its Regulation	✓	✓	✓
Acute Phase Response	✓	✓	✓
LPS and IL-1 Mediated Inhibition of RXR Function	✓		
NF-kappa B Signaling	✓	✓	✓
TGF-beta Signaling	✓	✓	✓
Nrf2 Mediated Oxidative Stress Response	✓	✓	✓
Hypoxia and HIF Signaling	✓	✓	✓
ELF2 Kinase Mediated Stress Response	✓	✓	✓
p53 Signaling	✓	✓	✓
Cell Cycle G1/S Transition	✓	✓	✓
Cell Cycle G2/M Transition	✓	✓	✓
Mitochondrial Oxidative Phosphorylation	✓	✓	✓
Thyroid Hormone Synthesis, Regulation and Release	✓	✓	

Notes:

Required Instrumentation and Software

Arrays	Instrumentation	Software
GeneChip® Rat ToxFX 1.0 Array	GeneChip® System GeneChip® Scanner 3000 7G Scanner	GeneChip® Operating Software v1.4 or higher
GeneChip® Rat Genome 230 2.0 Array	GeneChip® Fluidics Station 400 or 450 GeneChip® Hybridization Oven 640	Affymetrix® Expression Console™ ToxFX™ Study Builder

Notes:

Supporting Products and Literature

Part Number	Product Name	Description
900493	GeneChip® One-Cycle Target Labeling and Control Reagents ¹	Sufficient for 30 Reactions Contains: GeneChip® IVT Labeling Kit GeneChip® One-Cycle cDNA Synthesis Kit GeneChip® Sample Cleanup Module GeneChip® Poly-A RNA Control Kit GeneChip® Hybridization Control Kit
¹ Individual Kit components may be ordered separately.		
900720	GeneChip® Hybridization, Wash and Stain Kit ²	Sufficient for 30 Reactions Contains: Hybridization Module - Pre-Hybridization Mix - 2X Hybridization Mix - DMSO - Nuclease-free Water Stain Module - Stain Cocktail 1 - Stain Cocktail 2 - Array Holding Buffer Wash Buffer A ³ Wash Buffer B ³
² GeneChip® Hybridization, Wash and Stain Kit is not included in GeneChip® Rat ToxFX 1.0 Array with ToxFX Analysis and Reagents (P/N 900997) and must be purchased separately.		
³ Wash Buffers A and B are also available for purchase individually		
Additional Analysis Certificates are available for purchase directly from Iconix Biosciences at www.toxfx.com .		

Literature

702389 *ToxFX™ Analysis Suite User Manual*
702390 *ToxFX™ Analysis Suite Tutorial*
For additional information, please visit www.toxfx.com

To Order Analysis Certificates:

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Ordering Information

GeneChip® Rat ToxFX 1.0 Array with ToxFX Analysis

GeneChip® Rat ToxFX 1.0 Array with ToxFX Analysis

900994 Contains 2 Arrays

900995 Contains 6 Arrays

900996 Contains 30 Arrays

GeneChip® Rat ToxFX 1.0 Array with ToxFX Analysis and Reagents[†]

900997 Contains 30 Arrays and GeneChip® One-Cycle Target Labeling and Control Reagents Sufficient for 30 Reactions

GeneChip® Rat Genome 230 2.0 Array*

900505 Contains 2 Arrays

900506 Contains 6 Arrays

900507 Contains 30 Arrays

[†]The GeneChip® Hybridization, Wash and Stain Kit is not included and must be purchased separately.

*Analysis certificates for use with Rat Genome 230 2.0 Arrays must be purchased directly from Iconix Biosciences. Please visit www.toxfx.com for more details.

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