MicroRNAs (miRNAs) are effectors of environmental influences on gene expression. Thus miRNAs play an important role in the cellular response to injury, exposure to toxicants, and disease. Insight into the mechanisms through which these small molecules function may lead to development of therapeutics that target miRNA pathways. Identification and quantitation of miRNA expression is an important first step in understanding the potential mechanisms involved in these cellular and molecular responses. Here, we present an advanced microfluidic biochip technology that was developed to enable comprehensive miRNA expression profiling. Detection of miRNA using an array offers the opportunity to examine all known and/or predicted miRNA transcripts in a single experiment providing a comprehensive view of all miRNAs that may be involved in the system being investigated. The detection probes are in situ synthesized using PGR (Photo-Generated Reactant) chemistry to afford the high yield and complete sequence flexibility, and modified nucleotides are incorporated to enhance the binding to short miRNAs without sacrificing specificity. Application examples will be provided to demonstrate how the technology is enabling breakthrough discoveries.

What are miRNAs’ functions in toxicogenomics?
1. miRNAs are effectors of environmental influences on gene expression. Thus miRNAs play an important role in the cellular response to toxicants and disease.
2. The expression of miRNAs, like many of the genes important in toxicology, can be regulated by xenobiotics and DNA methylation.
3. Xenobiota-mediated miRNA expression has been directly linked with downstream protein expression and cell proliferation.

Biomarkers for Exposure Assessment – miRNA profiling in response to toxic compounds can provide toxicant-specific profiles
1. miRNA expression profiles distinguish the carcinogenic effects of chemical toxins in specific organs.
2. miRNA expression can be used as biomarkers of chemical exposure in risk assessment of chemical carcinogenesis.

Molecular Basis for Susceptibility & Resistance – miRNAs play a fundamental role in toxicological phenomena such as cellular responses to xenobiotic stress, susceptibility and resistance.
1. miRNAs can suppress resistance to anticancer cytotoxic therapy.
2. Differences in the susceptibility to carcinogenesis may be determined by the variations in miRNA expression response to toxins.
3. miRNA expression profiling can be used to identify genetic susceptibility to pollutants.

Therapeutic Potential of miRNA – Identification of miRNAs that play essential roles in disease to act as drugs or possible therapeutic targets.
1. Drug combinations can sensitize cancer cells via miRNA pathways.
2. Restoring miRNA expression is a novel therapeutic approach to sensitizing and suppressing the growth of resistant tumors.

miRNA Detection and Expression Profiling
Which method to choose?
qRT-PCR – Fast, quantitative and very specific, but low throughput – Choose when the specific miRNAs of interest are known.
RNA-Seq – No prior sequence knowledge required, but expensive and low throughput – Choose for discovery applications, with unusual species samples or when difference in expression between isoforms is required.
Microarray – Fast, high-throughput, inexpensive, global expression profile, but alternatives are better suited for discovery or absolute quantitation. Choose for global comparisons of miRNA expression profiles in model species.

μParaflo™ Microfluidics Chip Platform
μParaflo™ miRNA arrays are in situ synthesized directly in a microfluidics chip, not spotted on a glass slide. This advanced in situ elongonuclease synthesis technology ensures tight process control, highly uniform spots and high reproducibility across lots of arrays, yet permits total customization of contents on each individual array. Three components are at the core of the μParaflo™ technology:

Programmable DLP Photolithography - Digital Light Projection drives light directed chemical reactions at specific sites in an array format and eliminates the need for expensive, inconvenient microfabricated photomasks. A computer generates the digital mask and a digital light projector (DLP) projects the light beam very accurately into the micro reaction chambers where a photogenerated reagent is produced.

Photo Generated Acid (PGA) Deprotection Chemistry - A novel solution photochemical approach employing light directed parallel synthesis and deprotection with a photogenerated reagent enables high-yield parallel synthesis using standard UMT protected phosphoramidites.

Microfluidic Reaction Devices Microfluidic chip [4K-30K reaction chambers] provides an enclosed system that facilitates the use of the solution photochemistry, promotes spot uniformity and reproducibility and enhances kinetics for chemical synthesis as well as various labeling/binding/array analyses.

Application Examples
Garcinol sensitizes human pancreatic adenocarcinoma cells to gemcitabine in association with microRNA signatures
Alterations in miRNA/miRNAs are of biological importance in the pathophysiology of cancers, including pancreatic cancer (PaCa). It has been demonstrated that garcinol induced PaCa cell growth arrest and apoptosis in vitro.
Researchers evaluated the miRNA expression profile of gemcitabine-resistant PaCa-1 cells treated with garcinol and/or gemcitabine. They found that garcinol synergizes with gemcitabine to inhibit cell proliferation and induce apoptosis in PaCa cells with significant modulation of key cancer regulators including PARP, VEGF, MMPs, ILs, caspases, and NF-κB. They identified garcinol-specific miRNA biomarkers that sensitize PaCa cells to gemcitabine treatment, thus attenuating the drug-resistance phenotype.
These results prompt further interest in garcinol and gemcitabine combination strategy as a drug modality to improve treatment outcome in patients diagnosed with PaCa.

Restoring miR-342 - a novel therapeutic approach to sensitizing and suppressing the growth of tamoxifen resistant breast tumors
Tumor resistance to the selective estrogen receptor modulator tamoxifen remains a serious clinical problem especially in patients with tumors that also overexpress HER2. However, a unifying molecular mechanism of tamoxifen resistance has remained elusive.
Researchers analyzed multiple cell models of tamoxifen resistance derived from MCF-7 cells to examine the influence of miRNAs on tamoxifen resistance. They observed significant and dramatic downregulation of miR-342 in tamoxifen resistant MCF-7 variant cell lines. Restoring miR-342 expression in the cell lines sensitized these cells to tamoxifen-induced apoptosis with a dramatic reduction in cell growth.
These findings suggest that miR-342 regulates Tamoxifen response in breast tumor cell lines and our clinical data indicates a trend towards reduced miR-342 expression and tamoxifen resistance.