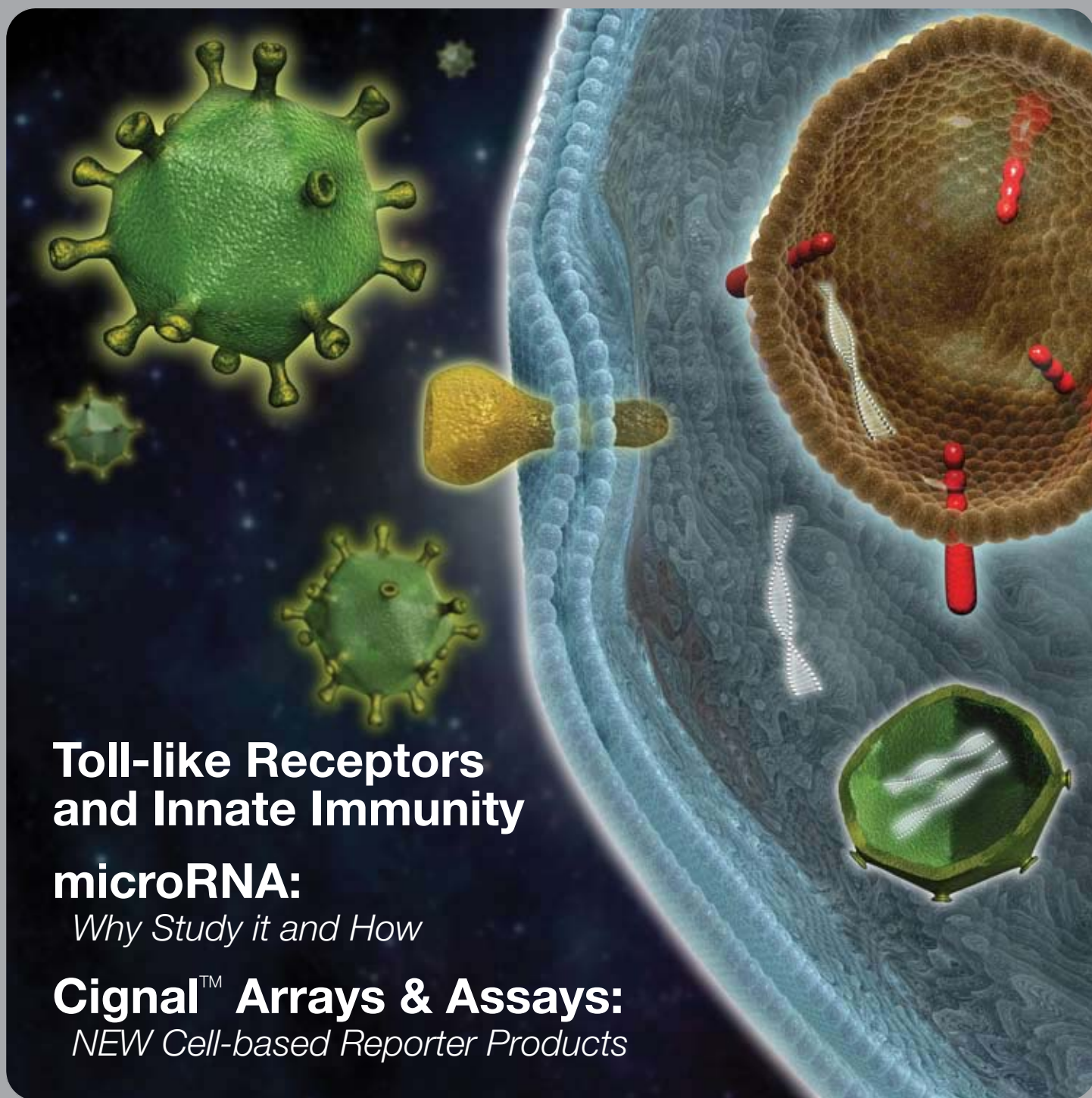


# PATHWAYS™

ISSUE 7



**Toll-like Receptors  
and Innate Immunity**

**microRNA:**

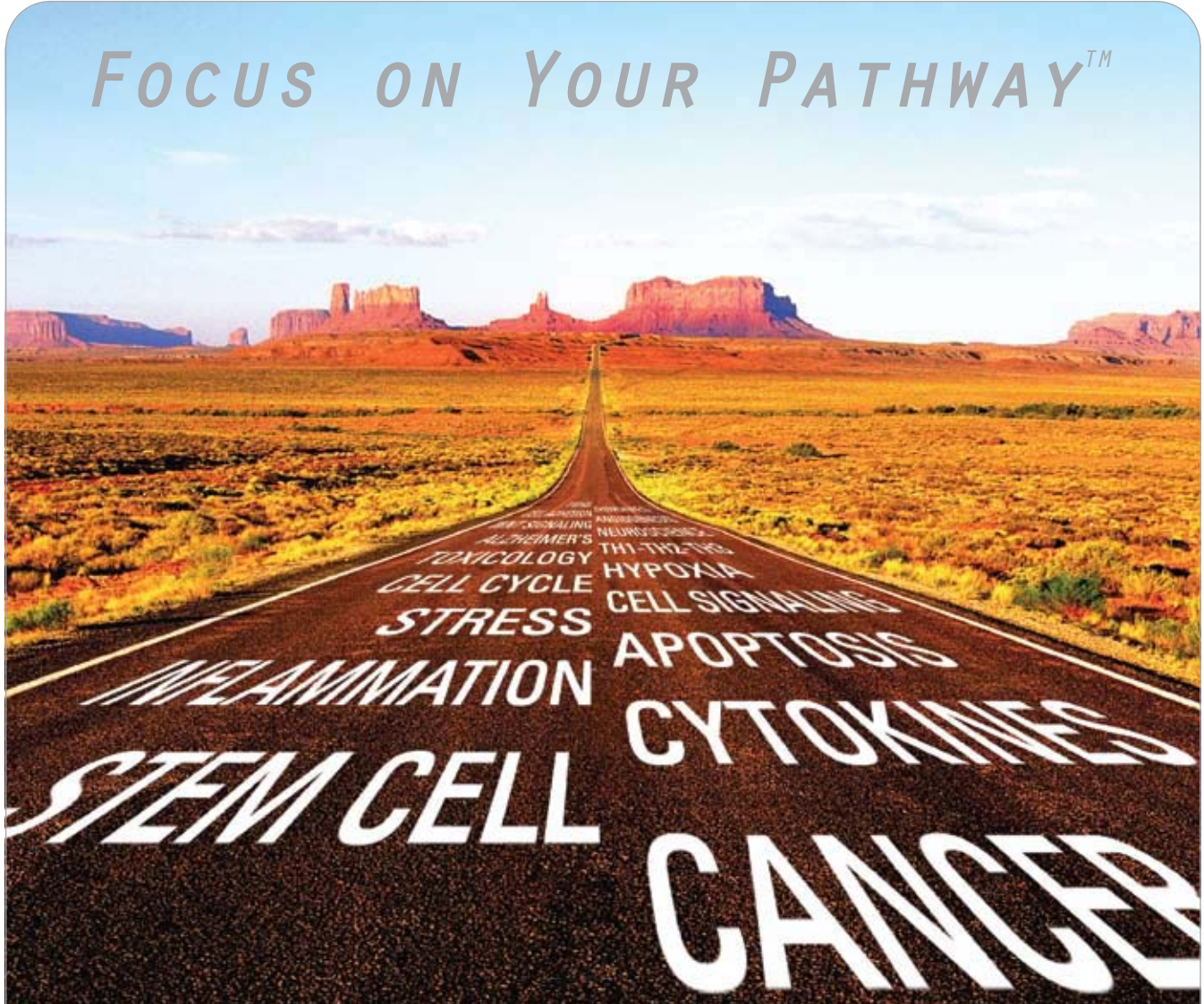
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Dear Scientists:

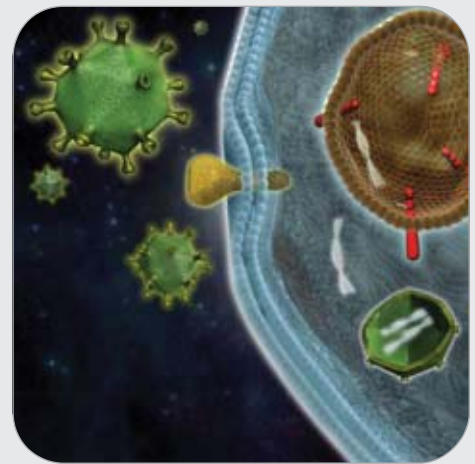
It has been an exciting 10 years since SuperArray Bioscience Corporation was founded. Our skilled team of staff scientists have rapidly expanded our product portfolio; providing system solutions from gene expression analysis to gene function, as well as protein and cell-based assays. To support the company's phenomenal growth; we have relocated to a newly constructed high-tech facility and changed our corporate name to **SABiosciences Corporation** effective July 18th 2008.

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Sincerely yours,

Jeffrey Hung, Ph.D.  
 Director of Marketing



PATHWAYS COVER IMAGE

**Most pathogens interact with cell surface toll-like receptors. In contrast, the nucleic acids present in viruses are recognized by intracellular toll-like receptors on the endosome. For a comprehensive review of toll-like receptor signaling pathways, please see page 2.**

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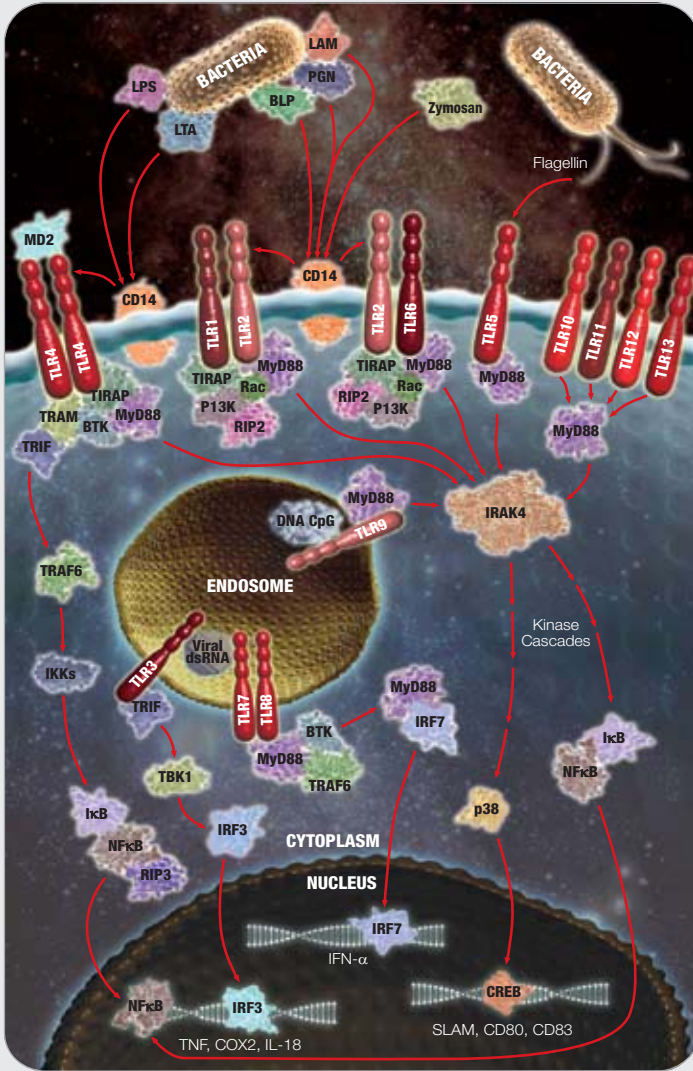
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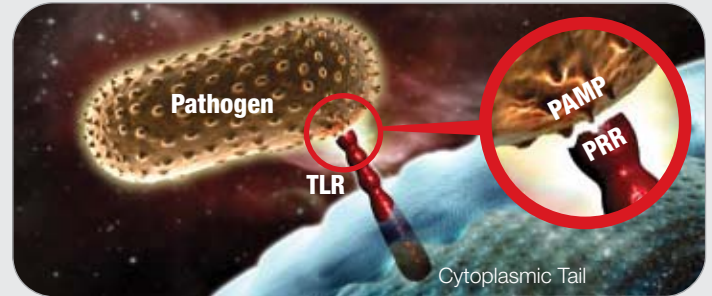
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# TOLL-LIKE RECEPTORS AND INNATE IMMUNITY



Toll-like Receptor Signaling Pathway

These highly conserved soluble and membrane bound proteins are collectively called Pattern-Recognition Receptors (PRRs), and it is the PAMP/PRR interaction that triggers the innate immune system. While the history of TLR-dependent observations goes back 100 years, most of the definitive work started about fifteen years ago. A tremendous amount of work has been done during this time, including the discovery of other PRR pathways. The cytosolic NOD (nucleotide oligomerization domain) proteins have been shown to be important innate immune response components.



**Figure 1: Binding of a Pathogen via Its PAMP (Pathogen Associated Molecular Pattern) to a TLR's PRR (Pattern Recognition Receptor) Domain. The Extracellular Leucine-Rich Repeats of the TLR, constitute the PRR Region.**

## Toll-like Receptors (TLR)

TLRs are transmembrane proteins expressed by cells of the innate immune system, which recognize invading microbes and activate signaling pathways that launch immune and inflammatory responses to destroy the invaders. Toll receptors were first identified in *Drosophila*. In mammals, the TLR family includes eleven proteins (TLR1–TLR11). Recently, two new members, TLR12 and TLR13 have been discovered in murine cells, but not much information is known about them. Mammalian TLRs consist of an extracellular portion containing leucine-rich repeats, a transmembrane region and a cytoplasmic tail, called the TIR (Toll-IL-1R (Interleukin-1-Receptor)) homology domain. Different TLRs serve as receptors for diverse ligands, including bacterial cell wall components, viral double-stranded RNA and small-molecule anti-viral or immunomodulatory compounds (Table 1).

Activation of TLRs occurs after binding of the cognate ligand to the extracellular leucine-rich repeats portion of the TLR. In humans, TLR1, 2, 4, 5 and 6 are outer membrane associated, and respond primarily to bacterial surface associated PAMPs. The second group, TLR3, 7, 8 and 9 are found on the surface of endosomes, where they respond primarily to nucleic acid based PAMPs from viruses and bacteria. Upon binding with their cognates, TLRs activate two major signaling pathways. The core pathway utilized by most TLRs leads to activation of the transcription factor NF-κB (Nuclear Factor-κB) and the MAPKs (Mitogen-Activated Protein Kinases) p38 and JNK (c-Jun N-terminal Kinase).

The second pathway involves TLR3 and TLR4 and leads to activation of both NF-κB and another transcription factor IRF3 (Interferon Regulatory Factor-3), allowing for an additional set of genes to be induced, including anti-viral genes such as IFN-β (Interferon-Beta) and others (1). The innate immune response is a complex set of interactions that have evolved to optimize the response to pathogens. While the structure of the TLRs has been highly conserved, the innate immune response for each organism has selectively been driven to best protect against the pathogens found in the host's environment.

## Innate and Adaptive Immunity

The immune system is divided into two parts, the innate and adaptive systems. The adaptive immune response depends on B and T lymphocytes which are specific for particular antigens. This system involves clonal selection of antibody producing B cells to respond to foreign antigens, and works well, but has a major limitation in that it takes from 4 to 7 days to ramp up. In that time period, pathogens could overwhelm the organism.

In contrast, the innate immune system is immediately available to combat threats. There is no complicated method of selecting cells that react to foreign substances from those that react to self. There is no memory to change how the system responds to the same threat upon the second or third exposure. Instead, the innate immune system responds to common structures shared by a vast majority of threats. These common structures are called pathogen associated molecular patterns, or PAMPs, and are recognized by the toll-like receptors, or TLRs. In addition to the cellular TLRs, an important part of the innate immune system is the humoral complement system that opsonizes and kills pathogens through the PAMP recognition mechanism.

**Table 1: The TLRs and Their Pathogen Derived Activators (2).**

PAMP	PRR	Pathogen
Pam3CSK4, PGN, Zymosan et al	TLR1,2,6	Gram Positive Bacteria (eg <i>S. aureus</i> )
LPS, Lipid A	TLR4	Gram Negative Bacteria (eg <i>E. coli</i> )
Flagellin	TLR5	Bacteria, Flagellum
dsRNA	TLR3	Virus
ssRNA	TLR7,8	Virus
CpG DNA	TLR9	Bacteria, DNA

## Innate Defense Against Bacteria

As shown in Table 1, the defense against bacteria involves all the TLRs except for 3,7 and 8 which are virus specific. Gram positive and negative bacteria differ in their surface PAMPs and bind to different TLRs. The specificity of the TLRs for bacterial cell wall fragments has just recently been established, as early reports were later found to be due to trace contaminants.

The signal transduction pathway for TLR4 activation by LPS (Lipopolysaccharide) serves as a representative example of the surface bound TLRs (see the opening illustration of the pathway).

LPS first binds to the CD14 (Cluster of Differentiation-14) receptor, which then transfers it to TLR4. TLR4 homodimerizes and forms a complex with the protein MD2. Cells need both MD2 and TLR4 in order to recognize LPS. TLR4 activation engages a set of MyD88 (Myeloid Differentiation Primary-Response Protein-88) adaptor family members, including TIRAP, TRIF, TRAM (all three are TIR domains containing adapter proteins) and MyD88. This pattern of activation is general for cell surface TLRs, but the subsequent intracellular signal cascades, which include a number of transcription factor activations, are unique for each TLR. This results in a response that is appropriate to each threat (1).

TLR2 is activated by bacterial LAM (Lipoarabinomannan), BLP (Bacterial Lipoprotein), and PGN (Peptidoglycans). LAM and PGN act on TLR2 through the CD14 receptor, similar to the process followed by the TLR4 with a similar downstream effect. BLP mediates both apoptosis and NF- $\kappa$ B activation through TLR2. TLR2 is also responsible for the recognition of the Yeast cell-wall particle Zymosan. Zymosan acts through the CD14 receptor to influence TLR2. The phagocytosed TLR2 vesicle signals the production of TNF (Tumor Necrosis Factor), through the NF- $\kappa$ B pathway. TLR6 associates with TLR2 and recognizes diacylated MALP2 (Mycoplasmal macrophage-Activating Lipopeptide-2 kD). Like TLR4, they also signal through MyD88 and TIRAP. PI3K (Phosphatidylinositol-3 Kinase), RIP2 (Receptor-Interacting Protein-2) and Rac (Ras-Related C3 Botulinum Toxin Substrate) are also involved in TLR6-TLR2 mediated signaling. TLR1 also associates with TLR2 and recognizes the native mycobacterial 19-kDa lipoprotein along with TLR2. TLR1-TLR2 also signals through MyD88, TIRAP, PI3K, RIP2 and Rac. TLR1 and TLR6 may participate in the activation of macrophages by gram positive bacteria. TLR5 is a signaling mediator of bacterial flagellin, thus activating NF- $\kappa$ B and may play a role in resistance to Salmonella infection (3). Human TLR10 is an orphan member of the TLR family. Genomic studies indicate that TLR10 is in a locus that also contains TLR1 and TLR6, two receptors known to function as coreceptors for TLR2. TLR10 not only homodimerizes but also heterodimerizes with TLRs 1 and 2. It has been found to activate gene transcription through MyD88. TLR9 is responsible for the recognition of CpG islands of bacterial DNA. The extracellular CpG fragment may activate TLR9, thus inducing the endocytosis of the DNA along with TLR9, or perhaps the bacteria is phagocytosed and TLR9, which has separately formed on the phagosome, is activated by the CpG islands; whatever the exact method, TLR9 activates the NF- $\kappa$ B pathway from the endocytosed vesicle. Recently IRF8 (Interferon Regulatory Factor-8) has been shown to be activated by TLR9 through MyD88 (3).

Co-receptors on TLR-bearing cells play a critical role in the inflammatory response. In monocytes for example, the CD36 and CD14 co-receptors are necessary for the TLR2 response to gram positive bacteria.

The continued arms race between bacteria and immune defense mechanisms is demonstrated by pyloric bacteria, which have evolved a modified flagellum that evades detection by TLR5, helping this pathogen to establish residency in the mammalian digestive system.



**Figure 2: The Flagella of *H. pylori* (*Helicobacter pylori*) Does Not Activate TLR5 due to Sequence Changes in the Flagellar Protein That Prevent Detection by TLR5.**

In addition to the TLRs, two NOD (nucleotide oligomerization domain) proteins in the cytoplasm, have recently been found to play an important role in the innate defense against *E. coli* & *S. aureus*. The NOD proteins contain leucine-rich repeats very similar to those in TLRs that recognize specific components of these bacteria (diaminopimelic acid) and form a cytoplasmic signaling platform with other proteins known as the inflammasome. This signaling leads to IL-1 & IL-8 production.

Recently it has been argued that while it is well established that a strong innate defense response to bacteria is essential for survival, the most important role of this TLR activation in the long term, may be in the induction of the adaptive immune response. This is discussed further in the three sentinel cells section.

## Innate Defense Against Viruses

Viral nucleic acids contain PAMPs that are recognized by intracellular TLRs. These TLRs are located on the intracellular endosome membranes. The TLRs found on endosomes are TLR3, TLR7, TLR8 and TLR9. TLR3 activates immune cells in response to double-stranded viral RNA. The stimulation of the TLR3 triggers TRIF activation that ultimately activates the IRF3 transcription factor through TBK1, independent of MyD88. This leads to the secretion of IFN- $\beta$ . TRIF also activates RIP1 (Receptor-Interacting Protein-1) and TRAF6, which may further activate the NF- $\kappa$ B pathway. Small anti-viral compounds activate immune cells via the TLR7/MyD88-dependent signaling pathway. TLR7 binds MyD88 and activates IRAF and TRAF6. TRAF6 then activates TANK (also known as I-TRAF). TANK interacts with TBK1 and IKK- $\epsilon$  to activate IRF3. TLR7 or TLR8 may also activate IRF7 through interaction of MyD88, BTK and TRAF6, thus inducing anti-viral responses by producing IFN- $\alpha$  (Interferon-Alpha). Recently, Mouse TLR11 has been identified as a participant in defense against uropathogenic bacteria. The ligands for Mouse TLR12 and TLR13 are currently unknown.

It should be noted that only plasmacytoid cells use the TLR pathway for viral defense. Other cells use RIG1 (retinoic acid inducible gene1)-like helicases (RLHs) to recognize viral PAMPs which results in primarily an IFN response (4,5). The fact that plasma cells utilize TLRs suggests that the TLR-dependent response to viral infection is both important for immediate viral protection, as well as the activation of adaptive immunity via the inflammatory cytokines. A number of studies have shown that a weakened TLR response to particular viruses leads to poor antibody & Th1 responses, and the combination leads to persistent viral infections.

## Innate Defense Against Parasites

The study of TLR activation in parasitic diseases is just beginning, but these early results indicate a significant role. Polymorphisms in TLRs have been linked with the severity of systemic malarial infections. In contrast, an intact TLR signaling system has been shown to contribute to the severe cerebral malarial infection that is often lethal (6). This is an example of how a vigorous TLR response to a parasite can lead to a more severe disease.

In leishmaniasis, which affects 10 million people, TLR2 and TLR4 are required for proper parasite control, due to the activity of inducible nitric oxide (iNOS). A second factor induced by TLR4 activation, neutrophil elastase, is also important for the leishmanicidal activity of macrophages (7).

*Toxoplasma gondii*, the common parasite causing toxoplasmosis in humans, binds to the newly discovered TLR11, which has no other known ligands in humans.

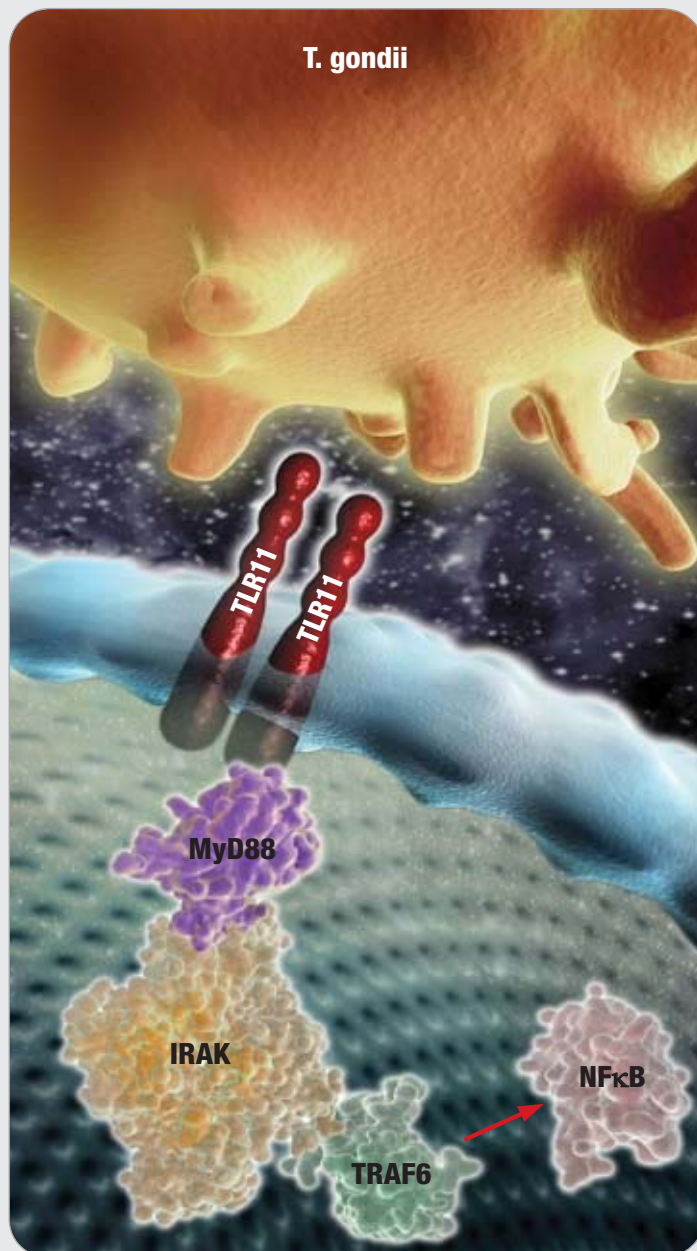


Figure 3: TLR11 Recognizes and Is Activated by the *T. Gondii* Parasite.

## The Three Sentinal Cells of Innate Immunity and TLRs

TLRs are primarily found on macrophages, mast cells and dendritic cells, the three sentinal cells of the innate immune response. It is interesting that the surface expression of TLRs in humans is highly variable and this has been linked to susceptibility to infections (8). It should be noted that the activation of TLRs on these cells begins a complex set of signaling cascades that are not yet completely understood. These interactions are not restricted to the innate immune response, but they also play an important role in adaptive immunity.

We are at an exciting point in immunological research, where our knowledge of the innate immune response, and how it guides adaptive immunity, may lead to more effective treatments of immunological diseases.

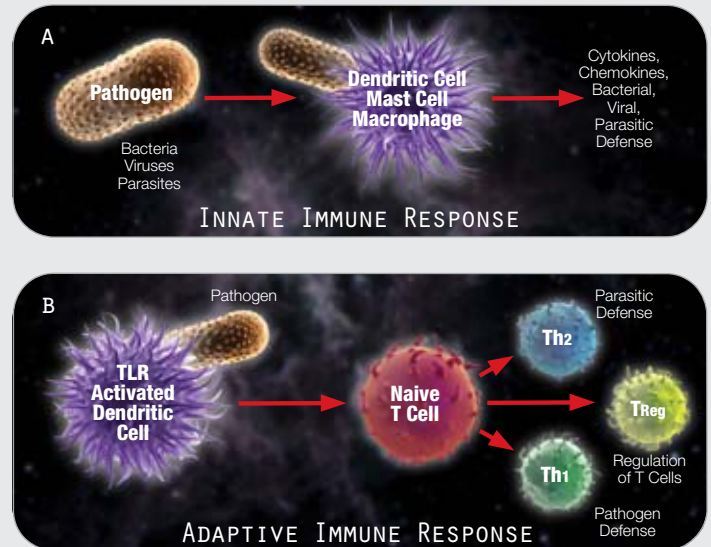


Figure 4: The 3 Sentinal Cells, Dendritic, Mast & Macrophages Protect Against Pathogens. Dendritic Cells Also Are Critical in the Adaptive Immune Response.

## Fine Tuning TLR Activation

Unchecked TLR activation by pathogens can lead to serious medical consequences, such as sepsis and autoimmune diseases. In the last few years, negative modulators of TLR activation have been identified, and their important role in reducing the inflammatory response has been demonstrated in animal models (9-11). The TAM family members are one example.

The TAM family, has been found to be central to the fine tuning of the TLR response. Loss of function of the three members of this family (Tyro3/Axl/Mer) in a triple knockout mouse results in a profound dysregulation of the immune response (10). This includes massive splenomegaly and lymphadenopathy, lymphocyte infiltration into all tissues, and high levels of autoimmunity. Even a single knockout of just Mer is sufficient to elevate susceptibility to LPS induced shock via the TLR4 signaling. These mice have elevated levels of dendritic cells, and the cells express elevated levels of activation markers, including MHC class II antigens. This effect was not restricted to TLR4, as hypersensitivity to the TLR3 activator polyIC was also observed. While the details of the mechanisms for this modulation of the innate immune response are not yet known, the TAM receptor ligands Gas6 and ProS, play an important role. The inhibitory effect requires the synthesis of SOCS1 (suppressor of cytokine signaling 1) which had been previously identified in the cytokine response. Further downstream, the transcription of STAT1 was shown to be essential, as was the IFN receptor IFNAR1.

## Summary

Innate immunity is recognized to play an important role in the response to challenge by pathogens.

The immune functions in which toll-like receptors play important roles include:

- Orchestration of the immediate tissue specific and global response of the innate immune system to pathogens. This orchestration is driven primarily by cytokine and chemokine production (TNF, Interferons, IL-1, IL-2, IL-6, IL-8 and IL-12 among others). Perhaps the most important of these early signals are the chemokines that draw the phagocytes to the site of infection.
- Transition from innate to adaptive immunity. In addition to the role in the innate immune response, TLRs have an important role in adaptive immunity by activating antigen presenting cells. The cytokine signaling cascade stimulated by TLR activation, begins a complex series of interactions that has evolved in each organism to maximize the odds for survival. Among the more important of these signals is T cell differentiation and regulation. TLRs on dendritic cells in particular, are essential in the T-helper-1 (Th1) versus Th2 pathways (12). An important early component of the Th1 response is the activation of cytotoxic T cells that helps to control the infection (Fig 4).

Our knowledge of the complex innate immune response is rapidly increasing. An organism's survival depends on a prompt response to pathogens, but it is equally important to avoid unregulated inflammation that can lead to dangerous pathologies such as sepsis and autoimmune disease. It is this fine balance between protection and self-damage that drives the complexity of the innate immune response.

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## SABiosciences Toll-Like Receptor Research Products

SABiosciences offers a number of research tools for the study of toll-like receptors and their signaling pathways:

Technology	Product	Catalog #
PCR Array	Toll-like Receptor Signaling PCR Array	Human PAHS-018
		Mouse PAMM-018
		Rat PARN-018
PCR Array	Interferons (IFN) & Receptors PCR Array	Human PAHS-064
		Mouse PAMM-064
PCR Array	Chemokines & Receptors PCR Array	Human PAHS-022
		Mouse PAMM-022
		Rat PARN-022
PCR Array	Inflammatory Cytokines PCR Array	Human PAHS-011
		Mouse PAMM-011
		Rat PARN-011
Oligo-Microarray	Inflammatory Cytokines & Receptors GEArray®	Human OHS-011
		Mouse OMM-011
		Rat ORN-011.2
siRNA Array	NFκB Signaling Pathway siRNA Array	Human SAH-025A
ELISArray	Th1, Th2, Th17 Cytokines Multi-Analyte Kits	Human MEH-003A
		Mouse MEM-003A
ELISArray	Common Cytokines	Human MEH-006A
ELISArray	Inflammatory Cytokines	Human MEH-004A
		Mouse MEM-004A
Cell-Based Assay	Signal NFκB Reporter Assay Kit ( <i>Luciferase</i> )	Human CCS-013L
Cell-Based Assay	Signal ISRE Reporter Assay Kit ( <i>Luciferase</i> )	Human CCS-008L
Cell-Based Assay	Signal GAS Reporter Assay Kit ( <i>Luciferase</i> )	Human CCS-009L
Cell-Based Assay	Signal SMAD Reporter Assay Kit ( <i>Luciferase</i> )	Human CCS-017L
Cell-Based Assay	Signal NFκB Reporter Assay Kit ( <i>GFP</i> )	Human CCS-013G
Cell-Based Assay	Signal SMAD Reporter Assay Kit ( <i>GFP</i> )	Human CCS-017G
Cell-Based Assay Array	Signal Finder™ Immune Response 10-Pathway Reporter Array ( <i>tube</i> )	Human CCA-002L
Cell-Based Assay Array	Signal Finder Immune Response 10-Pathway Reporter Array ( <i>plate</i> )	Human CCA-102L

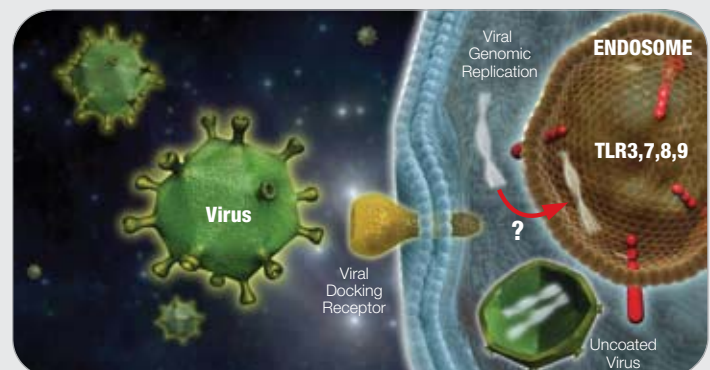


Figure 5: Viral Nucleic Acids Activate Endosomal TLR3,7,8,9.

# NEW PRODUCTS FROM SABIOSCIENCES:

Novel Gene & Protein Function Research Products

## SURESILENCING™ siRNA ARRAYS:

Pathway-Focused Gene Knockdown for Gene & Drug Function Studies

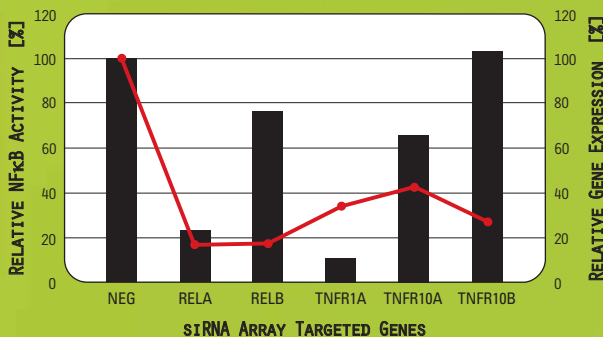
The SureSilencing siRNA Array is the latest technological innovation for conducting functional studies on your pathway of interest through RNA interference. Validated siRNAs for 42 key pathway-focused genes with appropriate controls are arrayed on a 96-well plate. Following a simple reverse transfection protocol with the siRNA, you can directly determine the phenotype of the cells on the same plate with colorimetric, luminescent, or fluorescent cell-based assays.

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SureSilencing siRNA Arrays are designed to study gene function with cell-based assays. The SureSilencing siRNA Arrays may be used for:

- **Accurate Pathway Analysis:** SureSilencing siRNA Arrays include positive and negative regulators as well as key members of a biological pathway. You can systematically analyze the pathway to identify the relevant genes to your biological research.
- **Functional Analysis of Novel Genes:** Determine the genetic and functional relationships of novel genes of interest to genes representative of a biological pathway on a SureSilencing siRNA Array via cotransfection.
- **Drug Target, Enhancer, or Inhibitor Validation:** SureSilencing siRNA Arrays can also be used to validate drug targets and identify enhancers or inhibitors of a drug's activity.

### ACCURATE PATHWAY ANALYSIS: HUMAN NFκB SIGNALING



**Figure: siRNA Array Showed that TNF Receptor 1A & RELA Are Required for TNF $\alpha$  Activation of NFκB Signaling.**

HEK-293H cells, containing the Signal NFκB Pathway Reporter Assay, were reverse transfected in the Human NFκB Signaling Pathway siRNA Array for 48 hours. After treatment with 50 ng/mL TNF $\alpha$  for 5 hours, relative NFκB activity was analyzed by luminescence (black bars), and gene knockdown was also determined by real-time RT-PCR (red points).

### SureSilencing siRNA Arrays

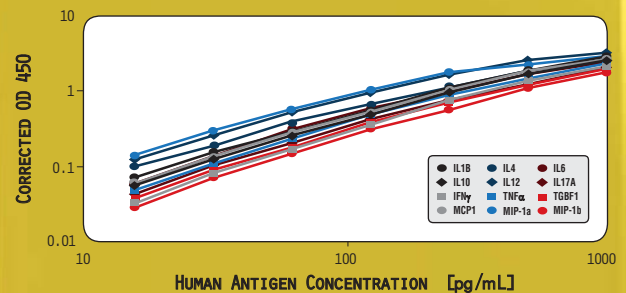
Pathway	Product	Human Catalog #
Apoptosis	Human Apoptosis Pathway Guide siRNA Array	SAH-012A
NFκB Signaling	Human NFκB Signaling Pathway	SAH-025A

## MULTI-ANALYTE ELISARRAY™ KIT:

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### SIMULTANEOUS ANALYSIS OF TWELVE CYTOKINES



**Figure: Similar Standard Curves Are Achievable for All Optimized Cytokine & Chemokine Assays in the Multi-Analyte ELISArray Kits.**

Cytokine antigen standard curves were generated using the Human Autoimmune Response Multi-Analyte ELISArray Kit. All standard curves have virtually identical slopes indicating that all twelve assays provide similar linear and sensitive responses under the same standardized conditions and development or incubation time.

### Single Analyte ELISArray Kits

Target Protein	Human Catalog #	Mouse Catalog #
G-CSF	SEH00723A	SEM02989A
GM-CSF	SEH00576A	SEM02990A
IFN $\gamma$	SEH00380A	SEM03121A
IL10	SEH00572A	SEM03017A
IL12	SEH00544A	SEM03019A
IL13	SEH00688A	SEM03021A
IL17A	SEH00537A	SEM03023A
IL1A	SEH00690A	SEM03010A
IL1B	SEH00171A	SEM03109A
IL2	SEH00172A	SEM02937A
IL23		SEM03763A
IL4	SEH00565A	SEM03013A
IL5	SEH00692A	SEM03014A
IL6	SEH00560A	SEM03015A
IL8	SEH00568A	
MCP1	SEH00192A	SEM03151A
MIP-1a	SEH00566A	SEM02949A
MIP-1b	SEH00563A	SEM02948A
TGFB1	SEH00508A	SEM02991A
TNF $\alpha$	SEH00341A	

### Multi-Analyte ELISArray Kits

Description	Human Catalog #	Mouse Catalog #
Th1/Th2/Th17 Cytokines	MEH-003A	MEM-003A
Inflammatory Cytokines	MEH-004A	MEM-004A
Autoimmune Response	MEH-005A	MEM-005A
Common Cytokines	MEH-006A	MEM-006A

# NEW PRODUCTS FROM SABIOSCIENCES:

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### ChIP-qPCR Assays:

Real-Time PCR Assays for Chromatin Immunoprecipitation Analysis

The SABiosciences ChIP-qPCR Assays are pre-designed and validated real-time PCR assays optimized to measure regulatory genomic DNA sequence enrichment within chromatin immunoprecipitation (ChIP) samples. Our ChIP-qPCR assays can quickly and quantitatively generate ChIP data for any gene promoter region in the human, mouse and rat genomes. Simply search & select the desired ChIP-qPCR assays from our 30 kb promoter tiling series for every RefSeq transcription start site (TSS).

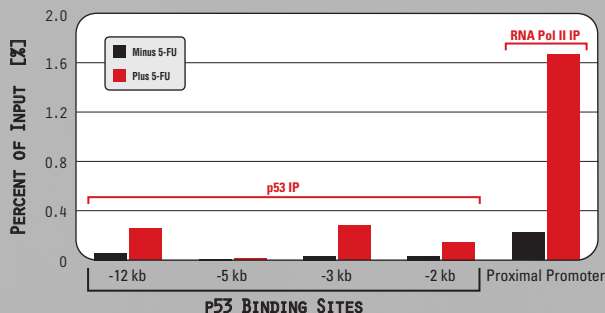
#### Why ChIP-qPCR Assays?

- Speed & Ease
- Save Time & Money
- Guaranteed Performance
- Comprehensive Coverage

#### How it Works

SABiosciences' ChIP-qPCR assays are designed to detect specific genomic DNA sequences within a 30 kb region around every transcription start site (TSS) in the RefSeq database. An average 1 kb assay tiling interval, when coupled with an average fragmentation size of 1 kb, provides a balance between sensitivity and resolution and provides a ChIP-target independent approach well-suited for rapidly establishing functional screening assays. Additionally, a custom qPCR array plate of ChIP-qPCR assays can be used to measure multiple target factor & promoter interactions on a single instrument run.

#### TREATMENT OF HCT-116 CELLS w/ 5-FLUOROURACIL INCREASES P53 & RNA POLYMERASE II BINDING TO THE CDKN1A PROMOTER



**Figure: Replicate HCT116 Cell Cultures Treated with Vehicle (DMSO, control, black) or 300  $\mu$ M 5-fluorouracil (5-FU, treatment, red) for 6 h.** After cell fixation and harvesting, chromatin DNA was isolated, sheared by sonication, and immunoprecipitated with monoclonal antibodies against p53 & RNA Polymerase II. The resulting enriched genomic DNA was purified and used in ChIP-qPCR Assays for CDKN1A promoter regions near the transcription start site (proximal promoter) and near the -2, -3, -5, and -12-kb p53 binding sites. The results are expressed as percent of input, or the fraction of the total input DNA co-immunoprecipitating with each factor. Treatment of these cancer cells with 5-FU increases p53 binding to its sites in the CDKN1A gene promoter (except the -5 kb site) by 4- to 9-fold and increases RNA Polymerase II binding to its proximal promoter by 75-fold. The increased p53 and RNA Polymerase II binding is consistent with the observed 12-fold up-regulation of CDKN1A expression as measured by real-time RT-PCR (data not shown).

### miRNA PCR ARRAYS & ASSAYS

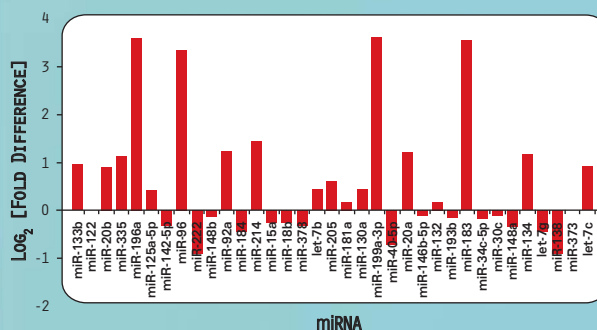
Detection of Genome-Wide or Pathway-Focused miRNA

MicroRNA (miRNA) is a class of endogenously expressed small single stranded RNA sequences that regulate the expression of messenger RNA sequences post-transcriptionally. They primarily inhibit translation, but can also promote message turnover. The discovery of miRNA has further complicated our understanding of how gene expression is regulated. The complete role played by each sequence has yet to be determined. In the absence of widely available functional analysis tools, researchers are currently correlating miRNA expression profiles with their biological phenotypes under study or the expression levels of their favorite proteins and genes. Let the RT<sup>2</sup> miRNA qPCR Arrays help you discover miRNA sequences regulating genes involved in your biological process of interest.

#### Cancer miRNA

The Human Cancer RT<sup>2</sup> miRNA PCR Array profiles the expression of 88 miRNA sequences whose levels, in published results, correlate well with the diagnosis, staging or progression, and prognosis of various cancers or tumors.

#### HUMAN COLON TUMOR VERSUS ADJACENT NORMAL TISSUE



#### Figure: Development of Cancer Biomarker Profiles.

Small RNA enriched from human colon tumor and matched adjacent normal tissue was characterized on Human Cancer RT<sup>2</sup> miRNA PCR Arrays. The log<sub>2</sub> fold-differences plotted for each miRNA indicate that many of these sequences have been up-regulated in this particular tumor.

#### miRNA PCR Arrays

Product	Catalog #
Human Cancer miRNA PCR Array	MAH-102
Human Cell Differentiation & Development miRNA PCR Array	MAH-103
Human Genome miRNA PCR Array (96-well)	MAH-100
Human Genome miRNA PCR Array (384-well)	MAH-3100
Human miFinder™ miRNA PCR Array	MAH-001

#### Required miRNA PCR Accessories

Product	Catalog #
RT <sup>2</sup> qPCR-Grade miRNA Isolation Kit	MA-01
RT <sup>2</sup> miRNA First Strand Kit	MA-03
RT <sup>2</sup> qPCR Master Mix w/ SYBR Green/ROX	PA-012
RT <sup>2</sup> qPCR Master Mix w/ SYBR Green/Fluorescein	PA-011
RT <sup>2</sup> qPCR Master Mix w/ SYBR Green Only	PA-010

# FEATURED PRODUCTS: PCR ARRAYS

*"If you have access to a real-time PCR instrument, you can use PCR Arrays."*

## What are PCR Arrays?

RT<sup>2</sup> Profiler PCR Arrays are the most reliable and sensitive gene expression profiling technology for analyzing a focused panel of genes involved in a signal transduction, biological process, or disease related pathway using real-time PCR.

## How are PCR Arrays Utilized?

The RT<sup>2</sup> Profiler PCR Arrays have been increasingly used in research on cancer, immunology, stem cells, toxicology, biomarker discovery & validation, and phenotypic analysis of cells & transgenic animals.

## Why PCR Arrays?

### • Simplicity

The simplicity of the PCR Arrays makes expression profiling accessible for routine use in every research laboratory with a real-time PCR instrument.

### • Performance

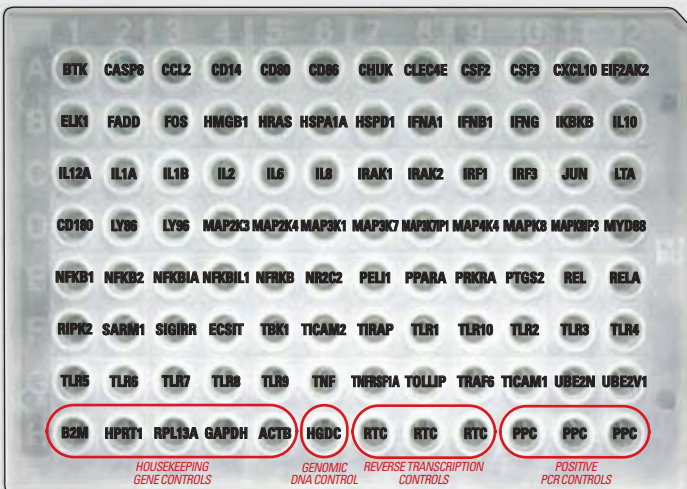
The PCR Arrays have the sensitive, reproducible, specific, and reliable performance to accurately profile multiple genes simultaneously in 96- or 384-well formats.

### • Relevance

PCR Arrays focus on profiling the genes relevant to the pathways or disease states of interest.

## Anatomy of a PCR Array

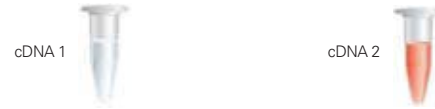
### Human Toll-like Receptor Signaling Pathway (PAHS-018A)



Each well in a PCR Array measures the expression of a gene related to a pathway or disease state. Plates are available in 96- and 384-well format.

## How PCR Arrays Work

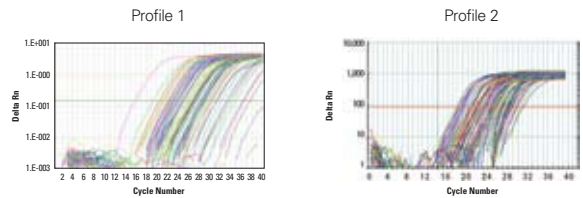
1. Convert Total RNA to cDNA.



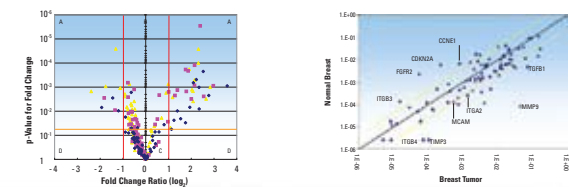
2. Add cDNA to RT<sup>2</sup> qPCR Master Mix & Aliquot Mixture Across PCR Array.



3. Run in Your Real-Time PCR Instrument.



4. Data Analysis.



## Complete PCR Systems

- RT<sup>2</sup> First Strand Kit: cDNA Synthesis

Eliminates Genomic DNA  
Insures RT Efficiency & Consistency  
20-Minute Protocol: Quick & Simple



- SYBR Green qPCR Master Mixes

Specific Amplification - No Primer Dimers  
Maximum Amplification Efficiencies  
Formulated for the Instrument in Your Lab



- Data Analysis: Convert Raw C<sub>t</sub> Data to Fold-Change Results

Easy-to-Use Web Portal

<http://www.SABiosciences.com/pcrarraydataanalysis.php>

## Newest & Most Popular PCR Arrays

Pathway / Topic Focus	PCR Array Catalog #		
	Human	Mouse	Rat
Alzheimer's Disease *	PAHS-057	PAMM-057	PARN-057
Angiogenesis	PAHS-024	PAMM-024	PARN-024
Angiogenic Growth Factors & Angiogenesis Inhibitors	PAHS-072	PAMM-072	PARN-072
Apoptosis	PAHS-012	PAMM-012	PARN-012
Breast Cancer and Estrogen Receptor Signaling	PAHS-005	PAMM-005	PARN-005
Cancer Drug Resistance and Metabolism	PAHS-004	PAMM-004	PARN-004
Cancer PathwayFinder	PAHS-033	PAMM-033	PARN-033
Cell Cycle	PAHS-020	PAMM-020	PARN-020
Chemokines and Receptors	PAHS-022	PAMM-022	PARN-022
Common Cytokines	PAHS-021	PAMM-021	PARN-021
DNA Damage Signaling Pathway	PAHS-029	PAMM-029	PARN-029
Drug Metabolism	PAHS-002	PAMM-002	PARN-002
Drug Transporters	PAHS-070	PAMM-070	PARN-070
EGF / PDGF Signaling Pathway *	PAHS-040	PAMM-040	PARN-040
Extracellular Matrix and Adhesion Molecules	PAHS-013	PAMM-013	PARN-013
Growth Factors	PAHS-041	PAMM-041	PARN-041
Heat Shock Proteins *	PAHS-076	PAMM-076	PARN-076
HIV Infection & Host Response *	PAHS-051	PAMM-051	PARN-051
Hypoxia Signaling Pathway	PAHS-032	PAMM-032	PARN-032
Inflammatory Cytokines and Receptors	PAHS-011	PAMM-011	PARN-011
Inflammatory Response & Autoimmunity *	PAHS-077	PAMM-077	PARN-077
Innate & Adaptive Immune Responses *	PAHS-052	PAMM-052	PARN-052
Interferons (IFN) and Receptors	PAHS-064	PAMM-064	PARN-064
JAK / STAT Signaling Pathway	PAHS-039	PAMM-039	PARN-039
MAP Kinase Signaling Pathway	PAHS-061	PAMM-061	PARN-061
Neurogenesis & Neural Stem Cells *	PAHS-404	PAMM-404	PARN-404
Neuroscience Ion Channels and Transporters	PAHS-036	PAMM-036	PARN-036
Neurotransmitter Receptors and Regulators	PAHS-060	PAMM-060	PARN-060
Neurotrophins and Receptors	PAHS-031	PAMM-031	PARN-031
NFκB Signaling Pathway	PAHS-025	PAMM-025	PARN-025
Nitric Oxide Signaling Pathway	PAHS-062	PAMM-062	PARN-062
Notch Signaling Pathway	PAHS-059	PAMM-059	PARN-059
Osteogenesis	PAHS-026	PAMM-026	PARN-026
Oxidative Stress and Antioxidant Defense	PAHS-065	PAMM-065	PARN-065
p53 Signaling Pathway	PAHS-027	PAMM-027	PARN-027
PI3K / AKT Signaling Pathway *	PAHS-058	PAMM-058	PARN-058
Signal Transduction PathwayFinder	PAHS-014	PAMM-014	PARN-014
Stem Cell	PAHS-405	PAMM-405	PARN-405
Stress and Toxicity PathwayFinder	PAHS-003	PAMM-003	PARN-003
TGFβ / BMP Signaling Pathway	PAHS-035	PAMM-035	PARN-035
Th17 for Autoimmunity and Inflammation	PAHS-073	PAMM-073	PARN-073
Th1-Th2-Th3	PAHS-034	PAMM-034	PARN-034
Toll-Like Receptor Signaling Pathway	PAHS-018	PAMM-018	PARN-018
Tumor Metastasis	PAHS-028	PAMM-028	PARN-028
Tumor Necrosis Factor (TNF) Ligands and Receptors	PAHS-063	PAMM-063	PARN-063
Wnt Signaling Pathway	PAHS-043	PAMM-043	PARN-043
Custom PCR Arrays	Inquire	Inquire	Inquire
PCR Array Gene Expression Analysis Service	Inquire	Inquire	Inquire

\* NEW Array

### Over 100 Pathways Available

## Supported PCR Instruments

### Applied Biosystems (ABI)

7000, 7300, 7500, 7500 FAST, 7700, 7900HT, StepOnePlus (96- & 384-well blocks)

### Bio-Rad

iCycler, iQ5, MyiQ, Chromo4, Opticon, Opticon 2

### Stratagene

Mx3000P, Mx3005P, Mx4000

### Roche

LightCycler 480 (96- & 384-well blocks)

### Eppendorf

Mastercycler ep realplex

## Complete the PCR Array System

### Product Pack Size

The RT<sup>2</sup> First Strand Kit is REQUIRED for use with PCR Arrays.

RT <sup>2</sup> First Strand Kit	12 Arrays
----------------------------------	-----------

PCR Array pack sizes of 12 & 24 plates offer the best value.

PCR Arrays	2 Arrays (96-well)
	12 Arrays (96-well)
	24 Arrays (96-well)
	4 Arrays (384-well)

RT<sup>2</sup> SYBR Green qPCR Master Mixes are necessary for use with PCR Arrays. The Master Mix is available with ROX, Fluorescein, or SYBR Green only.

Master Mix	2 Arrays
	12 Arrays
	24 Arrays

### Guaranteed Performance

# CIGNAL™ REPORTER ASSAY SYSTEM:

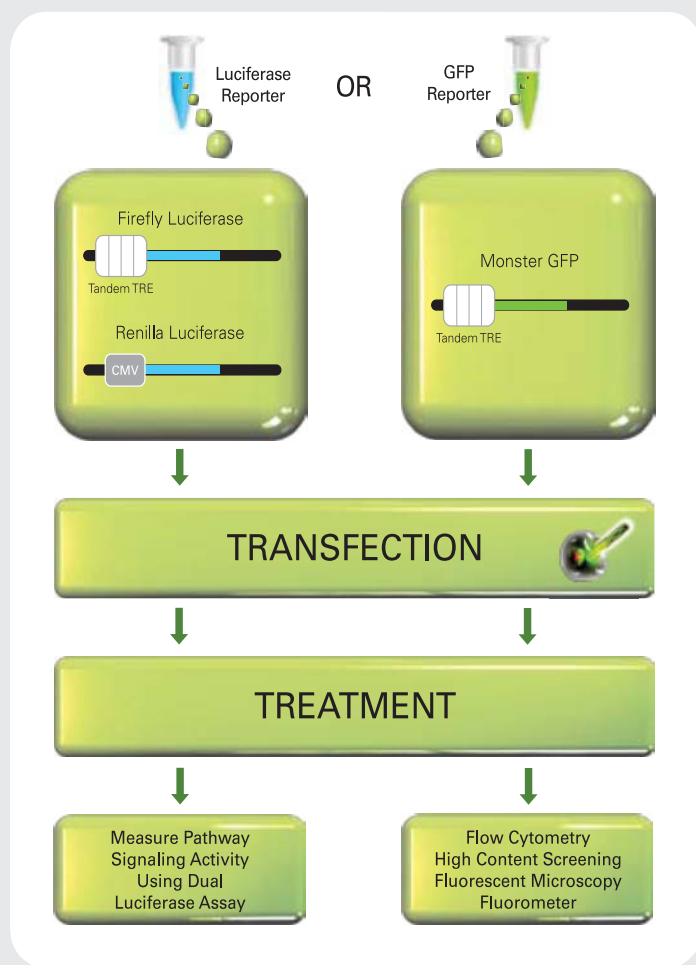
## Cell-Based Assays for Rapidly Analyzing Pathway Signaling Activity

### Cignal Reporter Assay System

The Cignal Reporter Assay System provides a comprehensive solution for monitoring diverse signaling pathways. The Cignal Reporter Assays provide a rapid, sensitive, and quantitative assessment of signal transduction pathway activation by measuring the activities of downstream transcription factors, using either dual-luciferase or green fluorescent protein (GFP) reporter systems.

Every reporter assay is individually engineered to exhibit outstanding sensitivity, specificity and signal-to-noise ratio. The Cignal Reporters are available as single pathway assays or as 10-pathway arrays, allowing you to monitor an individual pathway or obtain a comprehensive view of multiple pathways involved in a biological process. These reporter assays are valuable tools for understanding gene function, as well as determining the mechanisms of action for proteins, peptides, and small molecule compounds.

### How Cignal Reporter Assays Work



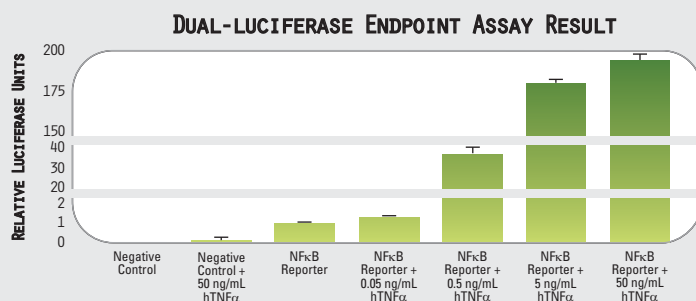
The Cignal Reporter Assays are available as transfection-ready constructs utilizing two reporter systems, either the dual-luciferase reporter or the GFP reporter. The GFP reporter allows the dynamic analysis of activity by fluorescent

microscopy or flow cytometry of individual cells, whereas the dual-luciferase assay provides end-point activity measurements with exceptional sensitivity and reproducibility. Appropriate controls are included to ensure that treatment outcomes are pathway-specific and not the result of nonspecific effects.

### Dual-Luciferase Reporter Assays

Exceptional sensitivity, reproducibility, specificity and signal to noise ratio are provided by key technical features:

- Proprietary TRE structures maximize the specificity & response of the assay.
- Reproducibility is greatly enhanced by the use of the dual-luciferase assay, where the firefly luciferase activity is normalized against the Renilla luciferase.
- Use of destabilized luciferase increases the signal-to-noise ratio.



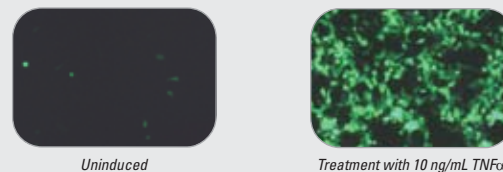
**Figure 1: Dual-Luciferase Endpoint Assay Result.** NFκB reporter for monitoring NFκB pathway activation.

### GFP Reporter Assays

The use of GFP as a reporter allows dynamic live cell expression analysis with single cell resolution using fluorescent microscopy or flow cytometry.

- Real-time live cell pathway analysis
- Single cell resolution
- Readout versatility - can use flow cytometry, fluorescent microscopy, or fluorometry

### GFP DYNAMIC PATHWAY ACTIVATION ASSAY RESULT



**Figure 2: Fluorescent Microscopy.**

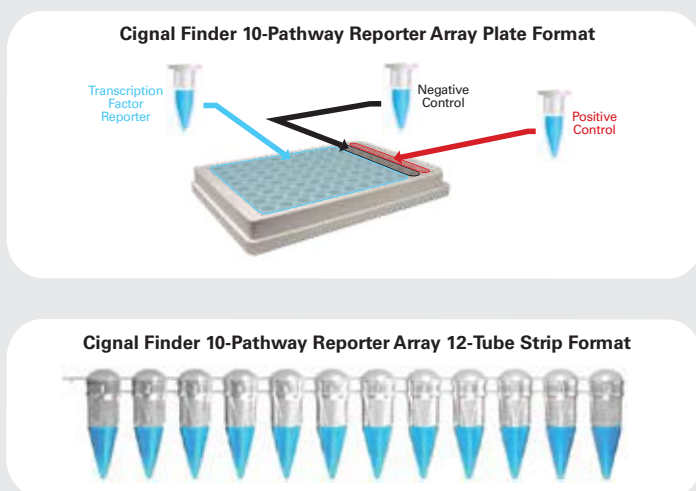
Cignal NFκB-GFP reporter showed that Human Tumor Necrosis Factor Alpha (hTNFα) activated the NFκB signaling pathway. 293-H cells were transfected with the Cignal NFκB-GFP reporter or negative control (for transfection protocol refer to our user manual). After 16 hours of transfection, medium was changed to assay medium (Opti-MEM + 0.5% FBS + 0.1 mM NEAA + 1 mM sodium pyruvate + 100 μg/mL penicillin + 100 μg/mL streptomycin). After 24 hours of transfection, cells were treated with 10 ng/mL hTNFα. After 18 hours of treatment, fluorescent images were taken of the cultures. Uninduced cultures and the treated cultures are indicated.

## Cignal Finder™ 10-Pathway Arrays (Luciferase)

In the post-genomic era, it is becoming ever more important to measure signaling pathway interactions. The 10-Pathway Arrays provide comprehensive pathway analysis in four research areas:

- Cancer
- Immunology
- Development
- Pharmacology / Toxicology

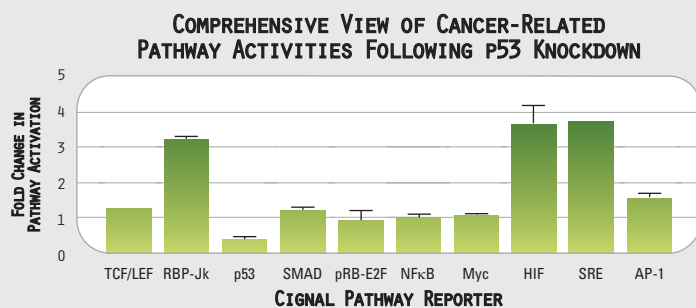
The Cignal Finder 10-Pathway Reporter Arrays are valuable tools for progressing from identifying genes, proteins and small molecules to understanding their function. These system-wide studies will reveal regulatory interactions that more closely reflect physiological interactions, and will lead to new insights into abnormal regulation in disease.



**Figure 3: Cignal Finder 10-Pathway Reporter Arrays Are Available in Two Formats.**

The tube format is delivered in a twelve tube strip with the negative and positive controls. The plate format is delivered in a 96-well cell culture plate. In the plate format, each of the 10 reporter assays (with negative and positive controls) is dried down in one row of the array (8-wells per reporter assay /control).

### Application Example of the Cignal Finder 10-Pathway Reporter Array



**Figure 4: Inhibition of the p53 Signaling Pathway by p53 siRNA Treatment Results in Activation of the Notch, Hypoxia, & MAPK/ERK Pathways.**

## Summary

SABiosciences has identified the shortcomings of currently available reporter assays, and has overcome them with a number of technical innovations that are incorporated into the Cignal Reporter Assays. The development of these reliable cell-based assays for measuring transcription factor activities is a much-needed technology. The Cignal Reporter Assay System enables researchers to carry out a rapid and comprehensive analysis of how their gene, protein, or small molecule of interest impacts critical cell signaling pathways.

## Cignal Products Currently Available

### Cignal Dual-Luciferase Reporter Assays

Pathway	Transcription Factor	Catalog #
C/EBP	C/EBP	CCS-001L
cAMP/PKA	CREB	CCS-002L
Cell Cycle	E2F/DP1	CCS-003L
p53/DNA Damage	p53	CCS-004L
Estrogen Receptor	Estrogen Receptor (ER)	CCS-005L
Glucocorticoid Receptor	Glucocorticoid Receptor (GR)	CCS-006L
Hypoxia	Hypoxia-Inducible Factor-1 (HIF-1)	CCS-007L
Type I Interferon	STAT1/STAT2	CCS-008L
Interferon Gamma	STAT1/STAT1	CCS-009L
MAPK/ERK	Elk-1/SRF	CCS-010L
MAPK/JNK	AP-1	CCS-011L
c-myc	Myc/Max	CCS-012L
NFκB	NFκB	CCS-013L
Notch	RBP-Jk	CCS-014L
PKC/Ca <sup>++</sup>	NFAT	CCS-015L
Retinoic Acid Receptor	Retinoic Acid Receptor (RAR)	CCS-016L
TGFβ	SMAD2/SMAD3/SMAD4	CCS-017L
Wnt	TCF/LEF	CCS-018L

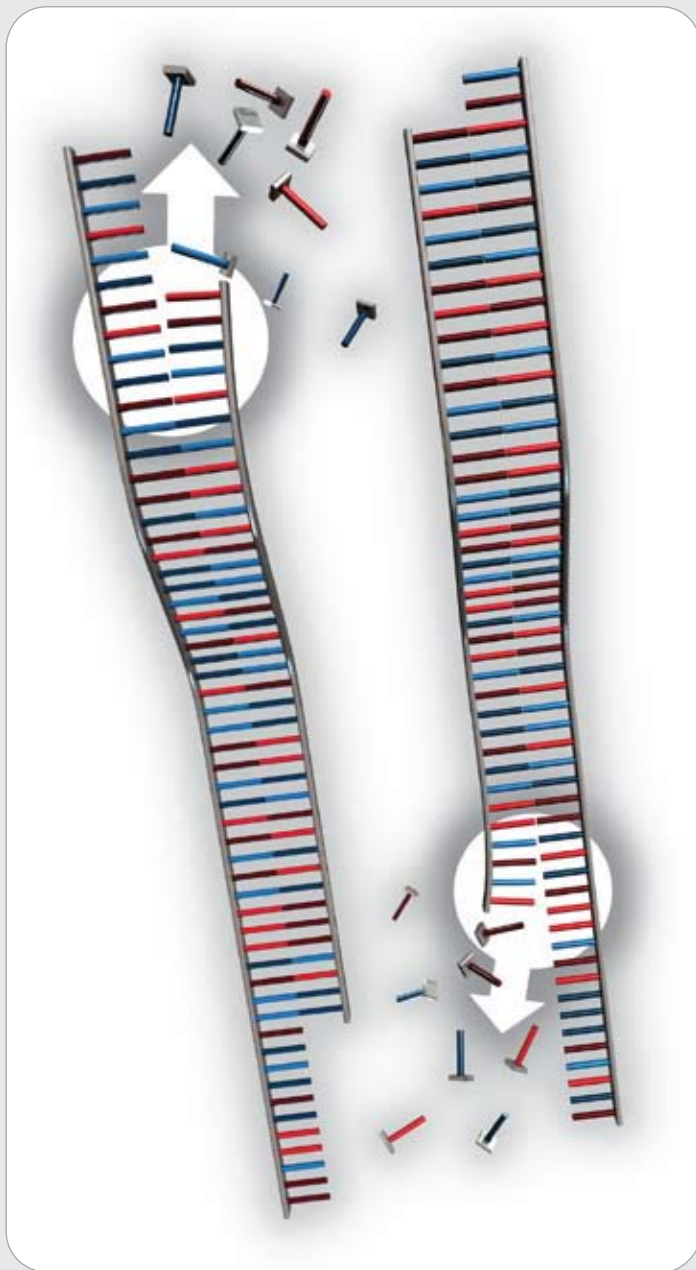
### Cignal GFP Reporter Assays

Pathway	Transcription Factor	Catalog #
cAMP/PKA	CREB	CCS-002G
MAPK/ERK	Elk-1/SRF	CCS-010G
MAPK/JNK	AP-1	CCS-011G
NFκB	NFκB	CCS-013G
TGFβ	SMAD2/SMAD3/SMAD4	CCS-017G
Wnt	TCF/LEF	CCS-018G

### Cignal Finder 10-Pathway Reporter Arrays (Luciferase)

Pathway	Catalog #
Cancer Reporter Array (tube format)	CCA-001L
Cancer Reporter Array (plate format)	CCA-101L
Immune Response Reporter Array (tube format)	CCA-002L
Immune Response Reporter Array (plate format)	CCA-102L
Development Reporter Array (tube format)	CCA-003L
Development Reporter Array (plate format)	CCA-103L
Toxicity Reporter Array (tube format)	CCA-004L
Toxicity Reporter Array (plate format)	CCA-104L

# DESIGNING & VALIDATING REAL-TIME PCR PRIMERS: Systematic Guidelines



## Introduction

This article outlines the parameters that are crucial in designing and validating real-time RT-PCR assays, based on SABiosciences' experience with over 14,000 genes. The primer design algorithm and the reaction conditions must work together to provide optimal results. The tests required to identify potential problems with each critical aspect of primer design and their solutions will be described. Designs of good primers for RT-PCR must meet at least two critical performance parameters.

- Single-Amplicon Specificity
- Consistently High Amplification Efficiencies

## Design Algorithm

Primer design needs to include several important thermodynamic and sequence criteria (Table 1).

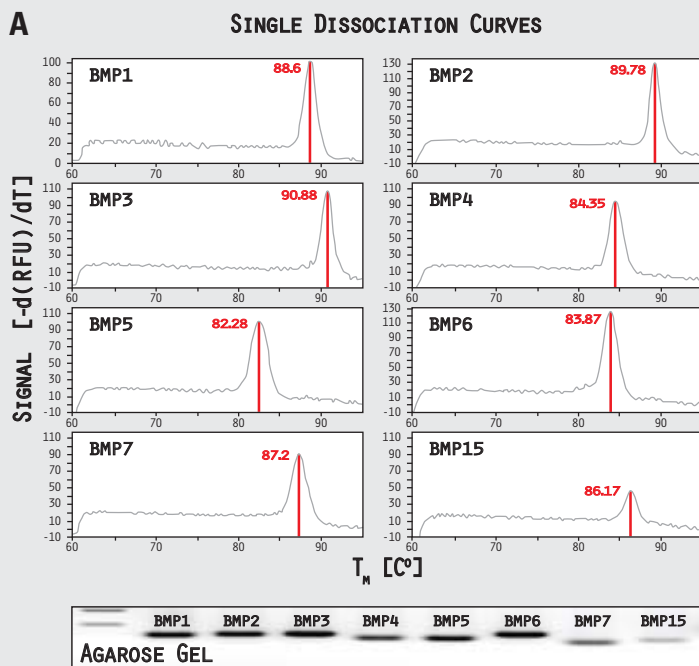
**Table 1: The Design Algorithm for the RT<sup>2</sup> qPCR Assays from SABiosciences Utilizes More Than TEN Thermodynamic and Sequence Alignment Criteria.**

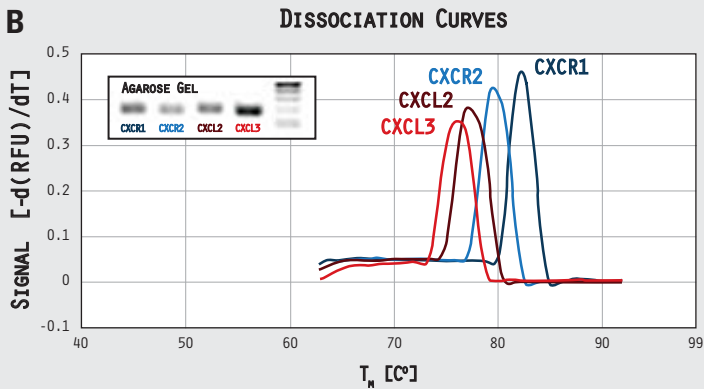
<b>Amplicon Length</b>	50 - 210 bp
<b>Primer Length</b>	19 - 23 nucleotides
<b>GC Content</b>	35 - 65%
<b>T<sub>m</sub></b>	60 - 68 °C
<b>3'-End Stability</b>	Composition of last 3 base pairs
<b>Complementaries</b>	Avoid primer self- or cross- annealing stretches > 4 bp
<b>Specificity</b>	BLAST versus entire mRNA RefSeq database
<b>SNP Database</b>	Primer sequences do not include known SNP

All algorithm's primer designs must also be experimentally validated with two important quality control assays. First, a melt curve analysis, confirmed by agarose gel electrophoresis, must verify that a single gene-specific product is produced. The second criterion to pass is an amplification efficiency > 90 %.

## Specificity

All real-time PCR primers must generate a single amplicon of the correct size on agarose gels. Alternatively, a default melting program can be run on real-time PCR instruments at the end of the cycling program. As shown in Figure 1, dissociation curves (first derivatives of the melting curves) should each contain a single peak with no shoulders, and the agarose gels of the amplified product should reveal single bands corresponding to the predicted amplicon length.

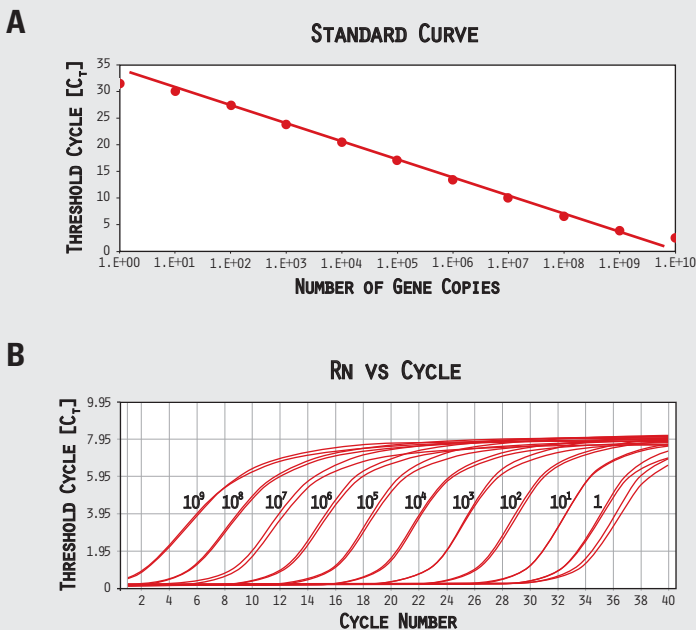




**Figure 1: RT<sup>2</sup> Profiler™ PCR Arrays and RT<sup>2</sup> qPCR Assays Amplify A Single Gene-Specific Product In Every Reaction.**  
 Human XpressRef™ Universal Total RNA was characterized on both the Human TGFβ / BMP Signaling Pathway (A) and the Human Common Cytokines (B) RT<sup>2</sup> Profiler PCR Arrays, followed by dissociation (melt) curve and gel electrophoretic analysis. Each RT<sup>2</sup> qPCR Assay specifically detects an individual gene. Assays for the displayed BMP and cytokine genes tend to be notoriously difficult to design.

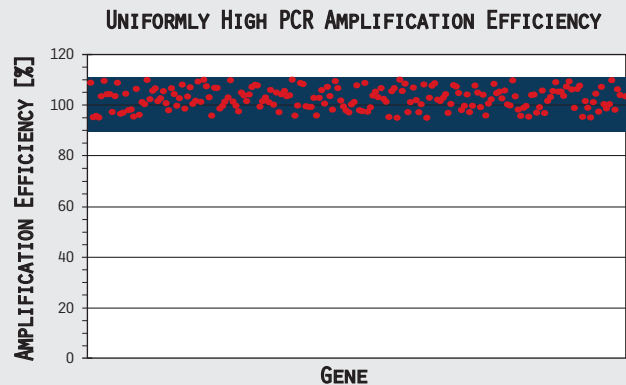
**Accuracy & Reliability**

The typical method to analyze real-time PCR data is the ΔΔC<sub>t</sub> method. Its mathematics assumes that the real-time PCR assay has a 100% amplification efficiency. As the primers deviate from this ideal, the error in the fold difference increases exponentially. The traditional method for determining amplification efficiency requires a calibration curve. Serially dilute an artificial template of known concentration. Plot the C<sub>t</sub> values versus the initial amounts of input material on a semi-log<sub>10</sub> plot, fit the data to a straight line (Fig. 2), and calculate the slope. The closer the slope is to -3.33, the closer the amplification efficiency is to the 100% ideal.



**Figure 2: RT<sup>2</sup> Profiler PCR Arrays and RT<sup>2</sup> qPCR Assays Have Sufficiently Wide Dynamic Ranges.**  
 A standard curve (A) was generated using duplicate ten-fold serial dilutions of purified template and the RT<sup>2</sup> qPCR Assay for the Human Nicotinic Acetylcholine Receptor Alpha 5 (CHRNA5, B). Our real-time RT-PCR assays have an eight-log linear dynamic range, from 10 to 10<sup>9</sup> copies of template.

Using this method, our observed average amplification efficiency over more than 4000 genes is 99% with a 95% confidence interval of ± 10% (Fig. 3).



**Figure 3: The RT<sup>2</sup> Profiler PCR Arrays & RT<sup>2</sup> qPCR Assays Yield The Most Accurate Results.**  
 A representative set of assays for 4,000 genes used in the RT<sup>2</sup> PCR Arrays demonstrate their average amplification efficiency of 99% and their 95% confidence interval about the mean from 90 to 110%. Consistently high amplification efficiencies enable PCR assays to accurately analyze gene expression using the ΔΔC<sub>t</sub> method.

**Summary**

As briefly outlined here, it is clear that the steps required for validating a gene expression assay often take longer than the experiment itself. With each additional gene, the validation requires the same amount of time. Whether starting with one gene or a set of genes, many labs do not have the time or resources to optimize each new real-time RT-PCR assay so that every assay can be performed together. How can you possibly achieve this level of quality control with the assays that you need today, as well as in the future?

To help researchers, SABiosciences is pleased to offer a genome-wide approach for gene expression analysis, providing performance guaranteed primer pairs with these characteristics for every gene.

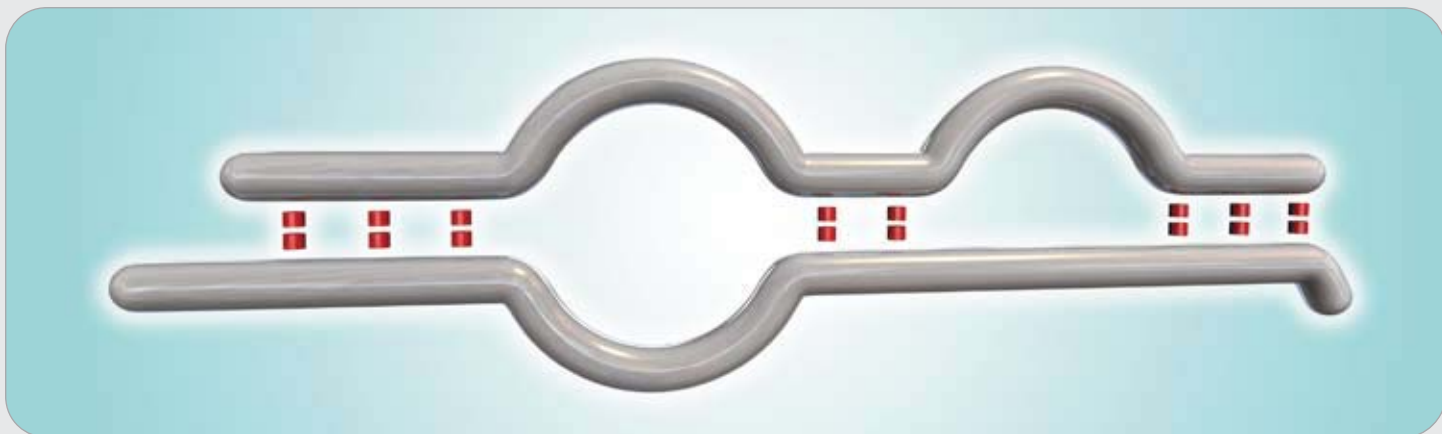
Product	Species	Catalog #
Genome-Wide qPCR Primer Assays	Human	PPH#####A-200
Genome-Wide qPCR Primer Assays	Mouse	PPM#####A-200
Genome-Wide qPCR Primer Assays	Rat	PPR#####A-200

Real-Time PCR primers are available genome-wide for human, mouse and rat genes. Find your gene(s) of interest using our gene search interface online at:

<http://www.SABiosciences.com>



# MICRORNA: WHY STUDY IT AND HOW



## MicroRNA (*miRNA*)

Regulation of transcription and translation in eukaryotes is complex. New layers of complexity are steadily found, such as a function for a part of the 'junk' DNA that is transcribed. MicroRNAs (miRNAs) were first discovered in 1993, when the miRNA *lin-4* was determined to downregulate expression of the gene *lin-14* in *Caenorhabditis elegans* [1][2]. However, since there is no homolog to *lin-4* in other species, this discovery was considered to be unique. Specific and potent silencing of genes by double stranded RNA (RNAi) was discovered in 1998 [3], and the discovery of the miRNA *let-7* in 2000 [4][5], with homologs in other species including humans, showed that miRNAs are quite common in eukaryotes. There are now known to be multiple types of small noncoding RNA (for review see [6]), with miRNAs being the largest family of noncoding RNAs involved in gene silencing.

## What is miRNA?

MicroRNA (miRNA), 19-25 nucleotides in length, are typically encoded within introns, and have been discovered in metazoans, plants and viruses, as well as a few in protists and slime mold, with more being confirmed every day. In mammals, miRNAs are first transcribed as a long RNA transcript (between hundreds of nucleotides and tens of kilobases) [7], called primary miRNA (pri-miRNA), which contains imperfectly base-paired hairpin structures. These pri-miRNA, which may contain sequences encoding multiple miRNAs, are cleaved in the nucleus into shorter precursor miRNA (pre-miRNA). This reaction is performed by a protein complex called Microprocessor, (Figure 1, page 6), which involves Drosha, the RNase III enzyme, and DiGeorge Syndrome Critical Region 8 Protein (DGCR8), a double-stranded RNA-binding domain protein. Pre-miRNA is a short stem loop ~70 nucleotides in length with a 2-nucleotide 3'-overhang. This pre-miRNA is exported from the nucleus by Exportin-5, and cleaved in the cytoplasm by Dicer, another RNase III enzyme, into the mature 19-25 nucleotide miRNA:miRNA\* duplex. The miRNA strand with lower base pairing stability (the guide strand) is loaded onto the RNA-induced silencing complex (RISC), composed of Dicer, TAR RNA binding protein (TRBP) and the Argonaute protein Ago2. The passenger guide strand, usually miRNA\*, is sometimes functional, but is usually degraded.

In vertebrates the RISC complex is guided to its mRNA target by the miRNA strand, which typically base pairs imperfectly to its target in the 3' untranslated region, signaling the target for translational repression through unknown mecha-

nisms. More than 500 miRNAs have been identified in humans [8][9], and each miRNA is proposed to have hundreds of mRNA targets due to the imperfect base pairing [10]. Therefore, the bioinformatic prediction that 30% of human genes are regulated by miRNA can be seen as a reasonable assumption [11]. Visit the following web address for a repository of all confirmed miRNA sequences:

<http://www.SABiosciences.com/miRNAsearch.php>

Small inhibitory RNA (siRNA) was discovered as a reagent that can be transfected into cells to transiently knockdown a specific protein. Many researchers are using this powerful tool to enhance their study of a gene of interest. Processing of siRNA is similar to miRNA, but varies from miRNA by its method of gene silencing; only 19-21 nucleotides in length, inhibition by siRNA requires an exact match to its single target mRNA, which differs from miRNA's imperfect basepairing; in addition, siRNA inhibits this target by triggering mRNA degradation, whereas miRNA triggers translation inhibition (Table 1).

## Relevance of miRNA to Human Biology

Before the discovery of miRNA, it had been known that a large part of the genome is not translated into proteins. This so called "junk" DNA was thought to be evolution's debris with no function. We now realize that a portion of this coding DNA is highly relevant in the regulation of gene expression.

The importance of the miRNA regulatory pathways is underscored by the impressive list of diseases which have recently been found to be associated with abnormal miRNA expression (Table 2).

### ● Cancer

miRNAs have been found to be downregulated in a number of tumors [10,25], and in some cases the reintroduction of these miRNAs has been shown to impair the viability of cancer cells. The value of miRNA profiles in tumor diagnostics is well established. For instance, strong up and down regulations of 16 miRNAs have been shown in primary breast tumors, and these markers may aid in the development of drug-resistance and treatment-selection tests [25]. Underlining the important role miRNA plays in oncology is the formation of several new companies which seek to expand development of miRNA-based therapeutics [25].

### Age-Related Diseases

Evidence is accumulating that many age-related diseases are associated with a decreased control of cell signaling that occurs in mid-life [25]. The miRNA control of such systems as the cell cycle, DNA repair, oxidative stress responses and apoptosis, has been shown to become abnormally expressed in mid-life. It is highly likely that continued research will reveal important associations with the aging process, and may lead to therapeutics that can improve the quality of life.

### Heart Disease

Two heart-specific miRNAs were deleted in mouse models resulting in abnormal heart development in a large proportion of the offspring [25]. While these lethal effects were expected, other studies show a more subtle role for miRNA in the heart. When miR-208 was eliminated, the mice appeared normal. Defects were revealed only when their hearts were stressed. These results show that comprehensive miRNA studies may be valuable in the diagnosis of heart disease.

### Neurological Diseases

Numerous reports have demonstrated the role of miRNAs in neural development. Evidence for a role in Parkinsons disease comes from animal model studies published last year, showing that loss of miRNAs may be involved in the development and progression of the disease. In cell culture experiments, transfer of small RNA fragments partially preserved miRNA deficient nerve cells [25]. While these results and others point to an important role for miRNA in neurodegenerative disorders, much more work is needed to delineate the exact role of miRNAs in this important area.

### Immune Function Disorders

Recent miRNA deletion studies have revealed a central role in the regulation of the immune response. The deletion of miRNA-155 impaired T and B cell differentiation in germinal centers, and greatly decreased antibody and cytokine production [24]. Two additional studies deleting miRNA-181 and 223 were found to control T cell response and granulocyte production, respectively [25]. As more roles for miRNAs in the immune response are found, the list of immune function disorders with a miRNA component is certain to expand also.

## Future Directions for miRNA

miRNA may also be involved in other processes besides translational gene silencing. Currently there are hints of this, because mature mammalian miRNAs can be imported into the nucleus [17] and secreted from the cell [18]. These results suggest that miRNA may regulate transcription or paracrine signaling. Unlike siRNA, miRNA is endogenous, and therefore has the potential to enhance the understanding of the regulation of particular genes. In addition, miRNA is now touted as an additional layer of gene regulation, which can be dysregulated in diseases. Currently the study of miRNAs requires large scale arrays, since few miRNA targets are experimentally confirmed and individual miRNAs may have overlapping functions. The relative lack of attention devoted to miRNA will change in the future, as scientists realize that their favorite gene may have an additional layer of regulation never touched upon. While siRNA is merely an important tool, miRNA is evolving into a whole new field of research.

**Table 1: Comparison of miRNA and siRNA.**

	Length	Where Found?	Target Recog	Mechanism
miRNA	19-25 nt	Endogenous	Imperfect Match	Translational Repression
siRNA	19-21 nt	Exogenous	Exact Match	mRNA Cleavage

**Table 2: Examples of miRNA Functions & Relevance of miRNA to Human Biology.**

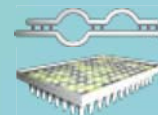
miRNA	Target	Function	Reference
miR-15/miR-16	Bcl2	Apoptosis	[19]
miR-1	GJA1/KCNJ2	Cardiac Arrhythmia	[20]
miR-146	IRAK1/TRAF6	Bacterial Infectious Response; TLR-NFκB	[21]
miR-520h	ABCG2	Stem Cell Differentiation	[22]
miR-106a	Rb1	Cancer Pathogenesis	[23]
miR-let7	Multiple	Cell Cycle Regulation	[25]
miR-155	-	Adaptive Immunity	[26]
miR-223	-	Granulocyte Regulation	[27]
miR-208	-	Stress Response (Heart)	[24]

## Complete System for miRNA Research from SABiosciences

SABiosciences' RT<sup>2</sup> miRNA PCR Arrays & qPCR Assays generate high-quality and genome-wide miRNA expression data with nothing more than a simple RT-PCR protocol. Our patented miRNA technology ingeniously integrates a universal tailing & reverse transcription reaction specific for miRNA with the accurate expression level measurement of distinct miRNA sequences that may only differ by a single nucleotide base. With this technology, you can easily get a comprehensive survey of miRNA expression in your cell line or tissue of interest.

### SABiosciences' complete miRNA PCR System includes:

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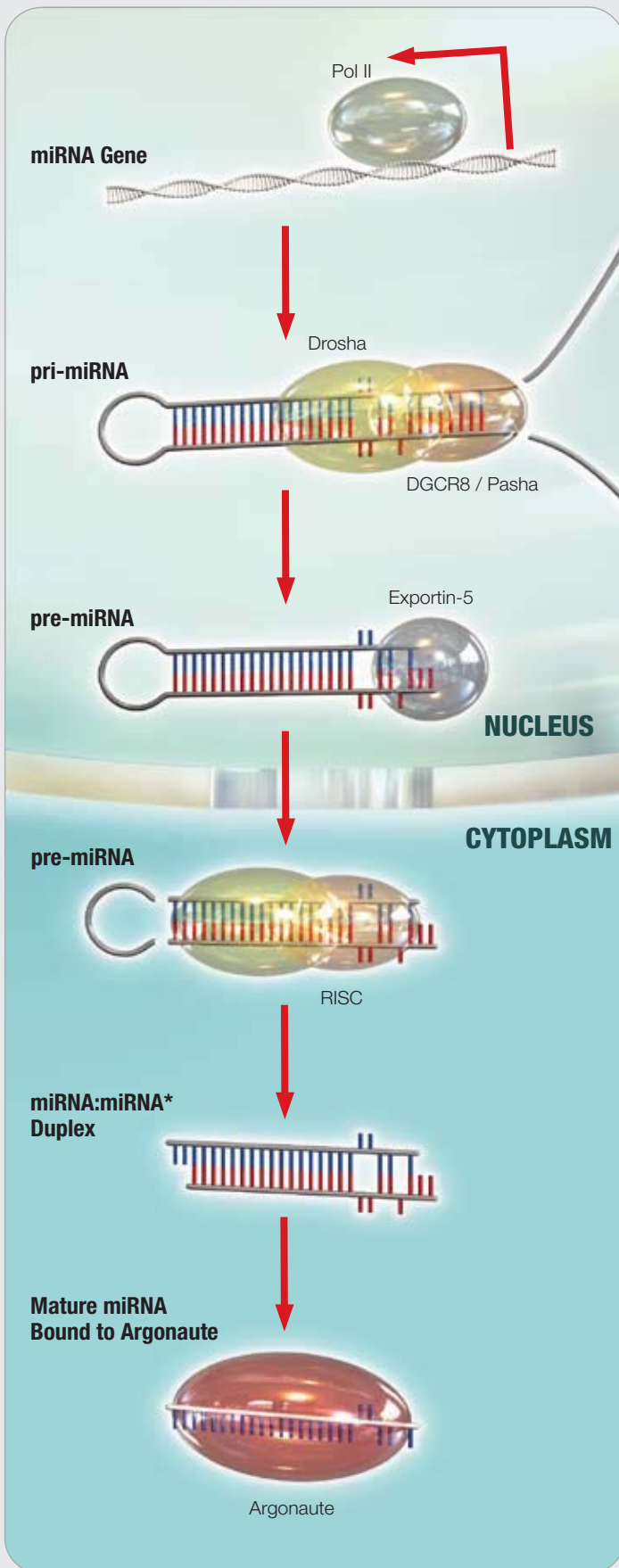


### What the System Offers:

Detecting every miRNA across the entire genome in a specific and sensitive way is a very challenging technology task. Many miRNA family members and otherwise distinct miRNA species have very similar sequences. Moreover, other RNA species such as snRNA, tRNA, mRNA, and rRNA can cause non-specific amplification, making the specific analysis of mature miRNA even more problematic. With SABiosciences' complete miRNA PCR System & expression analysis system, these problems are solved.

RT<sup>2</sup> miRNA PCR Arrays and Assays dramatically improve the specificity through patent pending primer design & proprietary reverse transcription chemistry. Our miRNA PCR Arrays include built-in control elements to insure the quality of your experimental data. The free data analysis software takes your raw threshold cycle data and automatically generates figures and tables ready for publication. With the RT<sup>2</sup> miRNA PCR Assay and Arrays, you can expect:

- **Sensitivity:** As little as 0.5 µg total RNA needed
- **Multi-Sequence Flexibility:** Analyze one to 376 sequences simultaneously
- **Simplicity:** As easy as a real-time PCR experiment



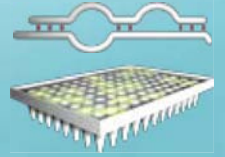
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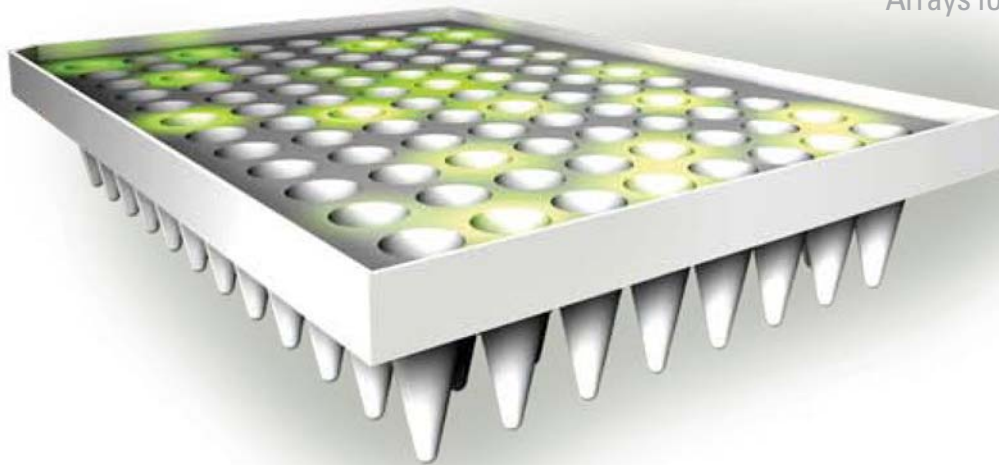
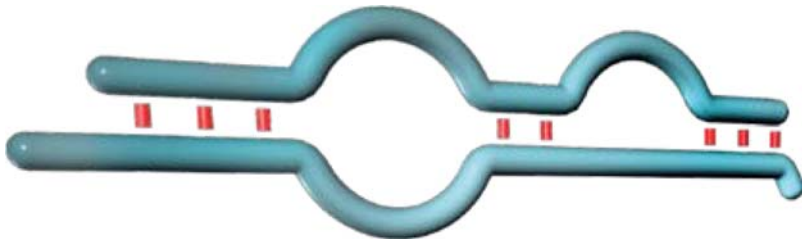
Figure 1: Biogenesis of miRNA.

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