

# InvivoGen Insight

An Insightful look at InvivoGen's Gene Delivery Products

This issue of InvivoGen Insight focuses on a pair of exciting new product lines encompassing two of the hottest topics in functional genomics: Toll-like receptors (TLRs) and small interfering RNAs (siRNAs).

TLRs are key regulators of innate immunity, sensing and responding to invading microorganisms. Understanding their complex role in host immunity offers tremendous potential for developing novel therapeutic drugs for more effective treatment of infectious and immune diseases. InvivoGen is fostering advancement in this field by

providing a comprehensive TLR product line.

Small interfering RNAs represent a very promising technology for studying loss-of-function phenotypes by inducing efficient silencing of a target gene. These mediators of RNA interference are short double stranded RNAs that induce the specific degradation of the corresponding mRNA. InvivoGen has developed the psiRNA system, a simple and efficient method for production of siRNAs in mammalian cells capable of switching off a target gene *in vitro* and *in vivo*.

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#### Toll-like Receptors:

The sentinels of the innate immune system

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## Toll-like Receptors: the sentinels of the innate immune system

Toll-Like receptors (TLRs) play a critical role in early innate immunity to invading pathogens by sensing microorganisms. These evolutionary conserved receptors, homologues of the *Drosophila* Toll gene, recognize highly conserved structural motifs only expressed by microbial pathogens, called pathogen-associated microbial patterns (PAMPs). PAMPs include various bacterial cell wall components such as lipopolysaccharides (LPS), peptidoglycans and lipopeptides, as well as flagellin, bacterial DNA and viral double-stranded RNA. Stimulation of TLRs by PAMPs initiates a signaling cascade that involves a number of proteins, such as MyD88 and IRAK<sup>1</sup>. This signaling cascade leads to the activation of the transcription factor NF- $\kappa$ B which induces the secretion of pro-inflammatory cytokines and effector cytokines that direct the adaptive immune response.

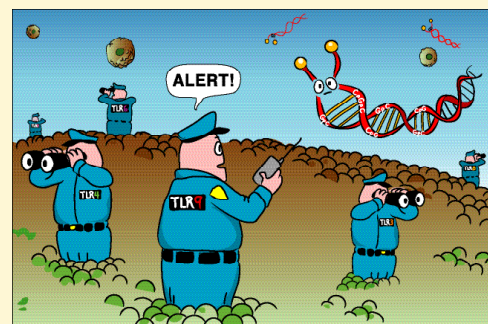
TLRs are transmembrane proteins characterized by an extracellular leucine-rich domain and a cytoplasmic tail that contains a conserved region called the Toll/IL-1 receptor (TIR) domain. TLRs are predominantly expressed in tissues involved in immune function, such as spleen and peripheral blood leukocytes, as well as those exposed to the external environment such as lung and the gastrointestinal tract.

Ten human and nine murine TLRs have been characterized so far and only five of them have had their natural ligands identified<sup>2</sup>. TLR2 is essential for the recognition of a variety of PAMPs, including bacterial lipoproteins, peptidoglycan, and lipoteichoic acids. TLR3 is implicated in virus-derived double-stranded RNA. TLR4 is predominantly activated by lipopolysaccharide. TLR5 detects bacterial flagellin and TLR9 is required for response to unmethylated CpG DNA. Recently, TLR7 and TLR8 have been shown to recognize small synthetic antiviral molecules<sup>3</sup>.

Furthermore, in many instances, TLRs require the presence of a co-receptor to initiate the signaling cascade. One example is TLR4 which interacts with MD2 and CD14, a protein that exists both in soluble form and as a GPI-anchored protein, to induce NF- $\kappa$ B in response to LPS stimulation<sup>4</sup>.

Current knowledge of the TLRs indicates that these receptors are essential elements in host defense against pathogens by activating the innate immunity a prerequisite to induction of adaptive immunity. The growing interest on TLRs should bring a more complete understanding of the role of TLR-mediated responses and increase our range of weapons to treat infectious and immune diseases.

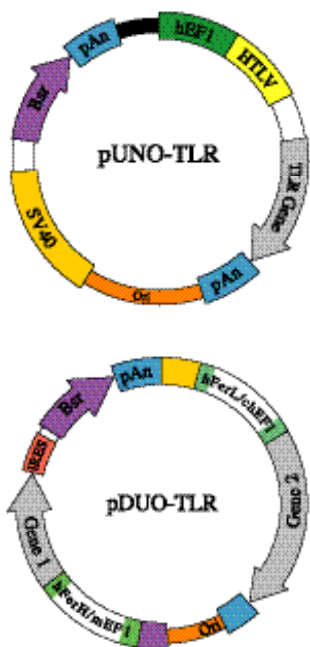
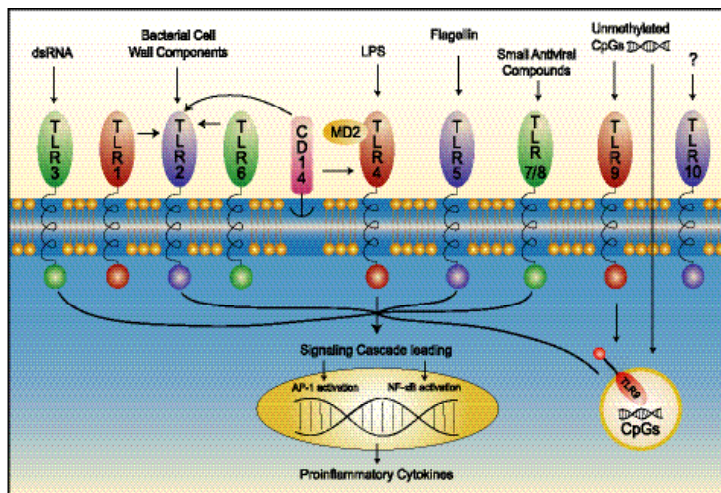
1. Medzhitov R. et al., 1997. A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature*, 388(6640):394-7
2. Underhill DM. and A. Ozinsky, 2002. Toll-like receptors: key mediators of microbe detection. *Curr Opin Immunol*, 14:103-10
3. Jurk M. et al., 2002. Human TLR7 or TLR8 independently confer responsiveness to the antiviral compound R-848. *Nat Immunol*, 3(6):499
4. Jiang Q. et al., 2000. Lipopolysaccharide induces physical proximity between CD14 and toll-like receptor 4 (TLR4) prior to nuclear translocation of NF- $\kappa$ B. *J Immunol*, 165(7):3541-4



# Toll-Like Receptors

## Unravel the Wonders of the TLRs

InvivoGen provides a comprehensive TLR product line that includes a large choice of genes, ligands and antibodies. These high-quality products allow you to generate cell lines that stably express one or several TLR genes, check their expression and test their functionality. InvivoGen's TLR product line will help jump-start your research of the TLRs whether it be studying the TLR metabolism, or discovering new therapeutic molecules.



## TLR Genes

Ten human and nine murine TLR genes have been characterized so far. Some TLRs have been shown to physically interact with other TLRs or TLR-related molecules. InvivoGen offers the TLR genes cloned either individually in the **pUNO** plasmid, or paired in the **pDUO** plasmid.

**pUNO** and **pDUO** offer the following features:

- Ready-to-use TLR expression vectors
- High levels of expression
- Convenient subcloning of the TLR genes
- Rapid selection of stable transfectants

**pUNO** and **pDUO** contain two transcription units, allowing the expression of one or several TLR genes. Both plasmids are selectable with blasticidin and can be used to stably transfect mammalian cells. TLR genes are driven by strong ubiquitous promoters: the EF1-RU5' promoter in pUNO, and the ferritin composite promoters which yield similar levels of expression in pDUO. Each TLR gene is flanked by unique restriction sites to facilitate their subcloning into another vector.

## TLR and TLR-related Genes Available

pUNO plasmid		
Human genes	TLR1, 2, 3, 4, 5, 6, 9, 10 MD1, MD2 RP105, Tollip CD14, sCD14	\$375
Murine genes	TLR1, 2, 3, 4, 5, 6, 7, 8, 9 MD1, MD2 RP105, Tollip	\$375
pDUO plasmid		
Human genes	TLR1/TLR2, TLR6/TLR2 CD14/TLR2, CD14/TLR4	\$450 \$450
Murine genes	TLR1/TLR2, TLR6/TLR2 MD2/TLR4	

## TLR Ligands

InvivoGen provides a selection of ligands known to activate a given TLR that can serve as controls in genetic and pharmaceutical studies on TLRs.

Product	Origin/Description	TLR Recognition	Quantity	Price
CpG oligonucleotides				
- ODN 2216	Human, type A	TLR9 ligand	100µg	\$140
- ODN 2006	Human, type B	TLR9 ligand	100µg	\$140
- ODN 1826	Murine	TLR9 ligand	100µg	\$140
Bacterial DNA				
- with endotoxins	<i>E. coli</i> K12	Multiple TLR ligand	100µg	\$70
- endotoxin-free		TLR9 ligand	100µg	\$140
Flagellin	Flagella component	TLR5 ligand	10µg	Inquire
Lipopolysaccharide	<i>E. coli</i> O11:B4, Purified LPS	TLR4 ligand	5 mg	\$80
Lipoteichoic acid	<i>B. subtilis</i>	TLR2 ligand	5 mg	\$55
Pam <sub>3</sub> Cys	Synthetic lipoprotein	TLR2 ligand	5 mg	\$80
Peptidoglycan	<i>Staphylococcus aureus</i>	TLR2 ligand	5 mg	\$80
Poly(I:C)	Synthetic analog of dsRNA	TLR3 ligand	10 mg	\$55
R848	Small antiviral molecule	TLR7/8 ligand	500µg	\$80
Zymosan	<i>S. cerevisiae</i> , cell wall	TLR2 ligand	100 mg	\$55

Product	Reactivity	Quantity	Price
Ab-TLR1	anti-human	40 µg	\$1.00
Ab-TLR2	anti-human	40 µg	\$1.00
	anti-mouse	40 µg	\$1.00
Ab-TLR4	anti-human	40 µg	\$1.00
Ab-TLR6	anti-human	40 µg	\$1.00
Ab-TLR9	anti-human	40 µg	\$1.00
Ab-TLR4/MD2	anti-mouse	40 µg	\$1.00
Ab-hRP105	anti-human	40 µg	\$1.00
	anti-mouse	40 µg	\$1.00

For more information on InvivoGen's TLR products, check our website at:  
[www.invivogen.com](http://www.invivogen.com)

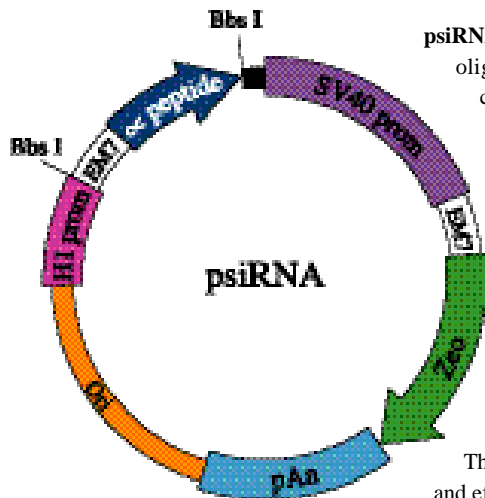
# psiRNA

## Transform Your Cell Line into a siRNA Factory...

In vivoGen provides a plasmid-based system developed to knockdown the expression of a wide variety of mammalian genes efficiently. This system represents a simple and affordable method to generate siRNAs by eliminating the need to synthesize RNA oligonucleotides. The key element of this system is psiRNA, a

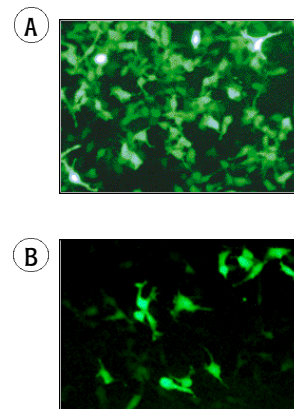
RNA polymerase III based expression vector that enables the endogenous production of small double-stranded RNAs for gene silencing applications. Furthermore, psiRNA can be stably transfected into a given cell line to achieve persistent down-regulation of a target gene.

### ... For Efficient Gene Silencing



psiRNA is specifically designed for the cloning of small synthetic oligonucleotides (around 50-mer) that encode two complementary sequences of 19 to 21 nt, homologous to a segment of the gene of interest, separated by a short spacer region of 5-7 nt. The insert is cloned downstream of a RNA polymerase III promoter, such as the human H1 promoter, and is transcribed into a short dsRNA with an hairpin structure consisting of a 19-21 bp double stranded region corresponding to the target sequence and a small loop formed by the spacer region.

psiRNA was successfully used to knockdown the expression of new alleles of the GFP and LacZ reporter genes in different cell lines of human and murine origins. The siRNAs generated intracellularly were able to selectively and efficiently downregulate the target gene.



**Knockdown of the LGFP gene in B16 cells.** Cells were transfected with a LGFP-expressing plasmid (A) and cotransfected with psiRNA-LGFP (B) at a ratio of 1:4. Two days after transfection, over 80% of the cells showed a downregulation of the LGFP gene.

#### Synthesis of two complementary oligonucleotides and hybridization

Both oligonucleotides are designed such that the first four bases create 5' overhangs compatible with Bbs I (TCCC for the sense strand and AAAAC for the antisense strand). In the sense strand, the 5' overhang is followed by an A (transcription initiation point of the human H1 promoter), then the target sequence of 19-21 mer, 5 to 7 bases for the spacer region, and the inverted 19-21 mer sequence. The sense strand ends with TT to reconstitute the T5 terminator sequence.

#### Ligation into psiRNA linearized with Bbs I

Digestion with Bbs I liberates the lacZ cassette and creates incompatible cohesive ends. This increases the number of recombinant clones with an insert in the proper orientation.

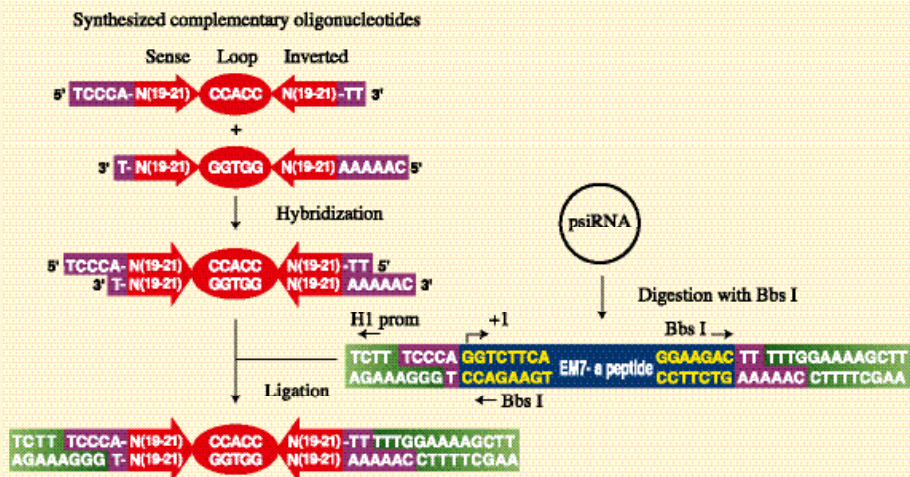
#### Transformation of *E. coli* GT116 strain

GT116 is an engineered *E. coli* strain compatible with hairpin structures.

#### DNA extraction and sequencing of the siRNA insert.

The psiRNA has been optimized so that analysis of only 5 white transformed colonies is sufficient to obtain the expected siRNA.

## psiRNA Cloning Strategy



# psiRNA

## A simple and innovative tool to create siRNAs

The components of the psiRNA system were developed to facilitate the generation of plasmid-based siRNAs. They are available either individually or as a kit.

- **psiRNA cloning vector**
- **E. coli GT116 ( $\Delta sbcC$ - $sbcD$  strain)**
- **OL381 sequencing primer**
- **E. coli Fast-Media®**

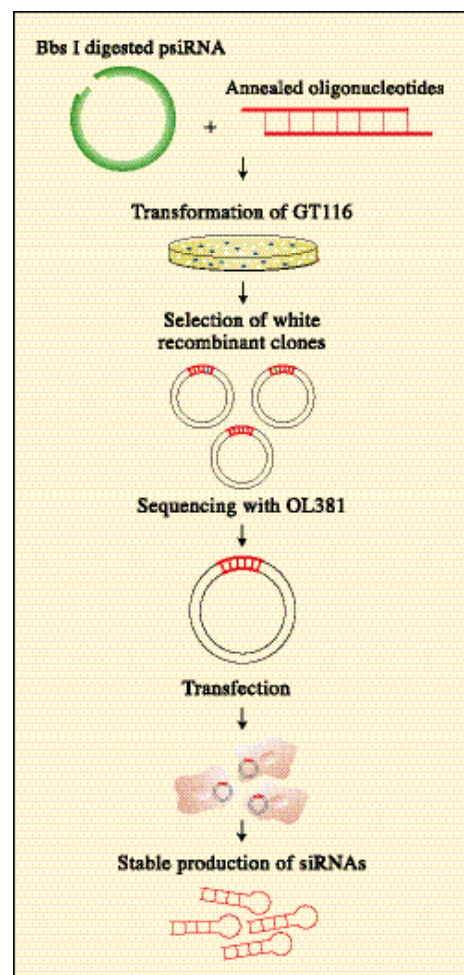
psiRNA uses Bbs I, an unusual restriction enzyme that generates assymmetric cohesive overhangs that are not compatible to eliminate the risk of self-ligation of the vector.

psiRNA exploits the white-blue selection system to further facilitate the cloning. The Bbs I sites flank the lacZ alpha-peptide allowing the discrimination between blue parental clones and white recombinant clones. Although 90% of the white clones have integrated a fragment, it is necessary to sequence the insert to verify the integrity of the sequence since it has been reported that only one base difference can lead to an inactive siRNA.

Hairpin structures are known to be unstable in *E. coli* due to their elimination by a protein complex called SbcCD that recognizes and cleaves hairpins. To increase their stability in *E. coli*, we developed **GT116**, a *sbcCD* mutant strain that significantly improves the number of recombinant clones.

Hairpin structures are also known to be difficult to sequence. Therefore, InvivoGen designed several primers located 5' or 3' to the insert and selected one, called **OL381** that allows the full reading of the insert sequence by using conventional sequencing methods.

psiRNA is available with two different selectable markers: Zeo<sup>R</sup> (zeocin resistance) or Neo<sup>R</sup> (Kan/G418 resistance) and can be cotransfected with another vector for silencing studies of exogenous genes. To facilitate the selection of recombinant clones, pouches of **Fast-Media®** Zeo Xgal or Kan Xgal are provided for the preparation in less than 5 minutes of high quality *E. coli* medium.



## Custom-Made siRNAs

### Let us design your siRNAs

InvivoGen provides a complete siRNA service to help you speed up your gene silencing experiments. Just give us the accession number of your gene of interest and we will take care of everything, from choosing the target sequence on your gene to sequencing the resulting siRNA.

- Full custom service
- Your psiRNA ready-to-use
- Cost effective
- Rapid processing

Product	Quantity	Price
psiRNAKit	*	\$315
psiRNA	20 µg	\$250
E. coli GT116	1 disk	\$75
OL381 primer	20µg	\$70
Fast-Media® Kan XGal	30 pouches	\$180
Fast-Media® Zeo XGal	30 pouches	\$225
Custom-made psiRNA	**	\$700

\* The psiRNAKit contains 20 µg of psiRNA, 1 disk of *E. coli* GT116 strain, 20µg of OL381 primer, 4 pouches of the appropriate Fast-Media® XGal.

\*\* Each custom-made psiRNA is provided as an *E. coli* GT116 clone on a paper disk.



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For more information  
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