

# InvivoGen Insight

An Insightful Look At InvivoGen's Innovative Products

Following the great feedback we received from the last InvivoGen Insight, we kept the focus of this new issue on small interfering RNAs (siRNAs) and Toll-like Receptors (TLRs).

siRNAs are now widely used to silence gene expression in mammalian cells. Their applications extend beyond functional genomics as a growing number of publications report their potential as therapeutic agents for the treatment of genetic or infectious diseases. In a continuous effort to provide the most innovative and convenient siRNA-related tools, InvivoGen offers a new generation of **psiRNA** plasmids and introduces the **siRNA Wizard**, an online resource for the design of siRNAs.

Our understanding of how TLRs respond to invading pathogens is steadily increasing. However, some questions remain unanswered such as how TLRs recognize their ligands, or what role they play in immunological disorders. To help you address these questions, InvivoGen is expanding its TLR product line to include TLR- $\Delta$ TIR genes, new TLR ligands, and engineered cells expressing a given TLR gene.

Finally, you will find in this issue the description of **LyoVec™**, the first lyophilized water-soluble transfection reagent developed for easy and efficient transfection of mammalian cells and optimized for **psiRNA** plasmids.

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## InvivoGen

Toll Free: 888.457.5873

Tel: 858.457.5873

Fax: 858.457.5843

Email: [info@invivogen.com](mailto:info@invivogen.com)

Web: [www.invivogen.com](http://www.invivogen.com)

3950-A Sorrento Valley Blvd  
San Diego, CA 92121 USA



## siRNA and shRNA Design Guidelines

The design of siRNAs and short hairpin siRNAs (shRNAs) remains an empirical process since the molecular mechanisms underlying RNAi are not yet sufficiently understood to allow for the rational design of siRNAs. However, based on the research from various laboratories including our own, InvivoGen has been able to develop **siRNA Wizard**, an online tool accessible from our homepage, that will help you find the best siRNA sequences on your target gene. The **siRNA Wizard** tool will also design the pair of oligonucleotides needed to generate shRNAs using InvivoGen's psiRNA plasmids. Below is the list of general rules, used by the **siRNA Wizard**, that have been revised to better suit the design of shRNAs.

The current guidelines recommend avoiding the first 50-100 nt located downstream of the Start codon and the 100 nt located upstream of the Stop codon, as well as 5' and 3'UTRs. These regions contain binding sequences for regulatory proteins that may affect the accessibility of the RNA target sequence to the RISC complex. However, we and others have successfully silenced the expression of several genes by targeting the 5' or 3'UTRs<sup>1,2,3,4</sup>. Therefore, 5' and 3'UTRs should also be considered when selecting a region on your target gene.

The first nucleotide of the siRNA sequence can either be an A or a G. Although we recommend choosing an A (see Selection criteria for Standard search), a G can also be used since in several examples siRNAs starting with a G and expressed from the human H1 promoter have worked<sup>4,5</sup>. The pyrimidines C and T should be avoided because expression of RNAs from RNA polymerase III promoters is only efficient when the first transcribed nucleotide is a purine. In cases where your siRNA sequence starts with a C or T, we recommend adding an A as the first nucleotide.

This addition will not affect the activity of your siRNA since it will generate a T at the end of the antisense siRNA strand that will be included in the termination signal maintaining its complementarity with the target sequence. This point is important since according to current knowledge recognition of the specific gene target is achieved by the antisense siRNA strand.

It is usually recommended to choose sequences with low GC content (between 30-55%). There are also many examples of active siRNAs with high GC content<sup>6,7,8</sup>. siRNA-mediated RNAi is based on using dsRNA < 30 nt to avoid nonspecific silencing. According to Hannon *et al.* siRNA of 25-29 nt are generally more effective than shorter ones. However, we and others found that hairpin siRNAs with duplex length of 19-21 nt are as effective as longer hairpin siRNAs<sup>6,9,10</sup>.

Several teams including ours have tested a variety of sequences for the loop between the two complementary regions of a shRNA, ranging from 3 to 9 nt in length. Similar effectiveness have been obtained for loops of 5, 7 or 9 nt. We use a 7 nt loop sequence (TCAAGAG) for the psiRNA vectors.

Despite the fact that this set of rules is still not well defined, sequences generated by the **siRNA Wizard** will likely work better than randomly selected sequences. However, because some candidate siRNAs are more active than others, it is recommended to vary the selection criteria and to compare a panel of three siRNAs to find the most efficient.

- 1- Yokota T. *et al.*, 2003. EMBO reports AOP.
- 2- Yu JY. *et al.*, 2002. PNAS 99(9):6047-6052
- 3- Rubinson DA. *et al.*, 2003. Nature Genetics 33:401-406
- 4- McManus MT. *et al.*, 2002. RNA 8:842-850
- 5- Tiscornia G. *et al.*, 2003. PNAS 100(4):1844-1848
- 6- Kim MH. *et al.*, 2002. BBRC 296:1372-1377
- 7- Hasuwa H. *et al.*, 2002. FEBS Letters 532:227-230
- 8- Bertrand JR. *et al.*, 2002. BBRC 296:1000-1004
- 9- Yu JY. *et al.*, 2003. Molecular Therapy 7(2):228-236
- 10- Song E. *et al.*, 2003. Nature Medicine 9(3): 347-351



psiRNA - The Champion of Gene Knockout

# psiRNA System

psiRNA is a plasmid-based system developed for the production of short hairpin RNAs (shRNAs) for gene silencing experiments. This system represents a simple and affordable method to generate shRNAs by eliminating the need to synthesize RNA oligonucleotides. It includes a choice of easy-to-use cloning vectors available with different selection markers allowing their stable transfection into a given cell line. Furthermore, unlike synthetic siRNAs, psiRNA plasmids are stable and easy to handle and will assure you an unlimited supply of your shRNA of interest.

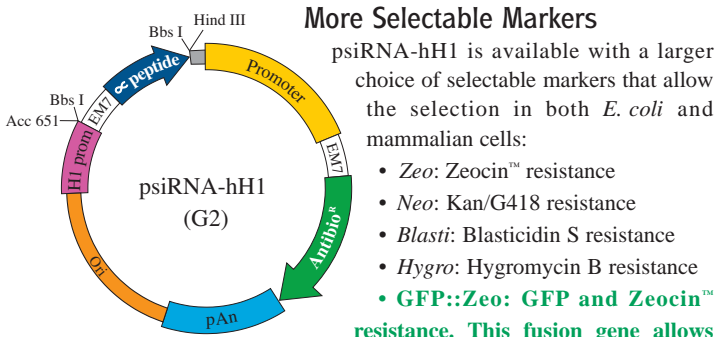
## psiRNA-hH1

### The New Generation

#### More Cloning Options

psiRNA-hH1 G2 plasmids offer two sets of restriction sites for the cloning of your siRNA insert downstream of the human H1 promoter:

- Acc 65I / Hind III
- Bbs I / Bbs I (although these sites are recognized by the same enzyme, they are different avoiding self-ligation of the plasmid)



#### More Selectable Markers

psiRNA-hH1 is available with a larger choice of selectable markers that allow the selection in both *E. coli* and mammalian cells:

- *Zeo*: Zeocin™ resistance
- *Neo*: Kan/G418 resistance
- *Blasti*: Blasticidin S resistance
- *Hygro*: Hygromycin B resistance
- **GFP::Zeo: GFP and Zeocin™ resistance. This fusion gene allows**

**you to evaluate the transfection efficiency by determining the percentage of green cells and therefore allow to normalize your silencing experiments.**

#### More Control psiRNA Plasmids

Two control vectors have been constructed containing siRNAs targeting reporter genes:

- siEGFP targets the enhanced green fluorescent protein gene.
- siLuc targets the firefly *Photinus pyralis* luciferase (pGL3) gene.

## LyoComp GT116

### Lyophilized Competent *E. coli*

*E. coli* GT116 is a *sbCD* mutant strain developed to facilitate the cloning of hairpin structures into psiRNA plasmids.

GT116 competent cells are now available lyophilized. **LyoComp GT116** are easy to handle and more stable than standard competent cells. They are shipped at room temperature eliminating costly dry ice shipping. They can be stored at 4°C for short term storage or at -20°C for long term storage. Their competency will allow you to successfully clone into psiRNA plasmids.

## siRNA Wizard Guide ([sirnowizard.com](http://sirnowizard.com))



### • Find siRNA sequences

The siRNA Wizard online tool offers two search options. The “Standard Search” uses default criteria to analyze your gene of interest whereas the “Advanced Search” lets you manually set the selection criteria. Here are our recommendations for finding a good siRNA candidate:

- **Motif size** - Choose a sequence between 19-21 nt.
- **Selected region on the target gene** - If searching for one siRNA sequence, select a region starting 100 nt downstream of the Start codon and ending 100 nt upstream of the Stop codon. If searching for several siRNA sequences, choose various regions far apart.
- **GC content** - Choose a sequence with a GC content of 30-50%.
- **First nucleotide of the siRNA sequence** - Choose a sequence starting with an A or G.
- **Specificity of the siRNA** - Using BLAST, choose a sequence presenting less than 15 adjoining nucleotides homologous to another sequence from the genome of the specie you are studying.

siRNA Sequence  $\begin{matrix} A \\ G \end{matrix} N(18-20)$

### • Design hairpin insert

This tool generates the sequence of two complementary oligonucleotides necessary to create the hairpin insert for psiRNA cloning vectors. You can choose the sequence of the loop and your cloning sites.

Example of siRNA oligonucleotides if using Bbs I/Bbs I

ACCTC -AN(18-20)-TCAAGAG -N(18-20)T -TT  
G -TN(18-20)-AGTTCTC -N(18-20)A -AAAAAC

Example of siRNA oligonucleotides if using Acc 65I/Hind III

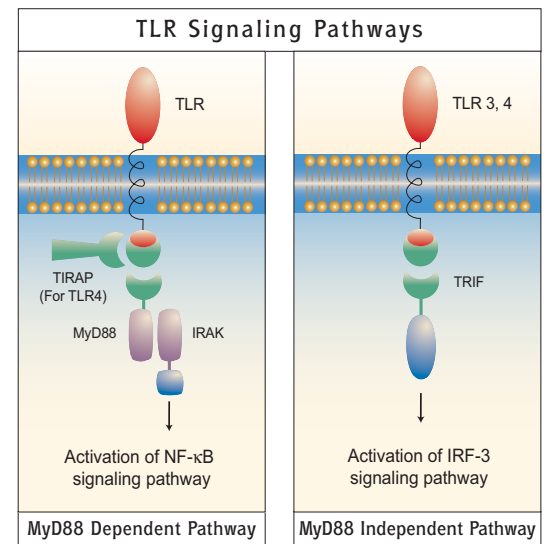
5'GTACCTC -AN(18-20)-TCAAGAG -N(18-20)T -TTTTTGAAA 3'  
3'GAG -TN(18-20)-AGTTCTC -N(18-20)A -AAAAACCTTTTCGA 5'

Product	Quantity	Price (\$/€)
psiRNA-hH1 G2 (zeo, neo, blasti, hygro)	20 µg	350
psiRNA-hH1 G2 (GFPzeo)	20 µg	400
psiRNA Kit G2 (zeo, neo, blasti, hygro)	*	500
psiRNA Kit G2 (GFPzeo)	*	550
psiRNA-hH1 control G2 (EGFP, Luc)	20 µg	325
LyoComp GT116	5 x 0.2 ml	180

\* Each psiRNA G2 Kit contains 20 µg of psiRNA plasmid, 1 disk of *E. coli* GT116 strain, 20 µg of sequencing primers, 4 pouches of the appropriate Fast-Media® XGal. Check our website for detailed catalog codes.

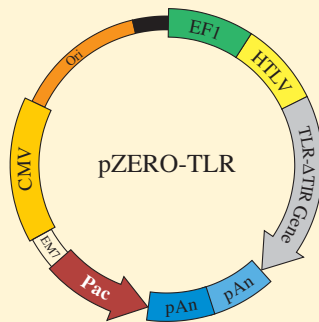
# Toll-Like Receptors

Toll-like receptors (TLRs) activate intracellular signaling pathways that share much in common with IL-1-R signaling, owing to their conserved TIR (Toll/IL-1R) domains present in the cytoplasmic tails. The TIR family also includes the adapters MyD88, TIRAP (MAL) and a new member TRIF (TICAM). Upon ligand binding, TLRs recruit MyD88 via their TIR domains. The TLR/MyD88 complex then activates IRAK eliciting a signaling cascade leading to the activation of NF-κB. Some TLRs also signal through a MyD88-independent pathway. Recently, TLR3 and TLR4 have been reported to interact with TRIF inducing the activation of the IRF-3 signaling pathway. Despite immense progress in the understanding of the TLR pathways, a lot remains unclear. To help you uncover the complex mechanisms governing TLR signaling, InvivoGen has engineered TIR domain-deleted TLR genes (TLR-ΔTIR). These mutant genes will serve as useful tools to study TLR signaling.



## TLR-ΔTIR Genes

**pZERO-TLR** plasmids express TIR-deleted TLR genes. All ten human and nine murine TLR-ΔTIR genes are available. Their expression is controlled by the strong EF1/HTLV composite promoter. **pZERO-TLR** plasmids are selectable with the potent antibiotic puromycin in both *E. coli* and mammalian cells.



### pZERO-TLR plasmid features

- Expression of TLR-ΔTIR genes
- Selectable with puromycin
- Cotransfectable with pUNO or pDUO plasmids
- Easy subcloning of the TLR-ΔTIR genes

**pZERO-TLR** plasmids are provided as lyophilized transformed *E. coli* cells with pouches of Fast-Media® Puro. Check our website for catalog codes.

## 293/TLR Cell Lines

Now you can obtain from InvivoGen 293 cells transfected with one or several TLR or TLR-associated genes. These cell lines are also available cotransfected with a NF-κB inducible SEAP reporter plasmid. Expression of SEAP can be readily quantified from the supernatant using a colorimetric assay.

293/TLR clones have been fully characterized. The expression of the TLR gene(s) has been verified by Western Blot analysis. The activation of the TLR signaling pathway has been confirmed in cells cotransfected with TLR and NF-κB reporter plasmids using the SEAP reporter assay. 293/TLR clones are grown in standard DMEM medium with 10% FBS supplemented with blasticidin (10 μg/ml) and/or Zeocin™ (100 μg/ml). Each vial of 293/TLR clone contains 1-5 x 10<sup>6</sup> cells and is shipped on dry ice.

Currently four different cell lines are available, with more to be added soon.

Cell Line	Catalog Code	Price (\$/€)
293/hTLR2	293-htlr2	600
293/hTLR2-SEAP	293-htlr2sp	1200
293/hTLR2/6	293-htlr2/6	675
293/hTLR2/6-SEAP	293-htlr2/6sp	1250

### Related Products

pUNO Vectors	#puno-<gene>
pDUO Vectors	#pduo-<gene>
pNiFty-SEAP	#pnifty-seap
SEAP Reporter Assay Kit	#rep-sap
Blasticidin	#ant-bl
Puromycin	#ant-pr
Zeocin™	#ant-zn

For updated information on TLR products, visit [www.invivogen.com](http://www.invivogen.com)

## TLR Ligands

InvivoGen continuously adds new TLR ligands to its already extensive list to provide the most comprehensive selection available for our customers convenience.

Product	Origin/Description	Catalog Code	Quantity	Price (\$/€)
<b>CpG oligonucleotides (TLR9 ligands)</b>				
- ODN 2216	Human 'type' CpGs, type A	tlrl-hodna	100 μg	140
- ODN 2216 control	Control	tlrl-hodnac	100 μg	140
- ODN 2006	Human 'type' CpGs, type B	tlrl-hodnb	100 μg	140
- ODN 2006 control	Control	tlrl-hodnbc	100 μg	140
- ODN 1826	Murine 'type' CpGs	tlrl-modn	100 μg	140
- ODN 1826 control	Control	tlrl-modnc	100 μg	140
<b>Small synthetic molecules (TLR7 ligands)</b>				
- R848	Imidazoquinoline compound	tlrl-r848	500 μg	80
- Loxoribine	Guanine analog	tlrl-lox	50 mg	80
<b>Heat-Killed Bacteria (TLR2, TLR2/6 ligands)</b>				
- HKLM	<i>L. monocytogenes</i> (Gram +)	tlrl-hklm	10 <sup>10</sup> cells	80
- HKAL	<i>A. laidlawii</i> (Mycoplasma)	tlrl-hkal	10 <sup>9</sup> cells	80

# LyoVec™

## The First Lyophilized Transfection Reagent

LyoVec™ is a new lyophilized formulation of LipoVec™, a cationic lipid-based transfection reagent. The major constituent of LyoVec™ is the phosphonolipid DMPTA. Phosphonolipids are a family of cationic lipids originally described by Floch *et al.* as efficient transfection reagents<sup>1,2</sup>. Their positive charge enables them to bind to DNA, and their phospholipid-like structure promotes fusion with cellular membranes for DNA delivery. DMPTA is coupled with DOPE, a neutral lipid that helps destabilizing membrane bilayers, therefore increasing the *in vitro* transfection efficiency of LyoVec™.

### Rapid and Simple To Use

- Hands-on time: No more than 15 min
- No preparation required the day prior transfection
- Works similarly at various lipid/DNA ratios
- Soluble in water up to 10X

### Maximum Transfection Efficiency

- Transfects with high efficiencies a broad spectrum of mammalian cells, including primary cells and non-adherent cells
- Effective for transient and stable transfections

### Minimal Cytotoxicity and Increased Stability

- Works in the presence of serum
- No need to wash cells after transfection
- Stable over one year when lyophilized and up to six months when rehydrated

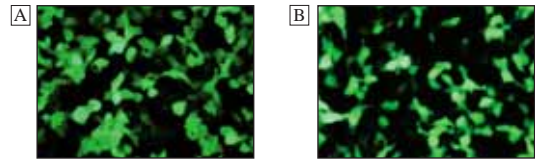
### Long Shelf-life of pDNA/LyoVec Complexes

In contrast with other cationic lipids, pDNA/LyoVec™ complexes remain fully active for transfection for at least two months at 4°C. Thus, preparation of large volumes of complexes can be made and reused repeatedly, saving you time. This stability allows us to provide a control pDNA/LyoVec™ complex with each order.

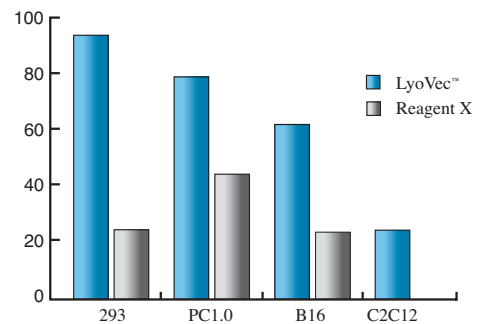
### Optimized for psiRNA Plasmids

Transfection efficiency is a key factor for successful gene silencing using plasmid-based siRNAs such as psiRNAs. LyoVec's formulation has been optimized to guarantee maximum transfection efficiency with psiRNA plasmids.

1- Floch *et al.* 1997. Cationic phosphonolipids as non viral vectors for DNA transfection in hematopoietic cell lines and CD34+ cells. *Blood Cells, Molec. & Diseases* 23: 69-87.  
2- Guillaume-Gable *et al.* 1998. Cationic phosphonolipids as nonviral gene transfer agents in the lung of mice. *Hum. Gene Ther.* 9: 2309-2319.



Expression of GFP::Zeo fusion gene in 293 (A) and B16 (B) cells transfected with psiRNA-hH1GFPzeo using LyoVec™ in the presence of serum.



Cell Line	Catalog Code	Quantity	Price (\$/€)
LyoVec™	lyec-1	8 ml lyophilized	90
	lyec-2	18 ml lyophilized	150



3950 Sorrento Valley Blvd, Suite A  
San Diego, CA 92121 USA

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