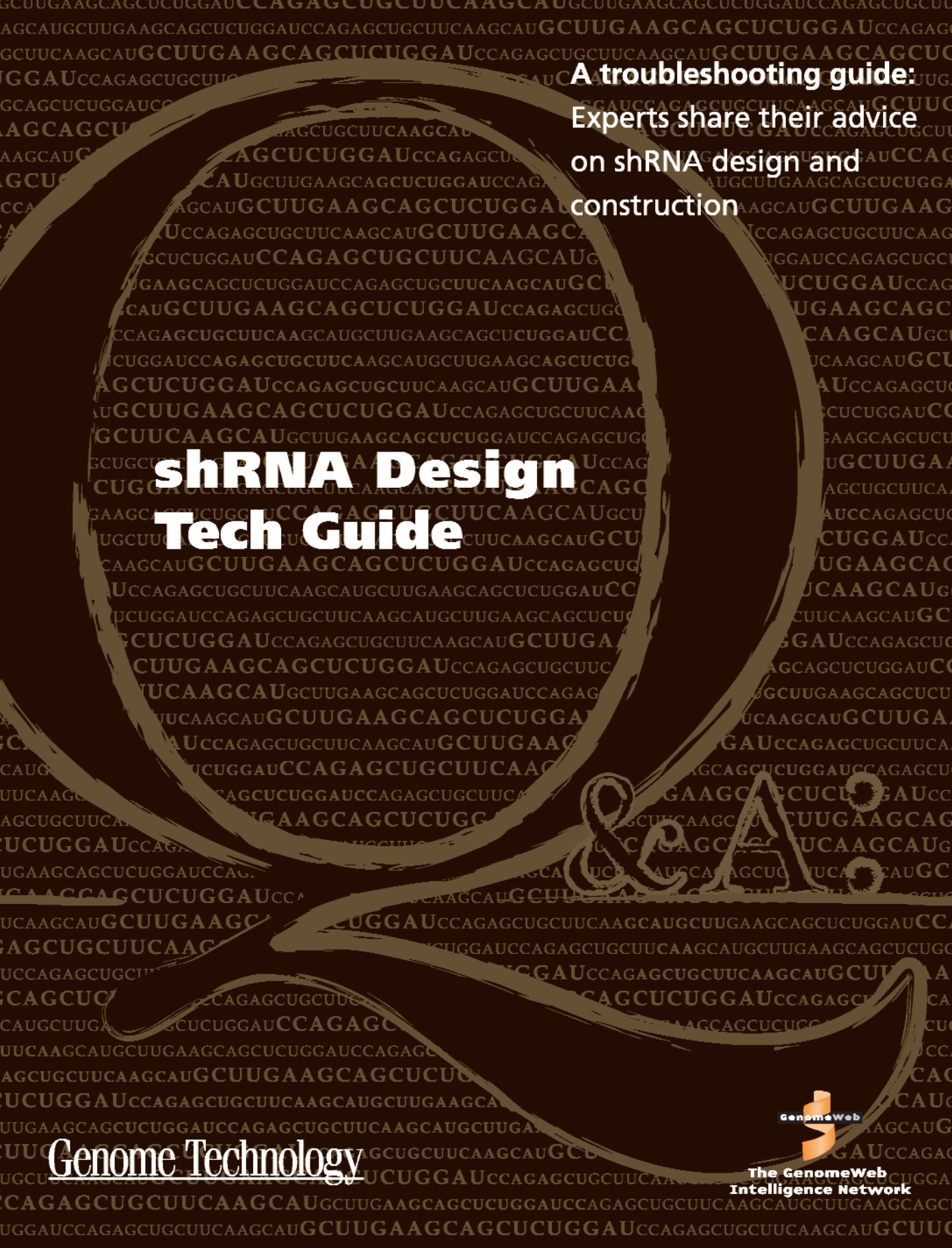
# shRNA Design Tech Guide

**A troubleshooting guide:**
Experts share their advice on shRNA design and construction

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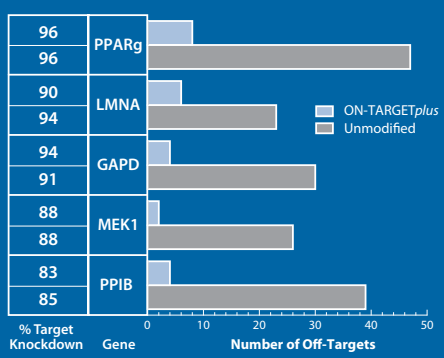


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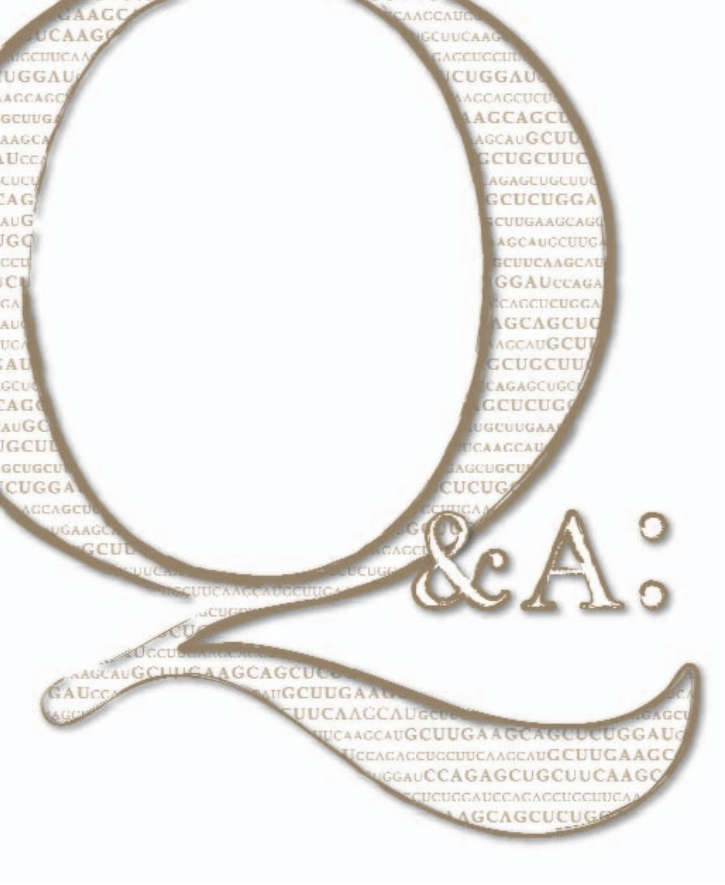


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# Letter from the editor

It took us a year, but we here at *Genome Technology* are pleased to present a second tech guide on the topic of RNA interference. If April is the cruelest month, then March must be the most silent, as we covered short interfering RNA design exactly one year ago in these pages. This time, however, we are pleased to present you with a guide to short hairpin RNA design and construction.

RNAi is a surgically precise way of silencing a gene, while shRNA and siRNA are a bit like the scalpels that get the work done. But whereas siRNA oligos are transfected to induce short-term silencing, shRNAs can be smuggled into a cell via a vector or virus to induce transient or stable expression in both dividing and nondividing cells. Since applications for shRNAs are both cost-effective and broad, it's no surprise that more and more researchers are availing themselves of this potent technology.

At this point, it's not clear whether the trade secrets that make for an effective siRNA oligo apply to shRNAs. For this reason alone, we thought that a guide exploring the topic would be interesting. We put several questions to an impressive team of shRNA experts, and they were each gracious enough to reply with thoughtful and detailed responses.

Keep this guide on hand for detailed advice on selecting highly effective shRNA hairpin regions, choosing promoters, avoiding unintended targeting, confirming suppression, and more. As always, we've included a resource guide at the end of the issue to ensure that your silencing experiments are a success.

*— Jennifer Crebs*

# Index of experts

Anne Cahill
University of Chicago

Andreas Hofmann
University of Bonn

Ming Jiang
University of York

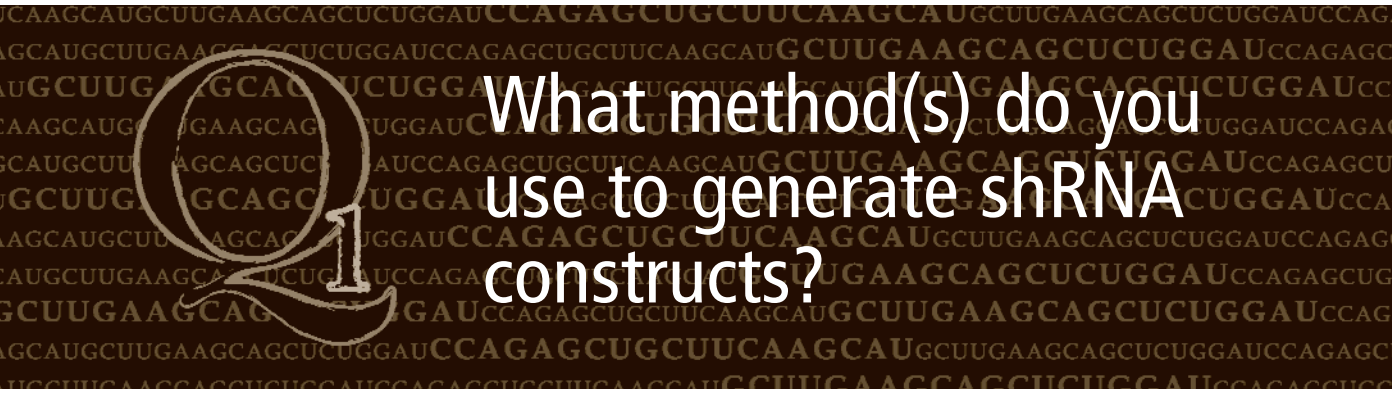
Tatsuo Kanda
St. Louis University

Alexander Pfeifer
University of Bonn

Randy Poon
University of Hong Kong

Ravi Sachidanandam
Cold Spring Harbor Laboratory

Debra Taxman
University of North Carolina
at Chapel Hill



# What method(s) do you use to generate shRNA constructs?

To generate an shRNA-expressing plasmid, I design a pair of deoxyoligonucleotides that are complementary except for different 5'-overhangs that anneal to the sticky ends of an shRNA plasmid digested with an appropriate pair of restriction enzymes. Each ~66 base deoxyoligonucleotide contains (5' to 3'): a restriction-site compatible overhang, the selected target sequence, a loop sequence, the reverse complement of the target sequence, and an RNA polymerase III termination sequence (five T's). This design is illustrated in Fig 1B of Cahill *et al.*, 2006. The PAGE-purified deoxyoligonucleotides are synthesized commercially, phosphorylated in my lab with T4 polynucleotide kinase, annealed, and ligated into the appropriately digested shRNA plasmid.

*— Anne Cahill*

I use the vector pENTR/U6 from Invitrogen.

*— Ming Jiang*

shRNA oligonucleotides were designed to contain a sense strand of 19-nucleotide sequences from targets, followed by a short spacer (TTCAAGAGA), the reverse complement of the sense strand, and five thymidines as an RNA polymerase III transcriptional stop signal. After oligonucleotides are annealed with the reverse strand, for an example, we clone shRNA oligonucleotides into the BamHI and HindIII sites of pRNAT-H1.3/Hygro (GenScript Corporation, Piscataway, NJ).

*— Tatsuo Kanda*

We design shRNA constructs by either sense-loop-antisense or antisense-loop-sense orientation and add ´TTTTT´ as termination signal. Then, we order these sequences as forward and reverse oligonucleotides with restriction sites at the 5´ and 3´ end. After annealing of the oligonucleotides, we ligate the double-stranded fragments into an expression vector containing a suitable polymerase III promoter, like the H1 (Brummelkamp *et al.*, *Science* 2002) or the U6 (Lee *et al.*, *Nature Biotechnology* 2002) promoter. Finally, we transfer the complete shRNA expression cassette into a lentiviral vector (Naldini *et al.*, *Science* 1996) and generate high titer lentiviruses (Pfeifer *et al.*, *Proceedings of the National Academy of Sciences* 2001 and *Journal of Clinical Investigation* 2006).

*— Alexander Pfeifer and Andreas Hofmann*

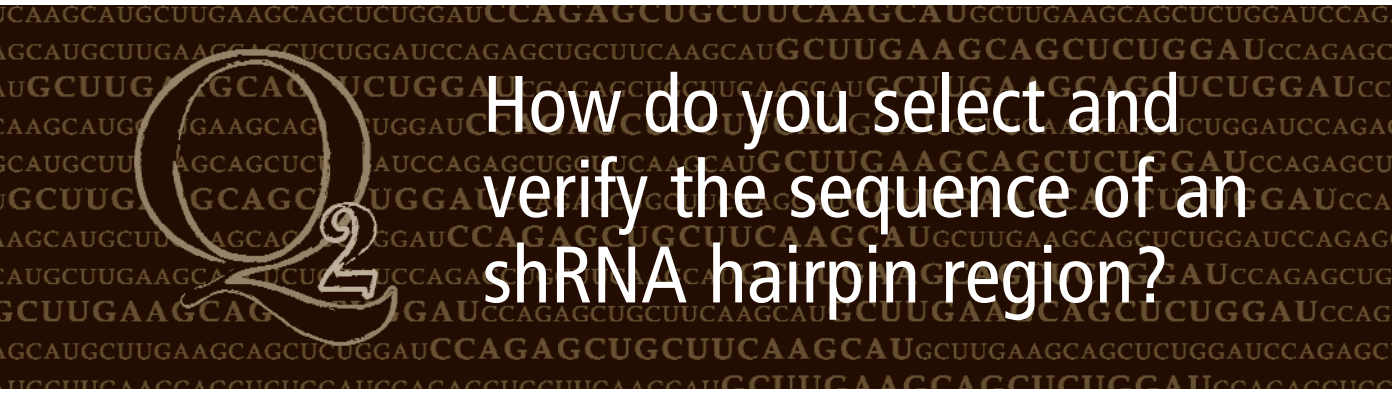
My group uses a pair of oligonucleotides (56-mers each) to generate each shRNA construct. The overhangs produced after the annealing of the two complementary oligonucleotides allow direct ligation into the vector. Our favorite vectors are mU6pro and pKAR1.

*— Randy Poon*

We start from siRNA designs that are then inserted into a microRNA context (mir-30). We also have an shRNA/siRNA designer that people can use on our website.

*— Ravi Sachidanandam*

We use one of two different methods to generate shRNA. Both methods seem to work well. In the first, we design two complementary oligos. The oligos are approximately 50 nt to 60 nt each, and encompass the hairpin *(continued on p.14)*



# How do you select and verify the sequence of an shRNA hairpin region?

I usually select an shRNA target using the siSearch website. I like this site because it returns the scores based on a number of different sets of published criteria for design of effective siRNAs. However, I don't believe that any single website or any set of criteria for design of effective siRNAs is perfect yet. (I once tested eight siRNA design sites with the same input sequence and tried to find a consensus target site, but it was fairly hopeless.) So I don't just blindly use the top ranked target suggested by siSearch. I try to evaluate several suggested targets based on other criteria such as the predicted secondary structure of the antisense strand of the siRNA (Patzel *et al.*, 2005) and the accessibility of the target site in the predicted mRNA (Sfold [as described in] Ding *et al.*, 2004). I also look at validated shRNAs suggested by the RNAi Consortium or RNAi Codex, if these are available for my gene of interest.

To verify the sequence of an shRNA hairpin I have all shRNA plasmids sequenced before use. The sequencing facility at my university initially had some problems getting good sequences through the hairpin region, but lately they have had good success. I have occasionally found "mistakes" in the hairpins, so it is a good idea to routinely do several plasmid minipreps and have the plasmids sequenced before proceeding.

*— Anne Cahill*

I use the loop sequence TCAAGAG based on the literature. There are a few choices such as GAAGCTTG.

*— Ming Jiang*

We use the Ambion site to select siRNA sequences. Then we check specificity of siRNA using NCBI Blast database. After checking secondary structure of targets, we choose single-stranded regions as targets.

*— Tatsuo Kanda*

The selection of effective target sites is the prerequisite for efficient RNA interference (RNAi). In the World Wide Web one can find several online shRNA-design programs (e.g. http://www.genelink.com/sirna/shRNAi.asp). Furthermore, several companies offer design services for shRNAs (e.g. Invitrogen, MWG Biotech, and B-Bridge).

The manual selection of target sites is difficult and time-consuming. Dozens of publications describe characteristics for an ideal siRNA: Reynolds *et al.* (*Nature Biotechnology* 2004) describe eight criterias for rational siRNA design. Schwarz *et al.* (*Cell* 2003) point out that "stability of the base pairs at the 5´end of the two siRNA strands determine the degree to which each strand participates in the RNAi pathway." And the group of J.J. Rossi (Heale *et al.*, *Nucleic Acid Research* 2005) found that the mRNA secondary structure has major influence on the efficacy of RNAi. The loop sequence between sense and antisense strand is another element of uncertainty. Several sequences for short hairpin loops are published until now (e.g. Brummelkamp *et al.*, *Science* 2002). The loop of choice is dependent on the RNAi target sequence and should not generate additional stop signals and repetitive sequences in the shRNAs.

Taken together, there is no optimum formula to find an effective shRNA and sometimes a lot of sequences have to be tested by the trial-and-error


method until an efficient knockdown of the desired gene can be achieved.

*— Alexander Pfeifer and Andreas Hofmann*

The shRNA targeting sequences are selected based on the guide described by Tuschl's group. In addition, we also look for shRNAs with a relatively low binding energy for the 5' end of the antisense strand. The sequence of the shRNA coding region is verified by DNA sequencing.

*— Randy Poon*

For selecting regions, we try and use features that are known and published in the literature. We also try to use experimentally validated constructs to constantly improve our design methods.

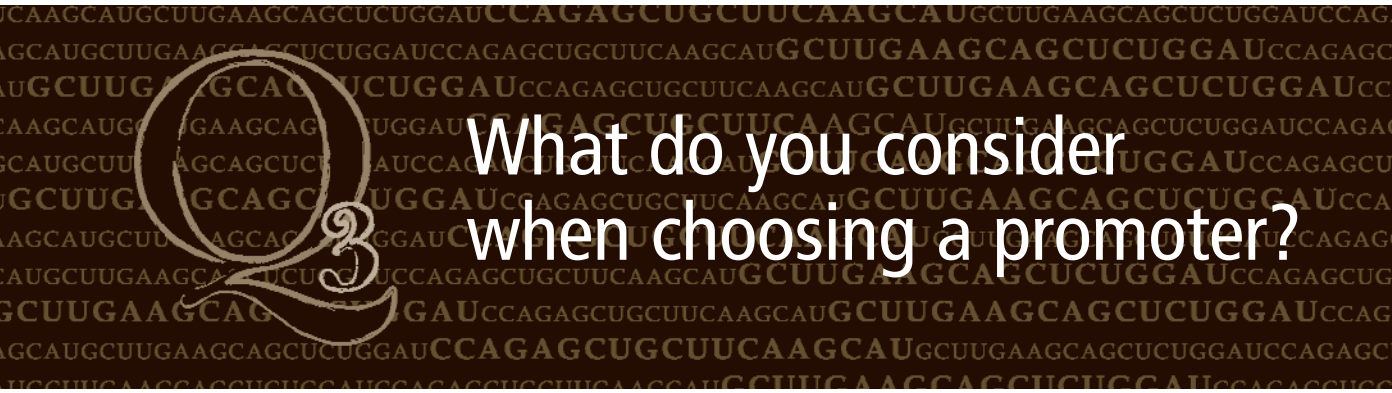
*— Ravi Sachidanandam*

> "It also is important in selecting a target sequence to choose an exon that is used by all of the splice forms of the gene."
>
> *— Debra Taxman*

We select the shRNA target sequence using a modification of the Ui-Tei algorithm that we have found to be effective in making functional shRNAs. This was first described in our January 2006 *BMC Biotechnology* manuscript, and we are currently in the process of creating a Web-based program so that other researchers can make use of our algorithm modification. The program is tentatively scheduled to be available in April 2007, and will be located at shRNAdesigner.med.unc.edu. In our manuscript, we found that restricting the Tm for 6 central bp of the target improved the efficacy outcome for shRNAs. In addition to the parameters described in our study, we avoid repetitive sequences and runs of four or more of the same nucleotide, and we aim for approximately 30 percent to 60 percent G/C content. It also is important in selecting a target sequence to choose an exon that is used by all of the splice forms of the gene. The target sequence is screened by Blast analysis to make sure that there are no matches to other genes of the same species of 16 or more nucleotides, and then is screened for miRNA-type imperfect matches between the seed region of the target (nt 2-7) and the 3'UTR of non-targeted genes. This can be done easily using a program developed by Dharmacon. We pre-screen several different targets and then select the ones with the fewest seed region matches.

It can sometimes be difficult to sequence through the shRNA due to the secondary structure of the hairpin. However, we have had very good success sequencing if we add DNA relaxing agents to the sequencing reaction. PCRx Enhancer (Invitrogen) works well, but DMSO also usually is sufficient for the majority of the hairpins. For really difficult-to-sequence hairpins we arrange with the sequencing facility to have 1:10 ABI Prism dGTP BigDye Terminator Ready Reaction Mix added to the standard sequencing BigDye v1.1 mix, and this seems to take care of sequencing for shRNAs with even the most difficult secondary structure.

*— Debra Taxman*



# What do you consider when choosing a promoter?

I have only used the mouse U6 promoter because it worked when I first tried it. In several direct comparisons, the U6 promoter has been found to be more effective than others (Miyagishi *et al.*, 2004; Wooddell *et al.*, 2005; Makinen *et al.*, 2006).

*— Anne Cahill*

I chose U6 because Pol III promoter is ideal for producing precise short RNAs.

*— Ming Jiang*

We usually use H1.3 promoter to express shRNAs. We will also try to use other promoters.

*— Tatsuo Kanda*

The most widely used shRNA expression cassettes are based on polymerase III promoters like the H1 or the U6 RNA promoters, but also polymerase II promoters (like CMV) have been used to knock down target mRNA (Stegmeier *et al.*, *Proceedings of the National Academy of Sciences* 2005). In accordance with several recently published studies, we have found that the U6 promoter gives higher levels of knockdown than the H1 promoter.

*— Alexander Pfeifer and Andreas Hofmann*

We generally use a mouse U6 promoter for shRNA expression, but we have not extensively compared different promoters. Our opinion is that the strength of the promoter is perhaps not the most critical issue in achieving RNA interference. Recently, we have also modified the mouse U6 promoter to allow inducible expression of the shRNA. In this connection, it is likely that inducible systems of miRNA driven by CMV promoters offer a more robust control.

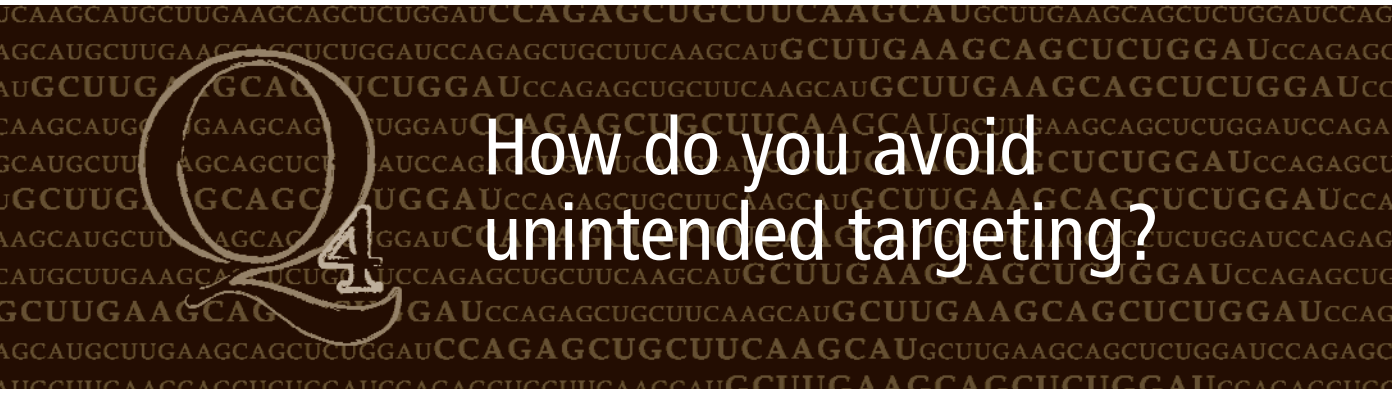
*— Randy Poon*

We have had good success with the H1 and U6 pol III promoters and their effective use has been well established in the literature. The 7S K promoter also seems like a good choice. There are a variety of newer promoters for shRNA, including modified CMV and U1 pol II promoters. However, the increased expression from these promoters has been associated with increased cytotoxicity in some cases. The choice of the promoter will depend on the desired expression level and the need for longevity of expression of the shRNA. The pol III promoters may have slightly less knockdown but may be expressed for longer with fewer side effects. Tet-inducible versions of the pol III promoters also seem like a good choice, but are leaky in some cases.

*— Debra Taxman*

"The choice of the promoter will depend on the desired expression level and the need for longevity of expression of the shRNA. The pol III promoters may have slightly less knockdown but may be expressed for longer with fewer side effects."

*— Debra Taxman*



# How do you avoid unintended targeting?

To avoid unintended targeting, I largely rely on the Blast searches available on several siRNA design sites, although I realize this may not be optimal. Off-target effects are of greater concern in genome-wide screens than in my studies targeting single genes, because I usually can do "rescue" experiments to demonstrate that the particular effect is due to knockdown of the targeted protein. Alternatively, specificity can be demonstrated by showing that the same effect is obtained using two different shRNAs to target a single gene.

*— Anne Cahill*

I carefully choose the target sequence and do a thorough search against the host genome. The guidelines for choosing shRNA target sequence are the same for choosing siRNA sequence.

*— Ming Jiang*

Use the procedures described in Q2 and Q5 to choose the sequences.

*— Tatsuo Kanda*

Every target sequence for RNAi has to be checked for unintended targeting, e.g by Blasting the sequence of the genome in which the experiments will be performed.

shRNA vectors may trigger an interferon (IFN) response. Hornung *et al.* show IFN-I response after uptake of siRNA molecules by immune cells — specifically plasmacytoid dendritic cells (*Nature Medicine* 2006). Interestingly, the authors identify a 9-bp consensus motif that induces high IFN-I production in the dendritic cells. Therefore, if siRNA molecules will enter the blood, the siRNA design should avoid such motifs. However, a recent study from the lab of J.J. Rossi described that stable expression of shRNAs (in human CD34+ progenitor cells) can avoid induction of interferon responses to siRNAs *in vitro* (Robbins *et al.*, *Nature Biotechnology* 2006). Nevertheless, we screen our lentiviral shRNA vectors for possible induction of IFN by quantitative PCR of interferon target sequences like OAS1 (Bridge *et al.*, *Nature Medicine* 2003).

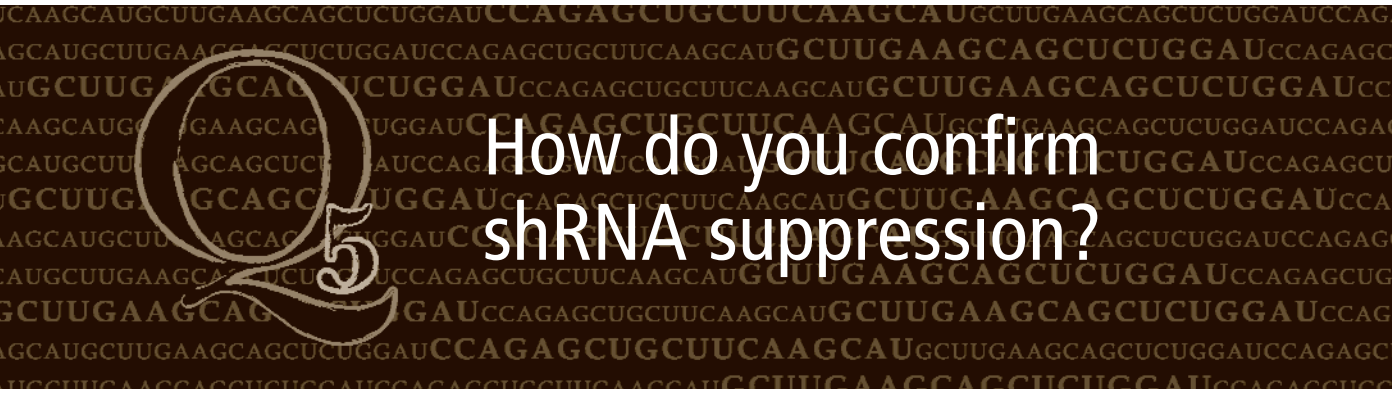
*— Alexander Pfeifer and Andreas Hofmann*

> "I largely rely on the Blast searches available on several siRNA design sites."
>
> *— Anne Cahill*

The candidate shRNA sequences are checked for their specificity using Blast. Various reports have indicated that the central region of the shRNA or the positions 2-8 of the antisense strand are most critical in determining the specificity. However, the ultimate control for shRNA experiment is the rescue of the RNAi effects by the expression of the target gene in a form refractory to the shRNA. This is usually achieved by introducing silent point mutations to the region of cDNA targeted by the shRNA.

*— Randy Poon*

To avoid unintended targeting, we check our collection of mRNAs to make sure that there are no close homologous targets other than the intended one. But, since the miRNAs *(continued on p.14)*



# How do you confirm shRNA suppression?

I confirm knockdown of the targeted protein by immunoblotting. The cell line I work with (PC12 cells) is not highly transfectable, so I generate stably transfected cell lines in which all the cells express the shRNA. The cell lines that survive selection are screened by PCR for insertion of the shRNA plasmid into genomic DNA, and PCR positive cell lines are then screened by immunoblotting. I find that the degree of knockdown can vary significantly among different cell lines transfected with the same plasmid, so I screen a number of cell lines.

*— Anne Cahill*

The shRNA suppression will be confirmed by decrease of protein level and mRNA level.

*— Ming Jiang*

First, we use bicistronic reporter including Renilla luciferase, target sequences, and Firefly luciferase gene. Next, we confirm the knockdown of gene expression using western blotting, RT-PCR, or real-time RT-PCR.

*— Tatsuo Kanda*

We measure knockdown of the desired mRNA by real-time PCR analyses. We prefer the TaqMan probe method rather than the SYBR Green method. This method allows the exact measurement of two different genes (the gene of interest and a gene used to determine RNA loading) in one and the same PCR reaction. In addition, western blot analyses can be performed if one has suitable antibodies of the desired gene product.

*— Alexander Pfeifer and Andreas Hofmann*

We are a bit quixotic and only use the suppression of the target protein as the criteria for assessing the effectiveness of the shRNA. Among different methods, immunoblotting is among the simplest and the most accurate, as it distinguished the targets with other cross-reactive proteins of different sizes. Occasionally, we screen the shRNA constructs by examining the expression of a co-transfected cDNA. This could be revealing when antibodies are not available or the endogenous proteins are difficult to detect. But ultimately the usefulness of an shRNA depends on its ability to reduce the expression of the endogenous protein.

*— Randy Poon*

"Among different methods, immunoblotting is among the simplest and the most accurate, as it distinguished the targets with other cross-reactive proteins of different sizes."

*— Randy Poon*

We confirm suppression by performing real-time PCR of RNA isolated on three different days. It's important to collect RNA on multiple days since we have found that knockdown levels can vary somewhat from day to day. It's important to design PCR primers that target the same splice variants as the shRNAs. We also do westerns if antibodies are available. For genes with a known function, we perform functional assays to confirm the expected effects of knockdown.

*— Debra Taxman*

# Q6 What are your tips for minimizing false positive rates?

To minimize the false positive, I normally use controls for the vector only, lamin A/C shRNA, and control shRNA (which does not target the host genome at all).

*— Ming Jiang*

Use the procedures described in Q2 and Q5 to choose the siRNA sequences.

*— Tatsuo Kanda*

We recommend using a scrambled shRNA — which has no target in the corresponding genome — in each experiment. Thus, one is able to show that the way of transduction (transfection, viral infection, etc.) and the shRNA expression system (expression plasmid, virus, etc.) have no influence on the RNAi pathway. In addition, several labs also use "nonsense" shRNAs that target e.g. the LacZ gene (Furumoto *et al.*, *Journal of Immunology* 2006).

*— Alexander Pfeifer and Andreas Hofmann*

Several approaches can be used to minimize false positives results. This includes using several shRNAs against the same gene, using the minimal concentration of shRNA constructs to obtain the desirable knockdown, and verifying that the expression of many unrelated proteins is not affected by the shRNA. The definitive control, however, remains the rescue of the RNAi effects by the expression of the target gene that is resistant to the shRNA. A caveat is that overexpression of the rescue protein may itself exert an effect on the cell. Hence a titration of the rescue cDNA may be needed to express the rescue protein to a level similar to the endogenous protein before knockdown.

*— Randy Poon*

To minimize false positives, it is essential that several constructs are tried out and the results replicated at least once by a distinct construct.

*— Ravi Sachidanandam*

"It is essential that several constructs are tried out and the results replicated at least once by a distinct construct."

*— Ravi Sachidanandam*

I think it's important to prepare two or more shRNAs for each gene. shRNA for other genes within the same pathway also help to verify specificity. It is important to include negative controls, such as empty vector and shRNA bearing scrambled sequences or targeting a different gene altogether. Other controls to minimize false positivity include adding back exogenous mRNA using either a form of the mRNA with a silencing mutation within the target or an orthologue of the gene from a different species; or showing functionality of the gene by a complementary methodology such as overexpression, dominant negative expression, or reconstruction of functionality using stimuli that act further downstream in the same pathway.

*— Debra Taxman*

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# List of resources

Our panel of experts referred to a number of publications and online tools that may be able to help you get a handle on hairpin silencing. Whether you're a novice or pro at shRNA design, these resources are sure to come in handy.

# Publications


Brummelkamp TR, Bernards R, Agami R (2002)
**A system for stable expression of short interfering RNAs in mammalian cells.**
*Science* 296(5567):550-3

Cahill AL, Herring BE, Fox AP (2006)
**Stable silencing of SNAP-25 in PC12 cells by RNA interference.**
*BMC Neurosci* 7:9

Ding Y, Chan CY, Lawrence CE (2004)
**Sfold web server for statistical folding and rational design of nucleic acids.**
*Nucleic Acids Res* 32: W135-141

Heale BSE, Soifer HS, Bowers C, Rossi J (2005)
**siRNA target site secondary structure predictions using local stable substructures.**
*Nucleic Acids Res* 33(3):e30

Ma HT, On KF, Tsang YH, Poon RYC (2007)
**An inducible system for expression and validation of the specificity of short hairpin RNA in mammalian cells.**
*Nucleic Acids Res*. [Epub ahead of print]

Makinen PI, Koponen JK, Karkkainen AM, *et al.* (2006)
**Stable RNA interference: comparison of U6 and H1 promoters in endothelial cells and in mouse brain.**
*JGene Med*, 8:433-441

Miyagishi M, Sumimoto H, Miyoshi H, Kawakami Y, Taira K (2004)
**Optimization of an siRNA-expression system with an improved hairpin and its significant suppressive effects in mammalian cells.**
*J Gene Med,* 6:715-723

Patzel V, Rutz S, Dietrich I, Koberle C, Scheffold A, Kaufmann SH (2005)
**Design of siRNAs producing unstructured guide-RNAs results in improved RNA interference efficiency**.
*Nat Biotechnol*, 23:1440-1444

Pfeifer A, Brandon E, Kootstra N, Gage F, Verma I (2001)
**Delivery of the Cre recombinase by a self-deleting lentiviral vector: Efficient gene targeting in vivo.**
*PNAS* 98(20):11450-11455

Reynolds A, Leake D, Boese Q, Scaringe S, Marshall W, Khvorova A (2004)
**Rational siRNA design for RNA interference.**
*Nat Biotechnol*, 22:326-330

Taxman DJ, Livingstone LR, Zhang J, Conti BJ, Iocca HA, Williams KL, Lich JD, Ting JP, Reed W.
**Criteria for effective design, construction, and gene knockdown by shRNA vectors.**
*BMC Biotechnol.* 2006 Jan 24;6:7.

Wooddell CI, Van Hout CV, Reppen T, Lewis DL, Herwiger H (2005)
**Long-term RNA interference from optimized siRNA expression constructs in adult mice.**
*Biochem Biophys Res Commun*, 334:117-127.

Yu JY, DeRuiter SL, Turner DL. (2002)
**RNA interference by expression of short-interfering RNAs and hairpin RNAs in mammalian cells.**
*PNAS*, 99(9):6047-52.


# Acknowledgements


Many thanks to Carl Novina for helpful advice when formulating questions for this guide.

We would also like to thank Ratna Ray for the input into the answers submitted by Tatsuo Kanda.

# Websites

**Ambion**
*http://www.ambion.com/techlib/misc/siRNA_finder.html*

**Dharmacon's Seed Locator**
*http://www.dharmacon.com/seedlocator/default.aspx*

**GenScript Corp**
*http://www.genscript.com/*

**Invitrogen**:
*http://www.invitrogen.com*

**shRNA Designer (as of April 2007)**
*http://shRNAdesigner.med.unc.edu*

**siSearch**
*http://sonnhammer.cgb.ki.se/siSearch/siSearch_1.7.html*

**siRNA User Guide (by Tom Tuschl)**
http://*www.rockefeller.edu/labheads/tuschl/sirna.html*

**sFold**
*http://sfold.wadsworth.org/*

**RNAi Consortium**
*http://www.broad.mit.edu/genome_bio/trc/rnai.html*

**RNAi Codex**
*http://codex.cshl.edu/scripts/newmain.pl*

## Q1: What method(s) do you use to generate shRNA constructs?

(continued from p.5)

sequence (a stem/loop which includes a target site and reverse complement of the target site separated by a loop), and a five-T termination sequence. The oligos are designed to leave restriction half sites on each end which can then be cloned 3' to a pol III promoter.

In the second method, we use PCR to amplify a fragment containing the entire promoter, the hairpin and the termination sequence. For this method we use a template containing the promoter and a universal forward primer to amplify the 5' end of the promoter. The 3' end of the promoter is amplified using a reverse primer of approximately 95 nt in length that we design specifically for each shRNA. This specific primer contains the terminator reverse complement (AAAAA), the stem/loop reverse complement, and approximately 20 nt of the 3' end of the promoter. The forward and reverse primers each are designed to include restriction sites on the end for cloning. PCR is performed using a high-fidelity polymerase (such as Pfx platinum polymerase; Invitrogen) and a DNA relaxing agent (such as PCRx Enhancer; Invitrogen). This helps significantly to reduce the number of mutations introduced during PCR. The PCR fragment is digested and cloned into the vector.

*— Debra Taxman*

## Q4: How do you avoid unintended targeting?

(continued from p.9)

show that the level of homology need not be great, this can be misleading sometimes. We can only avoid the obvious ones.

*— Ravi Sachidanandam*

We perform a Blast search and eliminate targets that have 16 nt or more match with other genes of the same species. We also search for imperfect matches between the seed region of the target and the 3'UTR of genes using the Dharmacon program. There are always some seed region matches, but we choose the target with the fewest number of multiple matches. Most importantly, since studies suggest that it is impossible to avoid all off-target effects, we include several controls in our experiments. We use two or more shRNAs for each gene since it is statistically unlikely that the same off-targets will be silenced. We also include irrelevant shRNAs as a negative control in our experiments (either scrambled sequences or shRNAs targeted against other genes). To further establish functionality of a gene, we prefer to use either a complementary method of knocking down the gene (such as dominant negatives), shRNA for other genes within the same pathway, or add-back of exogenous mRNA containing a silencing mutation so that it cannot be targeted by the shRNA. *— Debra Taxman*

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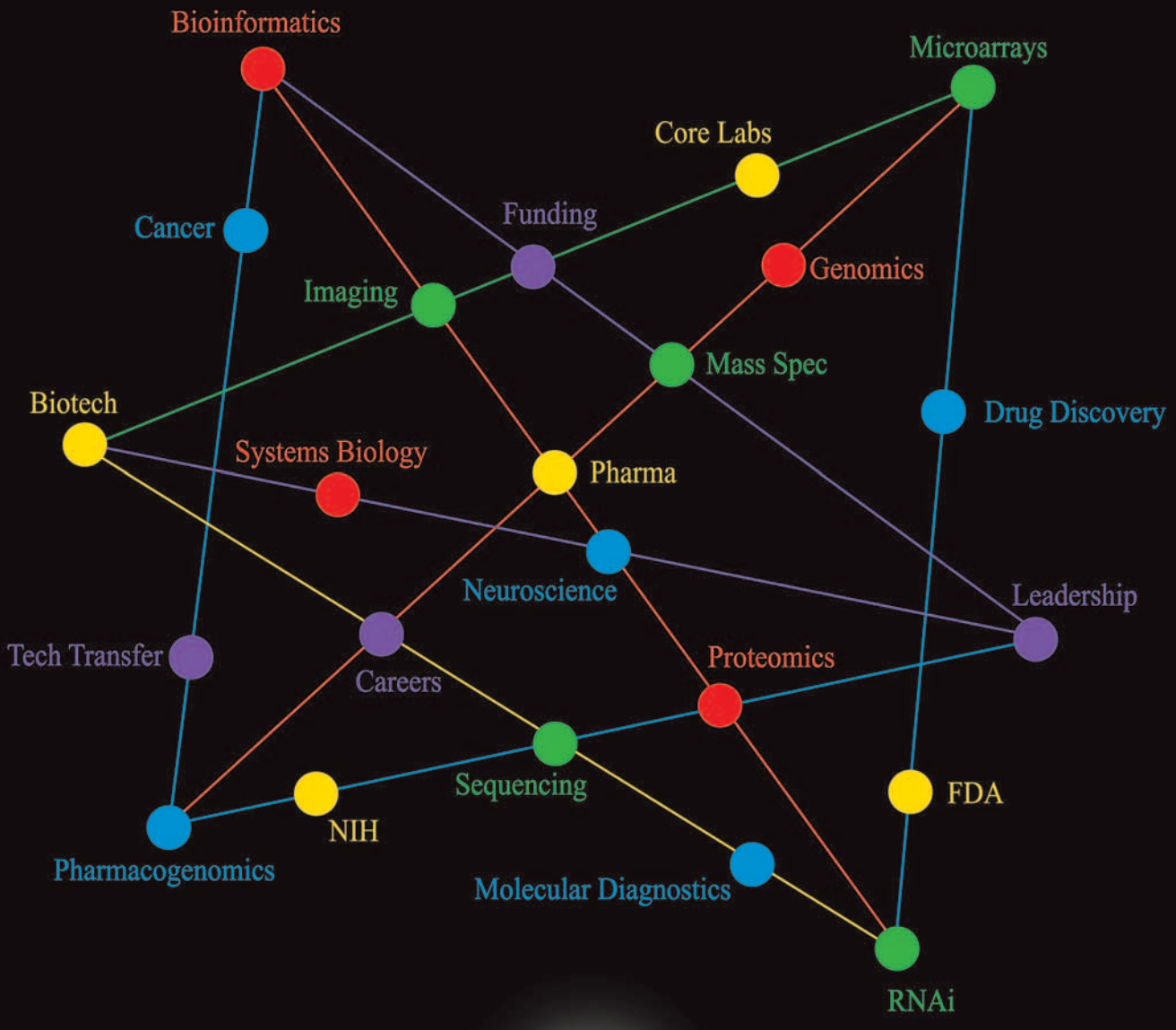
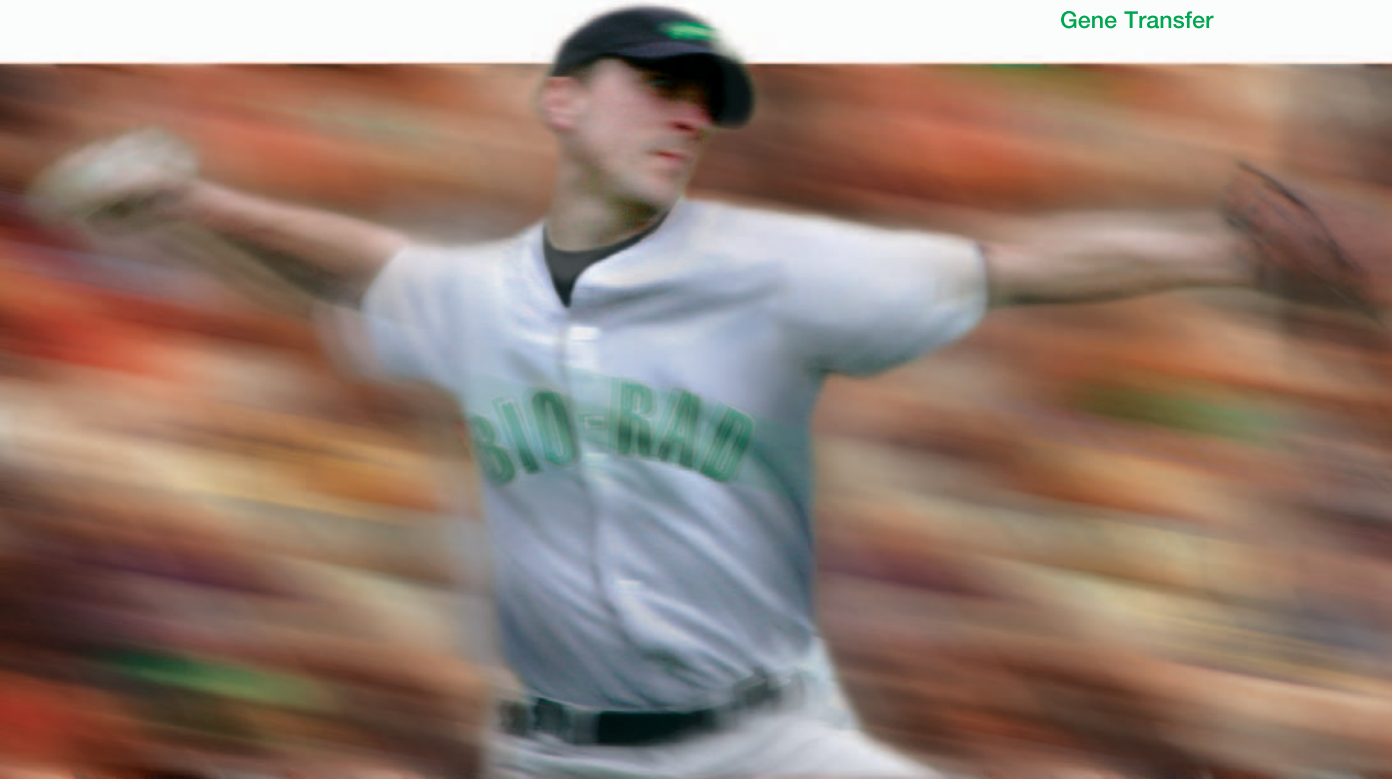



GenomeWeb



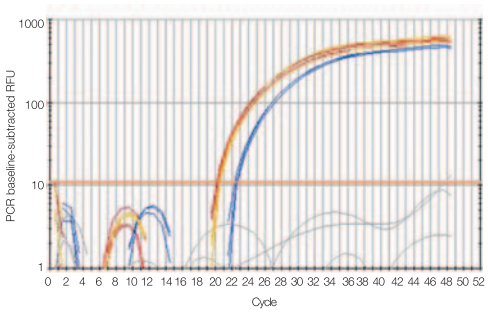

# Powerful shRNA Delivery

*High efficiency, lower cytotoxicity, and a simple protocol put Bio-Rad's TransFectin™ reagent in a league of its own.*

**Key Features of TransFectin Lipid Reagent**

- Effective delivery of vector-based shRNA for gene silencing in RNAi studies
- High efficiency and activity for a broad range of cells
- Lower cytotoxicity — maintains cell viability
- Excellent performance at any culture density
- Simple 3-step protocol

For more information, contact your Bio-Rad representative or visit us on the Web at **www.bio-rad.com/ad/transfectin/**




**GAPDH knockdown >75% in HeLa cells using TransFectin reagent for vector-based shRNA delivery.** HeLa cells were transfected in six-well plates using 2.5 µl TransFectin with 2.5 µg of each DNA sample: plasmid expressing GAPDH (■), plasmid expressing scrambled GAPDH (■), or plasmid expressing a nonspecific control (■). Cells were harvested 48 hr posttransfection, and RNA was extracted using an Aurum™ total RNA kit. cDNA was produced using an iScript™ cDNA synthesis kit, and quantitative PCR was performed.