Continued from Page 3

If you're working with a cell line that is refractory to transfection, or if you would like to make a stable cell line using your shRNA, then OriGene's pRS plasmid is ideal. A puromycin-N-acetyl transferase gene is located downstream of the SV40 early promoter, resulting in resistance to the antibiotic puromycin. Positive selection with puromycin after transfection will produce a stable cell line that has incorporated the shRNA cassette into the genome for continual expression of the hairpin construct. The HuSH pRS plasmid vector also contains both 5′ and 3′ long terminal repeats (LTRs) of the Moloney murine leukemia virus (MMLV), so that transfection of the plasmids into a retroviral packaging cell line produces replication deficient viruses that can be used to infect target cells.

OriGene is so certain that you will be happy with the results you see from using our HuSH-29 constructs that we're willing to guarantee performance. If using all four constructs individually and / or together against the target gene does not result in at least 70% knockdown of gene expression, OriGene's technical support staff will work together with you to troubleshoot the experiment. If the shRNA constructs are deemed ineffective for knockdown, new constructs will be designed and sent to you at no charge. So there's no risk – use HuSH-29 to knock down your gene of interest today!

To search for the HuSH-29 constructs against a particular gene, go to http://www.origene.com/rna/search.mspx and search by keyword, accession number, nucleotide or protein sequence, or gene family of your target. All standard HuSH-29 constructs are available at a cost of $800.00 USD (includes 4 unique constructs and two negative controls; custom constructs available for an additional fee), and are available three weeks from the date you place your order.

REFERENCES

1. Hannon GJ. (2002) RNA interference. Nature. 418:244-51.
2. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. (2002) Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 411:494-8.
3. Brummelkamp TR, Bernards R, Agami R. (2002) A system for stable expression of short interfering RNAs in mammalian cells. Science. 296:550-3.
4. Paddison PJ, Caudy AA, Bernstein E, Hannon GJ, Conklin DS. (2002) Short hairpin RNAs (shRNAs) induce sequence-specific silencing in mammalian cells. Genes Dev. 16:948-58.

HuSH-29 validation

RNA interference (RNAi) has garnered considerable attention and become a widely used research technique since its discovery just a few years ago (see article in this issue regarding 2006 Nobel Prize for Physiology or Medicine). OriGene has optimized its HuSH-29 product line to allow researchers to bring the power and excitement of RNAi to their labs. Here we demonstrate the efficacy of HuSH-29 by providing evidence of gene expression knockdown of two readily detectable cotransfected targets: green fluorescent protein (GFP) and luciferase.

Reporter constructs expressing luciferase or enhanced GFP (EGFP) were transfected into HEK293 cells with HuSH-29 constructs designed to silence the expression of the corresponding transcript. Equal masses of target and HuSH-29 DNA were cotransfected in each experiment, far exceeding the target expression expected from an endogenously expressed gene or even from a typical overexpression experiment. Twenty-four to forty-eight hours post-transfection, the cells were examined by luciferase assay or fluorescence microscopy to determine the level of gene expression knockdown. As evidenced from the figures that follow, OriGene's HuSH-29 constructs are powerful at knocking down overexpressed genes.

Continued on Page 21

Trivia Question

The average human body holds enough _____ to make 900 pencils, and enough _____ to make 2,200 match heads.

Send your answer to cDNA@origene.com. The first ten correct responses received will win a bag of free OriGene merchandise, including a "Nice Genes" T-shirt. Please include your full mailing address and your preferred T-shirt size (M, L, or XL).

Continued from Page 4

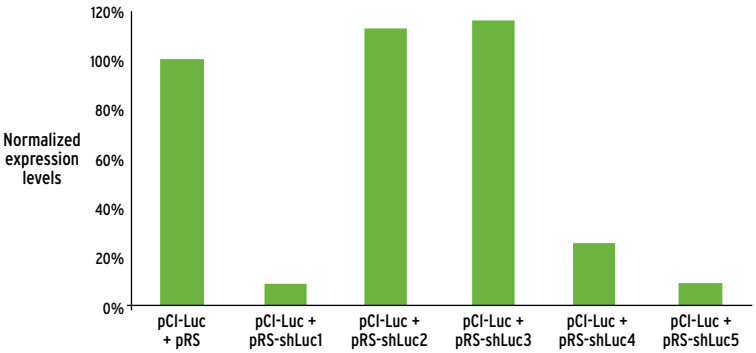

Figure 1. Expression of co-transfected luciferase is reduced by 85% on average by effective HuSH-29 constructs targeting that transcript. Percent expression is calculated relative to cells treated with the negative control HuSH-29 construct. Two non-effective constructs (sh-Luc2 and shLuc3) were identified, and are used to contrast the effectiveness of the other pRS-shLuc plasmids. Similar effects were seen with other promoter/luciferase constructs, such as pBRCA1-Luc (data not shown).

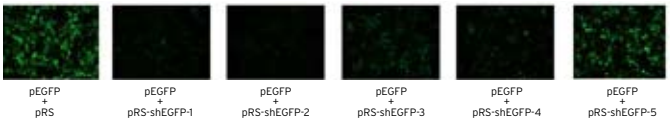

Figure 2. Expression of EGFP is markedly reduced by cotransfection with HuSH-29 constructs targeting EGFP. A non-effective construct (pRS-shEGFP-5) is identified, and used to contrast the effectiveness of the other pRS-shEGFP plasmids. Similar effects were seen in triplicate experiments (data not shown).

Materials and methods

LUCIFERASE

HEK293 cells were plated at a concentration of $2x10^4$ cells/well in 0.1 mL DMEM with 10% FBS and 1X penicillin/streptomycin into each well of a white polystyrene-coated 96 well plate (ISC Bioexpress, Kaysville UT). The following day, FuGENE6 (Roche, Indianapolis IN) was used as the transfection reagent at a ratio of 3:1 (uL FuGENE6:ug DNA). Equal masses of reporter plasmid pCI-Luc (Promega, Madison WI) and shRNA constructs (OriGene Technologies, Rockville MD) were mixed together, and combined with the transfection reagent in the serum free medium OptiMEM (Invitrogen, Carlsbad CA). After incubation, the transfection mixture was gently added to the cells, and the cells were incubated at 37°C, 5% CO_2. Twenty-four hours post-transfection, 30 uL of the BriteLite luciferase substrate (Promega) was added to each well and incubated for 2 minutes. Relative light units (RLU) were detected on a Victor3 multilabel plate reader (Perkin Elmer, Wellesley MA), and calculated as the mean of triplicate wells with standard deviation.

GFP

HEK293 cells were plated at a concentration of $5x10^5$ cells/well in 2 mL DMEM with 10% FBS and 1X penicillin/streptomycin into each well of a 6 well plate. Twelve hours later, FuGENE6 was used as the transfection reagent, at a ratio of 3:1 (uL FuGENE6:ug DNA). Equal masses of reporter plasmid pEGFP and shRNA constructs (OriGene Technologies) were mixed together, and combined with the transfection reagent in the serum free medium OptiMEM. After incubation, the transfection mixture was gently added to the cells, and the cells were incubated at 37°C, 5% CO_2. Forty-eight hours post-transfection, images were captured with a fluorescent microscope.

Catalog number
Product description
Quantity
Price (USD)

TR20003
(included in your order of any gene specific shRNA constructs)
pRS, empty cloning plasmid
200 ng
$150.00

TR30001
pRS-shGFP
200 ng
$150.00

TR30002
pRS-shLuc
200 ng
$150.00

TR30003
(included in your order of any gene specific shRNA constructs)
pRS-shGFP, non-effective (negative control)
200 ng
$150.00