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GeneDex

Editor: Michele Nealen

OriGene's HuSH-29 shRNA constructs solve your problems with gene-expression knockdown

problem: My current experiments show incomplete or undetectable knockdown.
solution: 29 basepair targeting sequences like those in HuSH-29 are up to 100 times more potent than the conventional 21 mer shRNA or siRNA. Retroviral infection and puromycin selection can effectively produce up to 100% transfection efficiency, and continuous hairpin expression can outlast long-lived transcripts and proteins that turn over slowly.

problem: Repeating a knockdown experiment or performing library screening with siRNA can be expensive.
solution: Much less shRNA is required for effective knockdown, so your per experiment cost is lower when using HuSH-29. Targeting multiple transcripts of loci at once decreases the number of individual reagents needed, and a plasmid is degraded more slowly than an oligo, allowing for longer effect with single application. The HuSH-29 plasmid can be stably integrated into the host genome, allowing for stable cell line

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production (eliminating the need for repeated transfections), and is a renewable resource via bacterial transformation and amplification. Four constructs corresponding to different regions of each target gene create optimal inhibition, and HuSH-29 comes with an efficacy guarantee for risk-free application.

problem: The large degree of off-target effects in my experiment masks my ability to see on-target effects of knockdown.
solution: HuSH-29 constructs have non-detectable amounts of interferon induction, and show minimal off-target effects.

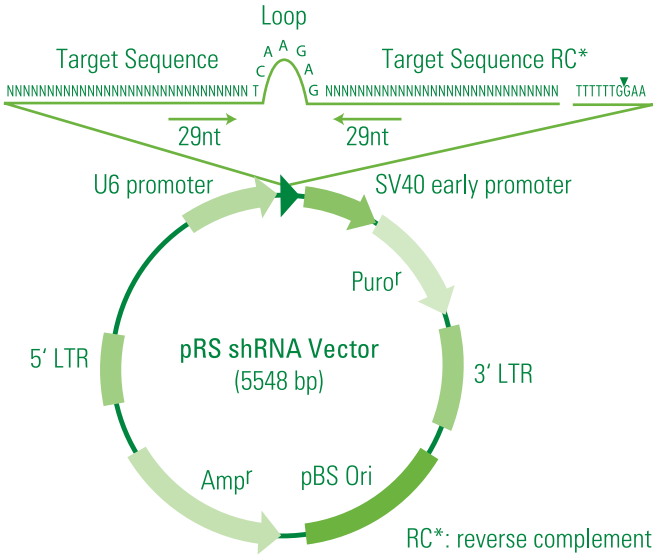
problem: Without knowing the targeting sequences, I can't be sure that the specific isoform I'm interested in will be knocked down.
solution: The gene specific sequences in all HuSH-29 constructs are published on our website, so you can be sure which isoforms will be targeted. Also, OriGene offers a custom design service (see below) that can help you target novel transcripts or only those isoforms you're studying.

problem: I'm having difficulty maintaining an RNase-free environment, so using siRNAs in my knockdown experiments is problematic.
solution: Since RNA is produced from the DNA HuSH-29 plasmid template after transfection, no RNase precautions are necessary for your experiments.

problem: I'm using primary cells or cells recalcitrant to transfection, and I'm experiencing an inefficient delivery of shRNA plasmid or siRNA oligo to the cells.
solution: Using retroviral infection of HuSH-29 constructs or positive selection of transfected cells using puromycin, you will see dramatic increases in the number of cells that take up the HuSH-29 plasmid. You can expect a large increase in the knockdown effect when using puromycin selection of transfected cells, or by using retroviral infection together with puromycin selection.

problem: I'm studying a novel transcript, for which there is no available shRNA or siRNA.
solution: Nearly every known human and mouse gene is targeted by a predesigned HuSH-29 construct. For those transcripts without a corresponding HuSH-29 product, OriGene will produce custom designed constructs. Please contact OriGene's Technical Support staff for more information.

The pRS plasmid is designed to be transfected into mammalian cells via standard methods, or to be packaged and used to produce retroviral particles for infection.



HuSH-29 shRNA constructs are the right tool for your experiment

HuSH-29 constructs have carefully designed, known target sequences to maximize their utility in gene knockdown experiments.

RNA interference (RNAi) is a cellular defense mechanism by which host cells use endogenous RNA transcripts to target homologous RNAs for destruction[1]. In mammalian cells, RNAi can be triggered by short interfering RNA constructs (siRNAs) that cause strong yet transient inhibition of gene expression of specific genes[2]. These siRNAs can be generated in vitro and transfected into mammalian cells, resulting in effective suppression of gene expression. Unfortunately, such suppression is transient, as the oligos are unprotected and therefore degraded by the host cell. In contrast, short hairpin RNA (shRNA) plasmids can repeatedly transcribe a similar dsRNA product and suppress gene expression over a prolonged period[3,4].

HuSH-29 shRNA constructs are the solution to your gene silencing problems. These highly effective constructs are available now for use in your knockdown experiments, whether you're transiently silencing the expression of a single gene, or creating stable cell lines that have diminished expression from a number of related transcripts. See how OriGene's pRS plasmid-based silencing constructs have the features you're looking for in a cost-effective product format.

OriGene created the HuSH-29 product line by engineering its own shRNA plasmid with features that make it ideal for any gene expression knockdown experiment. This retroviral silencing plasmid (pRS) contains murine retroviral long terminal repeats (LTRs) to allow for retroviral packaging, the puromycin resistance gene for stable cell selection, and a U6 small nuclear RNA gene promoter[1,2] to effectively express the inserted hairpin DNA. This plasmid achieves the RNA interference effect upon introduction into and subsequent processing by mammalian cells. The pRS plasmid has been validated for transient transfection and for the ability to inhibit target gene expression with GFP- and luciferase-specific hairpin DNA inserts (see "HuSH-29 validation" article in this issue). Moreover, OriGene's vector has been validated for retroviral expression of shRNA against EGFP, showing >90% suppression of overexpressed EGFP.

Each HuSH-29 plasmid was constructed using synthetic oligonucleotides cloned into the BamH I / Hind III cloning sites of the pRS vector. These plasmids have a U6 promoter followed immediately downstream by 29 base pair gene specific sequence, a 7 base pair loop, the 29 base pair sequence in reverse complement, followed by a TTTTTT termination sequence. Upon introduction of the plasmid into mammalian cells, the U6 promoter expresses the insert sequence, resulting in RNA forming a short hairpin (shRNA), which is processed by cellular machinery into a form that inhibits the expression of the target gene.

The OriGene HuSH-29 product line includes shRNA expression vectors targeting nearly all known human and mouse genes, such as protein kinases, phosphatases, oncogenes, tumor suppressor genes and other signaling molecules or structural proteins. For any given gene, four unique shRNA expression vectors are provided. These vectors are shipped as 200 ng of purified, lyophilized plasmid, each provided in a separate tube to allow individual screening. Negative control plasmids (one empty pRS cloning plasmid, and one pRS plasmid containing a non-effective hairpin cassette) are also included in the same package. All shRNA hairpin cassettes have been sequence-verified to match the targeted gene with 100% identity. The species specificity for all constructs is indicated on OriGene's website.

Positive control shRNA expression vectors against green fluorescent protein (GFP) and luciferase (Luc) genes are available for purchase (Catalog Numbers TR30001 and TR30002 respectively) to use as knockdown controls for your experiments. Both shRNA-GFP and shRNA-Luc are constructed in the same plasmid vector, pRS, and have been shown to inhibit their respective target genes by up to 90%. When cotransfected with GFP or luciferase expressing plasmids, these positive controls are readily assayed to determine your optimal transfection conditions for shRNA silencing.

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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.
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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.

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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).

Frequently Asked Questions... about HuSH-29

WHAT IS IN THE PLASMID OF THE HUSH-29 PRODUCT?

Answer: The pRS plasmid was designed to effectively express RNA hairpin insert sequences in transfected mammalian cells. These plasmids contain a U6 promoter, a 29 basepair (bp) gene specific sequence, a 7 bp intervening sequence, another 29 bp sequence that is the reverse complement to the gene specific sequence, and a transcription termination sequence. The expression of the insert sequence in mammalian cells results in the formation of a short hairpin RNA with a 29 bp stem and a 7 nucleotide loop. Such a structure has been shown to be able to be processed by mammalian cells into a form that can effectively knock down the expression of target genes. In addition, the pRS plasmid contains a puromycin selectable marker and a pair of retroviral integration sites flanking the gene silencing cassette and the selection marker. Both the selection marker and the retroviral integration functions have been validated.

WHAT IS THE U6 PROMOTER?

Answer: The human U6 promoter is a sequence that has been used extensively to express shRNAs in mammalian cells via its recognition by RNA polymerase III. This promoter has been validated in OriGene's HuSH-29 products in gene expression knockdown experiments.

WHAT IS THE 29 BASE PAIR GENE SPECIFIC SEQUENCE INSERTED IN THE PLASMID?

Answer: The gene specific sequences were designed using a specific algorithm (described later in this article) and the cDNA sequence of the target genes. All sequences of the shRNA expression cassettes were verified through DNA sequence analysis, matched against the target gene sequences, and confirmed to show no significant alignment to unrelated sequences. The sequence identity for each targeted locus is provided for users on OriGene's website and on the Certificate of Analysis included with each shipment.

HOW DOES THE TRANSCRIPTION TERMINATE?

Answer: A stretch of 6 thymidine nucleotides (dT_6) follows immediately after the reverse complement of the gene specific sequence. This dT_6 sequence leads to immediate termination of transcription by RNA Pol III.

HOW SHOULD I USE THE PRODUCTS?

Answer: OriGene recommends that the customers first transform into competent E. coli cells an aliquot of each of the HuSH-29 plasmids provided and perform a DNA miniprep. The purified DNA then can be transfected into a target cell line or used for retroviral particle production. After transfection or infection, cell lysates of the targeted cells can be obtained and used for Western blot analysis with an antibody against the target protein, or RNA can be harvested from transfected cells and used in quantitative RT-PCR experiments to determine the loss of gene expression.

WHAT PURPOSE DO THE NEGATIVE CONTROLS SERVE?

Answer: For any shRNA experiment, it is important to demonstrate that the effect of a targeting construct is gene specific, and not due to non-specific effects such as the interferon response or off-target silencing. Our negative control plasmids can be used to exclude these non-specific responses.

CAN THE EMPTY VECTOR BE USED TO SUBCLONE MY OWN SHRNA CASSETTE?

Answer: Yes, you can design your own oligos with BamH I and Hind III cloning sites on each end, and subclone your hairpin cassette into the empty vector.

HOW DO I CHOOSE WHICH OF THE FOUR HUSH-29 CONSTRUCTS TO USE FOR GENE SILENCING?

Answer: The functionally active shRNA expression plasmids need to be selected experimentally in transiently transfected cells. OriGene recommends testing each plasmid separately and together with the other constructs.

CAN I SELECT FOR HUSH-29 TRANSFECTED CELLS?

Answer: Yes. The pRS plasmid contains a puromycin resistance cassette, so transfected cells can be "positively selected" by growing the cells in culture medium containing puromycin (0.5-1 ug/ml) for 1-2 weeks.

HOW CAN I CREATE A STABLE CELL LINE USING THE HUSH-29 PLASMIDS?

Answer: Stable cell lines can be generated by two different approaches. First, target cells can be transfected with a functionally validated shRNA plasmid. Starting the day after transfection, the transfected cells can be selected with 0.5-1 ug/ml puromycin for a 1-2 weeks. Alternatively, retroviral packaging cell lines can be used to generate retroviruses, which can

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be used to infect target cells for stable cell line generation. See the article "HuSH-29 Protocols" in this issue for more details.

CAN I SCREEN ALL OF THE HUSH-29 CONSTRUCTS PROVIDED IN HEK293 CELLS, THEN PICK THE MOST EFFECTIVE ONE FOR RETROVIRAL INFECTIONS?

Answer: Absolutely. We recommend that you screen all the constructs independently and in combination to identify the most effective knockdown strategy. HEK293 (or NIH3T3 cells, for mouse shRNA) cells are a convenient and easily transfectable system for screening. Afterwards, the effective construct(s) can be used for retroviral infection of or for transfection into your target cells.

DO YOU RECOMMEND THAT WE LINEARIZE THE HUSH-29 PLASMIDS TO INCREASE TRANSFECTION EFFICIENCY?

Answer: We have no information to suggest that this step would increase transfection efficiency, and it is not known to us that a linearized plasmid with a larger Stokes radius would be efficiently transfected. If you wish to test this possibility, you may linearize the plasmid with Hind III, a unique cloning site in the HuSH-29 construct. It is possible that for stable cell line production, a linearized vector may integrate into the host genome more efficiently than a circularized plasmid, although we have no data to support this theory.

WILL YOUR HUSH-29 PRODUCTS WORK WITH ANY RETROVIRAL PACKAGING CELL LINE?

Answer: Our pRS vector has been designed for viral packaging in most retroviral packaging cell line commercially available. However, please make sure that the packaging line has not been previously transfected with plasmid(s) containing a puromycin resistance cassette. Furthermore, you need to ensure that the chosen cell line's viral particles are able to infect your target cell line (some cell lines have restricted species specificity). We have successfully used such packaging lines as PT67 (Clontech, Mountain View CA) and Phoenix (Orbigen, San Diego CA) for packaging.

shRNA plasmids are designed to repeatedly produce hairpin constructs for a longer lasting RNAi effect than siRNA.

WHY SHOULD I BUY A HUSH-29 CONSTRUCT RATHER THAN A SYNTHETIC SIRNA?

Answer: The HuSH-29 product offers customers an alternative strategy for the inhibition of target genes. There are several advantages to this expression plasmid-based approach:

1) The duration of gene silencing can be longer as the plasmid continuously expresses the shRNA. In contrast, synthetic siRNAs either become diluted during cell division or degraded.

2) When transfection efficiency is limiting, one can select successfully transfected cells with puromycin or use retroviral infection to ensure efficient gene silencing.

3) One can obtain stable cell lines through either plasmid transfection or viral infection via a packaging cell line.

4) The plasmids can be amplified and become an unlimited source for many experiments.

WHAT IS THE ADVANTAGE OF ORIGENE'S HUSH-29 PRODUCT OVER ANY OTHER COMPANY'S SHRNA PRODUCT?

Answer: Our algorithm for designing the target sequences engineered into each HuSH-29 construct is supported by published research to be highly effective. Additionally, the use of 29mer target sequences has been shown to be superior to 21mer sequences in knocking down gene expression. Finally, OriGene's guarantee on these plasmids (see below) reassures its customers that OriGene stands behind the effectiveness of the HuSH-29 product.

WHAT IS THE ORIGENE GUARANTEE ON THE HUSH-29 EXPRESSION PLASMIDS?

Answer: OriGene guarantees that the gene-specific sequences of the shRNA expression plasmids correspond with 100% identity to the target gene. If using all four constructs individually and / or together does not result in knockdown of gene expression, OriGene's Technical Support staff will work together with the customer to troubleshoot the experiment. If the shRNA constructs are deemed unsuitable for knockdown, new constructs will be designed and sent to the customer at no charge.

HOW DOES THIS PRODUCT DIFFER FROM YOUR PREVIOUS SHRNA CONSTRUCT, THE HUSH-21 PLASMIDS?

Answer: The HuSH-21 plasmids had a 21 bp gene specific sequence, a 10 bp intervening sequence, a 21 bp reverse complement to the gene specific sequence, and a transcription termination sequence. The 21 bp gene specific sequence was chosen through random library digestion. Recent data indicated that increasing the targeting sequence length, decreasing the loop length, and choosing the target sequence via our algorithm would significantly increase the effectiveness of gene expression knockdown. In our ongoing effort to improve our product offering and provide state of the art performance, we will no longer offer 19-21mer versions of HuSH products.

YOUR HUSH-29 PRODUCT IS STATED TO BE "LOCUS SPECIFIC". HOW DO I KNOW THAT IT WILL KNOCKDOWN THE EXPRESSION OF MY VARIANT OR ISOFORM?

Answer: Unless stated otherwise, HuSH-29 constructs are designed to be effective against most transcriptional variants at a particular gene locus. If you would like a knockdown construct against a specific transcriptional variant(s), OriGene can generate a custom HuSH-29 product that will selectively knockdown only the specified variants. Please contact OriGene's Technical Support staff for details.

I'VE SEEN DATA SUGGESTING THAT A PARTICULAR SIRNA SEQUENCE IS EFFECTIVE AT KNOCKING DOWN EXPRESSION OF MY GENE OF INTEREST. WILL YOU GENERATE HUSH-29 CONSTRUCTS WITH SEQUENCES THAT I GIVE YOU?

Answer: We are happy to clone a customer's specified sequences into our pRS vector as part of the hairpin insert. However, as these sequences are not designed using our algorithm, OriGene cannot extend our performance guarantee to these custom products. Furthermore, the specified sequence must be a 29mer. If you provide a 21mer sequence, we can assist you in choosing additional neighboring sequence to make the targeting sequence a 29mer. Please contact OriGene for price and delivery estimates for these custom HuSH-29.

YOUR WEBSITE LISTS 4 SEQUENCES FOR CATALOG NUMBER TR3XXXXX. DO WE GET ALL FOUR TARGETING CONSTRUCTS SEPARATELY, OR ARE THEY MIXED TOGETHER?

Answer: Each sequence listed represents a single targeting construct, which is provided in a separate tube as dried plasmid DNA. Your order includes 4 constructs plus two negative control plasmids, each packaged individually. While you can test the knockdown efficiency of all four constructs together, we provide the constructs separately so that you can test them individually as well.

Twenty-nine basepair targeting sequences have been shown to be much more effective at gene expression knockdown than twenty-one basepair sequences.

I WOULD LIKE A C-MYC, HIS, OR GFP TAGGED VERSION OF YOUR HUSH-29 CONSTRUCTS. CAN YOU GENERATE THESE FOR ME?

Answer: Unfortunately, we are currently unable to provide a custom subcloning service for our HuSH-29 constructs. We hope to be able to offer this service in the future.

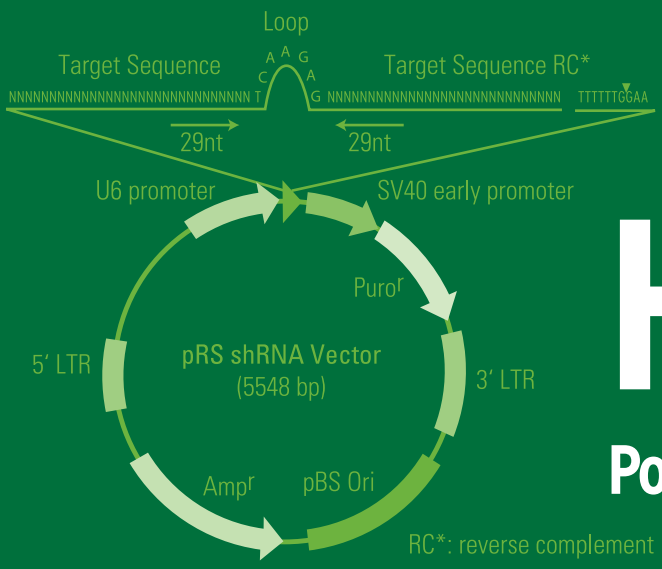
CAN I GET A HUSH-29 CONSTRUCT DESIGNED AGAINST A SPECIES OTHER THAN HUMAN?

Answer: Although designed against human and mouse gene targets, many of our current constructs are also homologous to other species' sequences. For those that are not homologous, OriGene can do custom HuSH-29 production for any sequence, regardless of species. When placing an order for a custom HuSH-29 product, please use the catalog number TR200000 and provide the NCBI accession number for the sequence you wish to target. Our Technical Support staff can offer you assistance in identifying the reference sequence for your target of interest.

HOW ARE THE TARGETING SEQUENCES CHOSEN FOR THE HUSH-29 CONSTRUCTS?

Answer: OriGene's newly optimized target sequence design strategy involves the avoidance of sequences with runs of identical bases, choosing regions with a GC content targeted at 55% (must be between 30% and 70%), a preference for sequences in which the GC content is higher at 5′ than at 3′, avoidance of internal palindrome sequences, and choosing sequences that target to as many transcriptional variants of a locus as possible.

For more information on the HuSH-29 product line, please visit OriGene's website at http://www.origene.com/rna/. You may also contact our Technical Support staff at techsupport@origene.com or 888-267-4436 (outside the US, dial 301-340-3188) with any questions.



HuSH-29:

Potent shRNA for human and mouse

OriGene, your source of over 30,000 expression-ready cDNA clones for over-expression studies, is now offering the most specific and effective shRNA for expression knock-down.

The most effective 29-mer duplex*

More potent than the traditional 21-mer shRNA or siRNA

Decreased off-target effects

Minimal interferon induction

Four constructs targeting 4 regions of each gene

Guaranteed knock-down

*1. Kim DH, Behlke MA, Rose SD, Chang M-S, Choi S and Rossi JJ 2005 *Nature Biotechnology* 23: 222-226
2. Siolas K, Lerner C, Burchard J, Ge W, Linsley PS, Paddison PJ, Hannon GJ and Cleary MA 2005 *Nature Biotechnology* 23:227-231

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HuSH-29 Protocols

Plasmid DNA amplification in E. coli

Transforming your HuSH constructs into competent cells allows you to create an eternal stock from which you can produce endless quantities of DNA for your transfection experiments. This simple protocol requires only 30 minutes of hands-on time to generate a glycerol stock and another 30 minutes to purify enough DNA for a transfection experiment.

STEP 1. RESUSPENSION OF LYOPHILIZED SHRNA CONSTRUCTS

Add 100 uL of sterile water into each of the tubes containing shRNA expression plasmids. Vortex the tubes gently or pipet up and down to resuspend the lyophilized DNA. This resuspension produces a DNA solution with an approximate concentration of ~2 ng/uL, which should be stored at –20°C.

STEP 2. TRANSFORMATION

Both electroporation and heat shock are appropriate methods of transformation for amplifying plasmid DNA; use the cells* normally employed in your lab for routine transformations. Example protocols are given below for transformations using chemically competent cells and electrocompetent cells. Be sure to follow the specific recommendations of your competent cell manufacturer.

*Most commercially available competent cells are appropriate for this purpose. Confirm the efficiency of your batch of cells by performing a parallel transformation with the supercoiled control DNA provided with the cells. OriGene recommends using cells with an efficiency of at least 10^6 CFU/ug DNA.

Transformation with chemically competent cells

Briefly thaw on ice a tube of competent cells. Aliquot into prechilled microfuge tubes the appropriate volume of cells for individual transformations (e.g., 10 uL of cells for 1-5 ng supercoiled DNA). Add 1-5 uL of each expression plasmid to an aliquot of competent cells, stir gently with the pipet tip, and incubate on ice for 30 minutes. Perform the heat shock by incubating the mixture of DNA and cells at 42°C for exactly 30 seconds, then removing the cells to ice immediately. Add 250 uL of recovery medium (such as SOC) to the cells, and incubate at 37°C for 1 hour with agitation. Plate several dilutions of the transformation mixture onto separate LB-amp agar plates (try 1%, 10%, and 50% of the transformation reaction on separate plates, each diluted up to 100 uL in SOC). Incubate the plates overnight at 37°C. Store any remaining transformation solution at 4°C in case further dilutions and replating are necessary. The following day, inoculate 2-3 single bacterial colonies from each transformation into individual sterile culture tubes containing 5 mL of liquid medium with 100 ug/mL ampicillin (LB-amp). Incubate overnight at 37°C with agitation.

Transformation with electrocompetent cells

Briefly thaw on ice a tube of competent cells. Aliquot into prechilled microfuge tubes the appropriate volume of cells for individual transformations (e.g., 10 uL of cells for 1-5 ng supercoiled DNA). Add 1-5 uL of each expression plasmid to an aliquot of competent cells, stir gently with the pipet tip, and transfer the mixture to a prechilled electroporation cuvette. Incubate cuvette on ice for 30 minutes. Perform the electroporation with settings optimized for your electroporator, and note the Tau value returned. (This value represents the time required for the current of electricity to cross the membranes of your competent cells, and is a good indication of the efficiency of the transformation process.) Add 250 uL of recovery medium (such as SOC) to the cells, and incubate at 37°C for 1 hour with agitation. Plate several dilutions of the transformation mixture onto separate LB-amp agar plates (try 1%, 10%, and 50% of the transformation reaction on separate plates, each diluted up to 100 uL in SOC). Incubate the plates overnight at 37°C. Store any remaining transformation solution at 4°C in case further dilutions and replating are necessary. The following day, inoculate 2-3 single bacterial colonies from each transformation into individual sterile culture tubes each containing 5 mL of liquid medium with 100 ug/mL ampicillin (LB-amp). Incubate overnight at 37°C with agitation.

STEP 3. CREATING A GLYCEROL STOCK

Remove 425 uL of each overnight liquid culture into a fresh microfuge tube. Add 75 uL sterile glycerol, and gently resuspend. Glycerol is quite viscous, so it's best to use a large bore pipet tip (you may even need to widen your pipet tips by cutting off the end with a sharp blade) or a transfer pipet. When the solution is homogenous, snap freeze the tube in liquid nitrogen or a dry-ice/ethanol bath. Store the glycerol stock at -80°C. If stored properly, this stock can be used for the next several years to inoculate a fresh liquid culture in order to amplify more DNA. Simply remove a small portion of the frozen glycerol stock (thawing the tube is not required) from

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the tube by scraping the surface with a pipet tip, and deposit it in a sterile culture tube containing LB-amp. The culture should be incubated overnight at 37°C with agitation before proceeding to step 4.

STEP 4. DNA PREPARATION

OriGene provides a plasmid preparation service for all of our HuSH-29 customers. To receive larger quantities of each plasmid, use catalog number DNA05 to receive 5 ug of each construct for the price of $100 USD each ($400 USD for all 4).

Miniprep method (for producing up to 40 ug plasmid from 5 mL liquid culture)

Isolate DNA from the liquid cultures by using your preferred method. OriGene routinely uses Marligen's High Purity Plasmid Miniprep Kits or similar systems. Follow the manufacturer's protocol for isolation, and elute the DNA in 50 uL of TE [10 mM Tris-HCl (pH 8.0), 1 mM EDTA]. Determine the concentration of each sample, and store the DNA at -20°C.

Midiprep method (for producing up to 500 ug plasmid from 100 mL liquid culture)

Add 5 mL of liquid culture grown several hours or overnight to a sterile flask containing 95 mL of LB-amp. Incubate overnight at 37°C with agitation. Isolate DNA from the liquid culture using your preferred method. OriGene routinely uses Marligen's High Purity Plasmid MidiPrep Kits or similar systems. Follow the manufacturer's protocol for isolation, and elute the DNA in 4 mL of TE [10 mM Tris-HCl (pH 8.0), 1 mM EDTA]. Determine the concentration of each sample, and store the DNA at -20°C.

HuSH-29 constructs are ideal for use on hard-to-transfect cells and cells lines, thanks to the option of retroviral infection.

Introduction of gene specific shRNA into mammalian cells via transient transfection

Transient transfection is the simplest method of introducing HuSH-29 constructs into your target cells. OriGene recommends transfecting each HuSH construct separately as well as all constructs together into an easily transfectable cell line (such as HEK293T or NIF3T3 cells) to determine the construct or pairing of constructs that produces the most complete knockdown effect, before proceeding to work with a more retractable cell line or primary culture.

STEP 1. PLATE CELLS

The day before transfection, passage cells into the desired cell container. Plate an amount of cells expected to achieve 50-80% confluency on the following day (see Table I for examples). Grow the cells overnight at 37°C in a 5% CO_2 incubator.

STEP 2. PREPARE TRANSFECTION MIXTURES

Dilute the transfection reagent* into serum-free medium without antibiotics (Invitrogen's OptiMEM solution is a good example). Do not let the transfection reagent come into contact with the side of the tube; instead, pipet the reagent directly into the medium. Gently flick the tube or pipet up and down to mix. Incubate for 5 minutes at room temperature. Follow the manufacturer's recommendations for ratios and volumes of reagent and DNA (see Table II for examples).

**Many commercially available transfection reagents are convenient for transient transfection. Choose a reagent that is compatible with your target cells. OriGene routinely uses lipid based transfection reagents such as FuGENE 6 or Lipofectamine 2000 on HEK293T cells for validation experiments, and the protocol here is based on those manufacturers' recommendations. Be sure to follow the specific protocol for your transfection reagent.*

Dilute the plasmid DNA into serum-free medium without antibiotics. Gently flick the tube or pipet up and down to mix. Combine the tube of reagent/medium with the tube of DNA/medium, and gently mix. Incubate for 15-45 minutes at room temperature.

TABLE I. SEEDING DENSITY OF TARGET CELLS 1 DAY PRIOR TO EXPERIMENT

Vessel type	Seeding density of cells	Volume of media
T-75 flask	10^6 cells	18 mL
T-25 flask	3 x 10^5 cells	16 mL
10 cm dish	7 x 10^5 cells	12 mL
6 well plate	10^5 cells	2 mL/well
12 well plate	5 x 10^4 cells	1 mL/well
24 well plate	2 x 10^4 cells	500 uL/well
96 well plate	4 x 10^3 cells	50 uL/well

TABLE II. VOLUMES RECOMMENDED FOR TRANSFECTION REACTIONS

Vessel type	OptiMEM	Transfection reagent	OptiMEM	shRNA plasmid	cDNA expression plasmid**
T-75 flask	400 uL	24 uL	400 uL	8.0 ug	80 ng
T-25 flask	125 uL	8 uL	125 uL	2.5 ug	25 ng
10 cm dish	300 uL	18 uL	300 uL	6 ug	60 ng
6 well plate	50 uL	3 uL	50 uL	1 ug	10 ng
12 well plate	25 uL	1.5 uL	25 uL	0.4 ug	4 ng
24 well plate	10 uL	0.6 uL	10 uL	0.2 ug	2 ng
96 well plate	2.5 uL	0.2 uL	2.5 uL	0.05 ug	0.5 ng

** The plasmid designed to overexpress the target gene may be included in some wells to directly compare the effects of overexpression with and without shRNA targeting. This is most useful when your target cells do not endogenously express the target gene, or express it at very low levels.

STEP 3. ADD TRANSFECTION MIXTURE TO CELLS

Remove culture vessel from incubator. For many transfection reagents, it is not necessary to change the medium to a serum-free solution prior to transfection, but check the manufacturer's recommendations for details. Slowly add the transfection mixture dropwise to the culture medium. Rock the plate gently to mix the solution into the media, then return the vessel to the incubator. For many transfection reagents, it is not necessary to change the media after transfection, but follow the manufacturer's instructions for your particular transfection reagent. Incubate the cells at 37°C in a 5% CO_2 incubator for 48 hours before harvesting for RNA analysis, or 72 hrs before harvesting for protein analysis.

Continued on Page 12

HuSH-29 constructs can be used for transient or stable transfection, or retroviral infection, of your target cells.

Continued from Page 11

Creating a stable cell line expressing gene specific shRNA via transfection

Stable integration of a HuSH-29 construct into a cell line allows you to study the effects of knockdown over a longer time course than transient transfection studies would allow. Stable cell lines can be clonally produced, assuring that every cell in the population contains the HuSH-29 plasmid.

STEP 1. TRANSFECTION

Transfect the cells using the protocol above for transient transfection. After transfection, do not change the medium until the next day.

STEP 2. SELECTION

The day after transfection, change the medium to fresh growth medium containing 0.5-1 ug/mL. Continue to grow and passage the cells as necessary, maintaining selection pressure by keeping 0.5-1 ug/mL puromycin in the growth medium. After 1-2 weeks, a large number of the cells will be killed by the puromycin, indicating that they did not take up or have lost the HuSH-29 plasmid with the puromycin resistance cassette. The cells that remain growing in the puromycin-containing medium have retained the expression plasmid, which stably integrates into the genome of the targeted cells.

STEP 3. CLONAL SELECTION

Select clonal populations of cells by transferring a well-isolated single clump of cells (the clonal ancestor and cells divided from it) into a well of a 24 well plate; repeat to select 5-10 clonal populations. Continue growing these cells in selection medium for 1-2 additional passages. At this time, each well contains a clonal population of stably transfected cells, which can be maintained in normal growth medium without the selection pressure of puromycin. These populations can be used for experiments or stored under liquid nitrogen in growth medium with 10% DMSO and 20% FBS for future use. You can verify the integration of the shRNA plasmid by isolating total RNA from the cells and performing RT-PCR to amplify the hairpin insert.

Infection of gene specific shRNA into cells via retrovirus

This protocol has been optimized to produce infectious, replication-incompetent retroviral particles that can efficiently transfer genes into a variety of mammalian cell types *in vitro* or *in vivo*. Retroviral infection allows for the delivery of shRNA plasmids to most dividing mammalian cell types, including many difficult-to-transfect cells.

STEP 1. PREPARE THE PACKAGING CELLS

The day before transfection, plate the retroviral packaging cells* of appropriate tropism at recommended dilutions. Distribute cells evenly about the plate, and incubate at 37ºC in 5% CO_2. Since subconfluent cells are best suited for transfection and potentially generate the highest viral titer, plan to grow the cells to approximately 60-70% confluency. Plating approximate 3 x 10^6 cells per 10 cm cell culture dish should achieve this level of confluence by the following day.

**Choose a packaging cell line whose species specificity is compatible with your target cell line. OriGene routinely uses PT67 (Clontech) or Phoenix (Orbigen) cells for this purpose.*

STEP 2A. TRANSIENTLY TRANSFECT THE PACKAGING CELLS

Transfect the shRNA plasmids** into the packaging cells by following the procedure above for transient transfection. Incubate for 2-3 days at 37ºC in 5% CO_2, and proceed directly to step 3.

***You may wish to do a parallel transfection with a marker plasmid (such as one that expresses EGFP) as a positive control for transfection efficiency.*

STEP 2B. STABLY TRANSFECT THE PACKAGING CELLS (SKIP IF YOU PERFORMED STEP 2A)

Transfect the shRNA plasmids individually (and separately, a plasmid without a puromycin resistance cassette) into the packaging cells by following the procedure above for transient transfection. One day after transfection, add 0.5-1 ug/mL puromycin to the medium for positive selection. Incubate the cells for 3-5 days at 37ºC in 5% CO_2. Confirm that all the negative control cells (those transfected with a puromycin sensitive plasmid) are dead before proceeding.

STEP 3. HARVEST VIRAL SUPERNATANT

When cells are 80-90% confluent, change the medium in the culture vessel to fresh medium normally used on your target cells (i.e., DMEM for HEK293 cells). Twelve hours later, collect the medium as the viral supernatant into 15 ml tubes and centrifuge at 2000 x g for 5 minutes or pass through a 0.45 uM filter to remove cell debris. The clarified supernatant is the viral stock, and can be aliquotted and frozen at -80ºC for future use, or used immediately to infect target cells.

STEP 4. INFECT TARGET CELLS

Add the viral stock with 4 ug/mL polybrene# (catalog number H9268, Sigma, St. Louis MO) as growth medium directly onto your target cells that have reached approximately 50% confluency. Incubate at 37ºC in 5% CO_2. 24 hours post-infection, replace the medium with fresh growth medium containing 0.5-1 ug/mL puromycin. Passage as needed, and maintain selection pressure for 1-2 weeks. Most uninfected cells should be killed by the puromycin within 1 week.

 # The efficiency of retroviral infection is enhanced significantly by including polybrene during the infection. This small, positively charged molecule binds to cell surfaces and allows the viral glycoproteins to bind more efficiently to their receptors by reducing the repulsion between sialic acid-containing molecules.

STEP 5. CLONAL SELECTION OF STABLY INFECTED CELLS (OPTIONAL)

Select clonal populations of cells by transferring a well-isolated single clump of cells (the clonal ancestor and cells divided from it) into a well of a 24 well plate; repeat to select 5-10 clonal populations. Continue growing the cells in selection medium for 1-2 additional passages. At this time, each well contains a clonal population of stably transfected cells, which can be maintained in normal growth medium without the selection pressure of puromycin. These populations can be used for experiments or stored under liquid nitrogen in growth medium with 10% DMSO and 20% FBS for future use. You can verify the integration of the shRNA plasmid by isolating total RNA from the cells and performing RT-PCR to amplify the hairpin insert.

You can download the application guide for HuSH-29 at http://www.origene.com/assets/Documents/AppGuideHuSH29.pdf.

TrueClone Citings

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Ong, HL, et al., The Journal of Biological Chemistry, Epub ahead of print, 2007

This column focuses on significant scientific discoveries by OriGene's valued customers through their use of OriGene's TrueClone full-length cDNAs. We recognize that such publications are the ultimate value statements coming from the research community.

STIM1 meets Orai1: The Store-operated Calcium Release Mystery: Solved?

It is widely known that calcium functions as an important second messenger in cell signaling, regulating cell metabolism, growth and division, and gene expression. The calcium required for these processes is usually released in a bi-phasic manner. The limited influx from channels within intracellular stores (store-operated channels, or SOCs) is followed by an increase from unknown plasma membrane channels (calcium release activated channels, or CRACs) that are regulated in some fashion by the store-operated channels.

RNAi-based screens revealed STIM1 as one regulator of store-operated calcium. The protein product of the STIM1 gene has an EF-hand domain, which acts as a calcium sensor within the lumen of the endoplasmic reticulum (ER). At low calcium concentrations, STIM1 is relocated into puncta at the cell periphery and signals to the CRAC channels. Recent studies discussed here further explain the interactions between the intracellular stores, STIM1 and the plasma membrane-bound channels.

Localization studies revealed that STIM1 does not enter the plasma membrane, and so must affect the calcium response through interactions inside the cell (Wu et al. 2006). Furthermore, the overexpression of STIM1 alone was insufficient to

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cause calcium release (Mercer et al. 2006). There must be some intermediate molecule interacting with STIM1 and the calcium channels in the plasma membrane.

SOC and CRAC activities had been known to require Orai1, a tetra-spanning membrane protein. In fact, the overexpression of Orai1 and STIM1 together stimulates a large response in SOC and CRAC activities. Recently, evidence of these proteins linking the two phases of calcium release was uncovered (Mercer et al. 2006, Soboloff et al. 2006). It was further shown that Orai1 and STIM1 do indeed co-localize after the initial store depletion within the ER and act in a coordinated fashion as the primary element of calcium influx (Luik et al. 2006).

While the mystery of how store-operated calcium regulation seems to be solved, the actual CRAC protein has yet to be discovered. It is postulated that Orai1 itself might be this channel, even though its transmembrane structure is not typical (Parekh et al. 2006). TRPC1 has very recently been implicated in the CRAC channel hunt (Ong et al. 2007); however, this search might be further accelerated using a comprehensive panel of ion channel expression clones.

Protein overexpression is a useful tool in determining gene functions and deciphering complex pathways. In fact, the studies above reveal that overexpressing two proteins at the same time is sometimes necessary to observe the biological perturbation. To assist the research community in this respect, OriGene has curated a comprehensive set of human cDNA clones already in mammalian expression vectors, the TrueClone collection. Having all clones in such a vector system enables the high-throughput screening of hundreds if not thousands of proteins. A technology which takes advantage of this unique collection and powerful vector is OriGene's GFC-Transfection Array (see http://www.origene.com/cdna/gfc-array/ for more details).

ORIGENE CLONES CITED IN THESE ARTICLES:

STIM1	TC118136	NM_003156
Orai1	TC124465	NM_032790
TRPC1	TC121934	NM_003304

ORIGENE HUSH KNOCKDOWN SHRNA CONSTRUCTS:

STIM1	TR309049	NM_003156
Orai1	TR301009	NM_032790
TRPC1	TR308629	NM_003304

IN SEARCH OF MORE "ORIGENE CITINGS"...

Published a paper recently using an OriGene product? If you're an author, send us a reprint or a PDF of the article and we'll send you a free OriGene "Nice Genes" T-shirt (please specify M, L, or XL).

Karl F. Kovacs, Ph.D.
OriGene Technologies, Inc.

Free Product and Good Data– it's a win/win situation!

Let us know how you'd like to use an OriGene product to further your research, and OriGene may make that possible... for free! We have several programs available to collaborate with scientists on research projects that would benefit from using HuSH-29 constructs, GFC-Transfection Arrays, TissueScan Oncology Panels, or other OriGene products. If you are interested in such a collaboration, and would like to receive free clones, arrays, HuSH constructs or other reagents, send an email to cDNA@origene.com with the subject line "collaboration". One of our highly trained scientists will contact you to discuss possible joint research ventures and the delivery of free OriGene products.

TissueScan

TissueScan Oncology Panel: Profile Cancers via Real-Time PCR

- **Gene Expression**
- **SNP**
- **Chromosomal Rearrangement**

TissueScan is a unique RT-PCR based system for fast and accurate gene expression profiling across a large number of cancer patient samples. High-quality cDNAs were prepared from 48 well-documented cancer biopsy samples, normalized and assembled into ready-to-use gene expression panels. This alleviates for researchers the tedious work of large sample collection and the meticulous work of RNA/cDNA preparation, while facilitating quick and reliable profiling of gene expression levels across cancer progression stages. TissueScan combines the high sensitivity and specificity of the RT-PCR methodology with a well-designed multi-sample format. It is an excellent tool for validation of potential cancer markers such as those obtained by microarray or differential display.

Expression levels in normal and cancerous tissues often yield the first clue about the function of a gene. Differentially expressed genes are intensely studied by cancer researchers for their potential value as diagnostic or/and therapeutic targets. The application of microarray technology has produced a large number of such candidate genes. Yet as a hybridization-based technology, microarray has limitations to its sensitivity and specificity. Also, the data generated through pair-wise differential screening need to be validated in larger, independent sample pools. To weed out false targets and to narrow the scope for further research, researchers await an innovation.

TissueScan technology was developed to meet the unmet need for quick target validation in the cancer biomarker field. TissueScan Oncology products are panels of cDNAs from well-documented biopsy samples. These panels currently represent 6 major types of cancers: breast, prostate, lung, colon, ovarian and thyroid.

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TissueScan
Oncology cDNA Panels

Validate Cancer Markers?

Expression or SNP profiling across 48 biopsy tissues in 2 hours

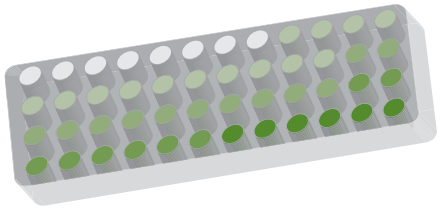
TissueScan is an RT-PCR based system for fast and accurate survey of expression level or SNP profiles. Normalized cDNAs from 48 archived cancer biopsy tissues (provided by Cytomyx) are assembled to a single ready-to-use panel. TissueScan combines the high sensitivity and specificity of the RT-PCR methodology with the well-designed multi-sample format. It is an excellent tool to validate the potential cancer markers, such as those obtained by microarray or differential display.

Convenient
No need for sample collection
No need for RNA/cDNA preparation
No need for cDNA normalization

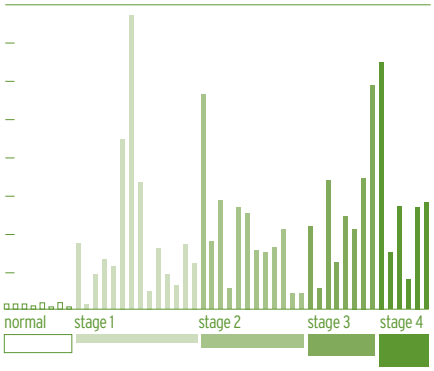
Comprehensive
Diverse cancer type representation
Thorough coverage on all progression stages
Detailed pathology report for each sample
Matching tissues are available from Cytomyx

Real-Time PCR format
High sensitivity, High specificity
Wide dynamic range

Currently Available
Prostate Cancer TissueScan - HPRT101
Breast Cancer TissueScan - BCRT101
Lung Cancer TissueScan - HLRT101
Ovarian Cancer TissueScan - HORT101
Colon Cancer TissueScan - HCRT101
Thyroid Cancer TissueScan - HTRT101



Layout of samples in a PCR plate



Scan results for topoisomerase II alpha with the Lung Cancer TissueScan Panel (HLRT101)

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Each sample included in the panels was obtained with informed consent and is accompanied by a detailed pathology report. For each cancer panel, 48 independent tissues were used to produce high-quality cDNA. The samples were designed to represent all progression stages, from stage 0 (pathologically normal tissue) to stage IV. The cDNAs were normalized by beta-actin levels and assembled into a single PCR plate. Researchers can run a real-time PCR assay by simply adding a PCR master mix and a pair of primers specific for the gene of interest. In a mere two hours, you can obtain an accurate expression profile of any gene across 48 disease-stage tissues.

Features & Benefits

48 TISSUES COVERING ALL CANCER PROGRESSION STAGES

- No need for tedious sample collection
- No need for laborious RNA/cDNA preparation
- Broad representation of the disease
- Increased statistical significance of the data

DETAILED DEMOGRAPHIC AND PATHOLOGY REPORT FOR EACH SAMPLE

- Clear background information for each data point

PRE-NORMALIZED CDNAS

- Simple data interpretation with a normalized baseline

REAL-TIME PCR-READY PANELS

- Highly sensitive and specific results
- Accurate quantification with no concern for signal saturation
- Data analysis begins two hours after opening product

CORRESPONDING TISSUES ARE AVAILABLE FROM CYTOMYX AS TISSUE BLOCKS OR AS TISSUE MICROARRAY

- All positive discoveries can be pursued further with original tissues
- Over 50% of the samples have corresponding clinical data

Each TissueScan kit includes two (or ten) identical PCR panels, beta-actin control primers and a copy of the Application Guide. The normalized cDNAs are lyophilized in the PCR plates.

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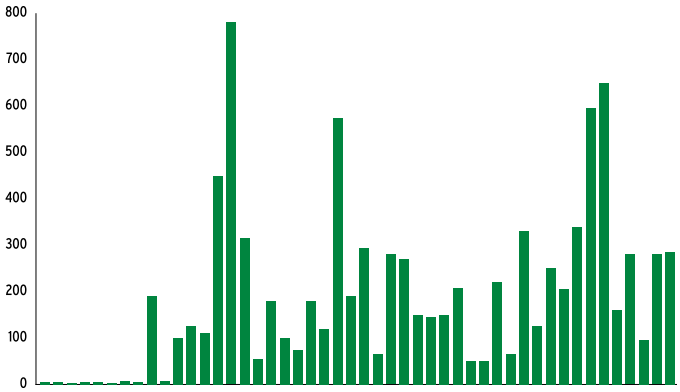

A. Normalized relative expression of Topo II alpha among 48 lung samples*

TissueScan Oncology panels contain 48 unique, prenormalized cDNAs ready for real-time PCR, removing the need for you to find, purchase, and prep numerous samples for analysis.

PROTOCOL AT A GLANCE

Before using TissueScan: Design a pair of real-time PCR primers specific for each gene of interest.

Step 1: Prepare RT-PCR master mix with gene-specific primers and aliquot into the PCR panel.

Step 2. Perform thermal cycling (see appendix for list of compatible thermal cyclers).

Step 3. Plot the expression profile of your gene of interest using Ct (threshold cycle) method.

APPLICATION EXAMPLE

To illustrate the validity of TissueScan, we used Lung Cancer Panel (HLRT-101) to evaluate the expression level of Topoisomerase II alpha, which has been known to be upregulated in a large percentage of lung cancers[1-2]. A pair of primers was designed to detect the transcript of this gene (NCBI accession #NM_001067). SYBR Green I based real-time PCR was performed in a Perkin Elmer thermal cycler (model #PE7700). The expression level plot calculated by the Ct method (Figure A) is shown at left.

In this experiment, the topoisomerase II alpha gene was showed to be expressed at very low levels in tissues at disease stage 0 ("normal" tissue; samples 1-8). In all but one diseased sample (well 10, tumor stage IA), the expression levels are markedly higher than in the normal tissues, supporting the notion that topoisomerase can serve as a valid biomarker for lung cancer. After primer design, the complete experiment took approximately 2 hours, with 20 minutes of hands-on time.

ADDITIONAL UTILITIES OF TISSUESCAN:

By using primers specific for a particular splicing variant or single nucleotide polymorphism (SNP), TissueScan can be used for:

- Comparing expression levels of transcriptional variants from a particular gene locus
- Determining SNP representation in normal and cancer tissues

PRODUCT OFFERING

BCRT101, BCRT501
TissueScan Real-Time Breast Cancer Panels

HPRT101, HPRT501
TissueScan Real-Time Prostate Cancer Disease Panels

HLRT101, HLRT501
TissueScan Real-Time Lung Cancer Disease Panels

HORT101, HORT501
TissueScan Real-Time Ovarian Cancer Disease Panels

HCRT101, HCRT501
TissueScan Real-Time Colon Cancer Disease Panels

HTRT101, HTRT501
TissueScan Real-Time Thyroid Cancer Disease Panels

Price:
A kit of two panels (101) $750 USD
A kit of ten panels (501) $2000 USD

For more information on TissueScan visit our website at http://www.origene.com/geneexpression/disease-panels/products.mspx. If you have questions about using TissueScan Oncology Panels, contact our Technical Support staff at techsupport@origene.com or 888-267-4436 (301-340-3188 outside the USA).

REFERENCE:

1. Differential expression of DNA topoisomerase II alpha and II beta genes between small cell and non-small cell lung cancer. Jpn J Cancer Res. 1998 Aug;89(8):855-61.
2. Topoisomerase II alpha gene expression is regulated by the p53 tumor suppressor gene in nonsmall cell lung carcinoma patients. Cancer. 2002 Apr 15;94(8):2239-47.

You can achieve SNP comparison or biomarker validation in just under two hours by using the TissueScan Oncology panels.

APPENDIX

Thermal Cyclers with known compatibility with TissueScan Oncology Panels:

- *ABI GeneAmp 2700/2720, 9600, and 9700*
- *ABI Prism 7000, 7300, 7500, 7700, 9500*
- *Biometra Uno, Uno II, T1, T3, Tgradient, and TRobot*
- *Bio-Rad MyiQ, iCycler, and MyCycler*
- *Eppendorf Mastercycler® Gradient and EP Gradient*
- *Ericomp SingleBlock and TwinBlock System, and Deltacycler I*
- *MJ Research PTC-200 DNA Engine, PTC-225 DNA Tetra/ PTC-220/221 DNA Dyad, and PTC-100 with 96 well block*
- *MWG Primus 96 and TheQ Lifecycler*
- *Stratagene Robocycler, MX4000, MX3000P*
- *TaKaRa TP 3000*
- *Techne TC-412/512, Touchgene Gradient, Flexigene, and Genius*
- *ThermoHybaid PCR Express, Px2, PxE, MultiBlock System and MBS, Touchdown, Omnigene, and Omn-E*

If your thermal cycler is not listed here, please contact OriGene's Technical Support to inquire.

Xuan Liu, Ph.D.
OriGene Technologies Inc.

2006 Nobel Prize awarded to **RNAi researchers**

The Nobel Assembly at Karolinska Institute has awarded the Nobel Prize in Physiology or Medicine for 2006 jointly to Andrew Z. Fire and Craig C. Mello for their discovery of "RNA interference – gene silencing by double-stranded RNA". This mechanism of gene expression knockdown has become a valuable scientific tool in the past nine years, and is at the heart of OriGene's HuSH-29 technology.

In 1998, the American scientists Andrew Fire and Craig Mello published their discovery of a mechanism that degrades mRNA from a specific gene. This mechanism, RNA interference, is activated when RNA molecules occur as double-stranded pairs in the cell. Double-stranded RNA (dsRNA) activates biochemical machinery, which degrades those mRNA molecules that carry a genetic code identical to that dsRNA. When such mRNA molecules disappear, the corresponding gene is silenced and no protein of the encoded type is made. RNA interference is widely used in basic science as a method to study the function of genes and has incredible potential to lead to novel clinical therapies in the future.

THE DISCOVERY OF RNA INTERFERENCE

Andrew Fire and Craig Mello were investigating how gene expression is regulated in the nematode worm Caenorhabditis elegans. Injecting sense or antisense mRNA molecules alone encoding a muscle protein led to no changes in the behavior of the worms. But when Fire and Mello injected sense and antisense RNA together, they observed that the worms displayed peculiar, twitching movements. (Similar movements were seen in worms that completely lacked a functioning gene for the muscle protein.) They theorized that a double-stranded RNA molecule silenced the gene carrying the same code as that particular RNA. Fire and Mello tested this hypothesis by injecting into the test animals double-stranded RNA molecules containing the genetic codes for several other worm proteins. In every experiment, injection of double-stranded RNA led to silencing of the gene containing that same sequence.

After a series of simple but elegant experiments, Fire and Mello deduced that double-stranded RNA can silence genes, that this RNA interference is specific for the gene whose code matches that of the injected RNA molecule, and that RNA interference can spread between cells and even be inherited. It was enough to inject tiny amounts of double-stranded RNA to achieve an effect, and Fire and Mello therefore proposed that RNA interference (now commonly abbreviated to RNAi) is a catalytic process.

Fire and Mello published their findings in the journal Nature on February 19, 1998. Their discovery clarified many confusing and contradictory experimental observations and revealed a natural mechanism for controlling the flow of genetic information, heralding the start of a new research field.

NEW OPPORTUNITIES IN BIOMEDICAL RESEARCH, GENE TECHNOLOGY AND HEALTH CARE

RNA interference opens up exciting possibilities for use in gene technology. Double-stranded RNA molecules have been designed to activate the silencing of specific genes in humans, animals or plants. Such silencing RNA molecules are introduced into the cell and activate the RNA interference machinery to break down mRNA with an identical code. OriGene's HuSH-29 constructs are designed to express these double-stranded molecules after transfection into a cellular target.

This method has already become an invaluable research tool in biology and biomedicine. In the future, it is hoped that it will be used in many disciplines including clinical medicine and agriculture. Several recent publications show successful gene silencing in human cells and experimental animals. For instance, a gene causing high blood cholesterol levels was recently shown to be silenced by treating animals with silencing RNA. Plans are underway to develop silencing RNA as a treatment for virus infections, cardiovascular diseases, cancer, endocrine disorders and several other conditions.

Adapted from the Nobel Prize press release at http://nobelprize.org/nobel_prizes/medicine/laureates/2006/press.html

Fire and Mello's seminal work led to the development of many molecular tools for RNAi, including OriGene's HuSH-29 constructs.

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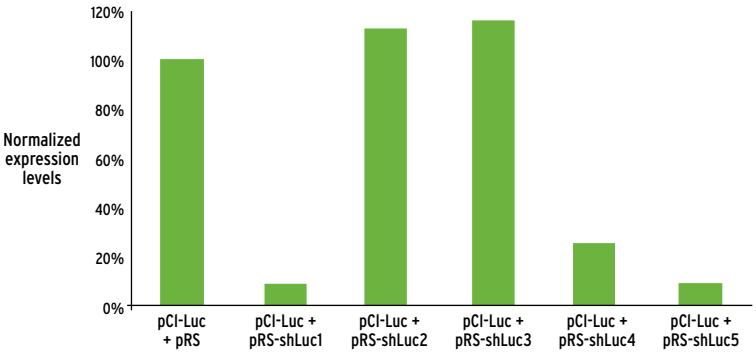

Figure 1. Expression of co-transfected luciferase is reduced by 85% on average by effective HuSH-29 constructs targeting that transcript. Percent expression is calculated relative to cells treated with the negative control HuSH-29 construct. Two non-effective constructs (sh-Luc2 and shLuc3) were identified, 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).

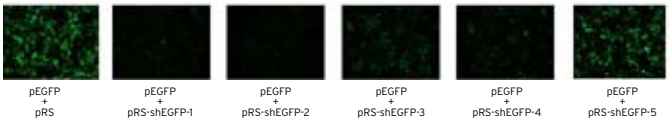

Figure 2. Expression of EGFP is markedly reduced by cotransfection with HuSH-29 constructs targeting EGFP. A non-effective construct (pRS-shEGFP-5) is identified, and used to contrast the effectiveness of the other pRS-shEGFP plasmids. Similar effects were seen in triplicate experiments (data not shown).

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

Ten kilobase ORF! OriGene reaches **Cloning Milestone**

OriGene's cDNA clone is the only clone for the huntingtin gene currently available and in stock on the research market.

In October 2006, OriGene proudly released the longest cDNA clone yet isolated in the company's history. A clone of the Homo sapiens huntingtin gene (implicated in Huntington's disease, represented by the NCBI reference sequence NM_002111) was isolated, fully sequenced and annotated across the open reading frame (ORF), and delivered to a very satisfied OriGene customer. This clone, whose ORF is nearly 10,000 basepairs

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Coming Soon to OriGene...

New and improved GFC-Transfection Arrays

OriGene's GFC-Transfection Arrays (Genome-wide Full-length cDNA Transfection Arrays) are a revolutionary tool for high throughput functional screening by protein overexpression. GFC-Transfection Arrays provide a set of expression ready cDNA clones in a convenient 96 or 384-well format optimized for high throughput transfection and rapid screening. Clones are selected from OriGene's large selection of human cDNA full-length clones (TrueClones™) in the robust pCMV6 mammalian expression vector. Extending the variety of GFC-Transfection Arrays and improving their quality is an ongoing mission at OriGene. As part of this effort we are updating our manufacturing procedures and adding important quality control features. Moving to high quality midi-and maxi-prep plasmid DNA will help us deliver a higher standard of quality and quantity of the arrayed clones and reduce manufacturing variations. A new detailed application guide is now available with new and improved optimization schemes (http://www.origene.com/assets/Documents/GFC-Array/OTIProductManual-GFC-Array002.pdf).

Coming in February is the new Transcription Factor GFC-Transfection Array. The array contains 704 common transcription factor cDNAs in two 384-well plates. Each well contains a standardized amount (60 ng) of plasmid DNA. The procedure is very simple: just add your reporter, transfection agent and cells, then score the array after 48 hours.

The TF-GFC-Transfection Array serves as a useful tool for discovery, and as a great introduction to high throughput functional screening. Components included in this kit allow anyone to successfully enter the world of high throughput functional screening at a very reasonable price, and to make new discoveries at the same time. If you ever asked the question "What activates my gene?", GFC-Transfection Arrays provide the most biologically relevant answer in just 48 hours. Having a standardized, ready-for-screening array will save you time and money, and allow you to focus on new discoveries.

Also coming soon are the Kinase GFC-Transfection Array of 352 known kinases followed by the Transmembrane GFC-Transfection Array.

Upgraded Quantities on Select cDNA Clones

Many OriGene customers appreciate the convenience of our SC clones, a 10 ug quantity of transfection-ready DNA available for each of our TrueClones. This DNA need not be transformed and purified from single colonies before use in mammalian transfection experiments, saving you several days of valuable time. Soon OriGene will begin shipping free upgrades to the SC clone on many orders of TrueClones. This is OriGene's way of saying "thank you!" for your trust in us as your primary cDNA clone provider.

long, has a slightly shorter CAG repeat segment than the NCBI sequence (encoding three fewer glutamine residues) but otherwise has 100% identity to the reference protein sequence.

This achievement is a yet another landmark for OriGene, which is striving to cover the entire human genome with high quality cDNA clones. Providing a clone for such an enormous open reading frame hints at the quality of the cDNA libraries from which all OriGene clones are derived, and is also an indication of the mastery of cloning techniques which OriGene scientists employ every day. No other company in the world currently has this clone available.

Are you struggling to clone a long or complex cDNA? Why bother? OriGene takes the struggle of cloning out of your hands, freeing you to focus on more important issues and experiments in your lab. Simply place an order with OriGene for the human cDNA clone you're seeking, and get back to the rest of your project while OriGene finds this clone for you. With numerous cDNA libraries generated from high quality human tissue, OriGene is sure to find the clone you need, at a fraction of the time and money you'd spend to clone it yourself. Visit OriGene's website at http://www.origene.com/cdna/trueclone/ to find your clone today, or call our Customer Care and Technical Support professionals at 888-267-4436 or 301-340-3188 to discuss your cloning needs.

OriGene's cDNA clone for the huntingtin gene is now available to all customers as catalog number TC303123.

Web resources

Looking for more information? OriGene's website is just brimming with information to help you find out more about a cDNA clone, choose which product is right for your experiment, or learn more about how genomics tools are being used to answer important scientific questions.

Would you like to browse through an application guide before purchasing a product, or need an extra copy to keep in the lab? You can download these manuals at http://www.origene.com/support/product/product_manuals.mspx.

Have a question about an OriGene product or how it's used? Check out our FAQ section on the website at http://www.origene.com/support/faqs/.

Wondering if anyone has published experimental results using an OriGene product? Many citations of peer-reviewed journal articles using OriGene clones or other expression products are listed at http://www.origene.com/support/citations/.

Want to find an shRNA construct or cDNA clone for your gene of interest? You can enter the accession number or keyword into the search box at the top of every page on the OriGene website, or search by nucleotide or protein sequence for shRNA at http://www.origene.com/rna/search.mspx or for cDNAs at http://www.origene.com/cdna/search/.

Need to see the vector map for a cDNA clone or HuSH construct? These maps can be viewed at http://www.origene.com/cdna/trueclone/vectors.mspx for TrueClones or at http://www.origene.com/rna/vector_information.mspx for HuSH-29. Vector nucleotide sequence can also be downloaded from these pages.

Looking for insert size of a cDNA clone, or sequence information? Just type in the catalog number or accession number into the search box at the top of each page of the OriGene website (http://www.origene.com), and press "Enter". Then click on the catalog number that corresponds to your product of interest, and click on the orange "Details and Pricing" button. After entering a valid email address, you'll be directed to a page containing the product's list price and availability, related products, vector identity, insert size, end sequence information, and reference sequence information.

Would you like to have the sequence information or species specificity of your HuSH-29 constructs, but can't find where you left your Certificate of Analysis? That information is easy to find online. Just type in the catalog number or accession number into the search box at the top of each page of the OriGene website (http://www.origene.com), and press "Enter". Then click on the catalog number that corresponds to your product of interest, and click on the orange "Details and Pricing" button. After entering a valid email address, you'll be directed to a page containing the product's list price and availability, a list of controls and related cDNA variants, hairpin sequence information, and target species identification.

Upcoming conferences—check us out!

American Association of Cancer Research
Apr 14-18, 2007—Los Angeles, CA
Booth# 1272

RNAi World Congress
Apr 24-25, 2007—Philadelphia, PA

Experimental Biology Annual Meeting
Apr 28-May 2, 2007—Washington, DC
Booth# 338

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