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GeneDex

Editor: Michele Nealen

ALSO IN THIS ISSUE:

Verify your RNAi constructs with **OriGene's Validation System!**

There continues to be some unpredictability in the use of RNA interference (RNAi) for gene silencing, which often requires time-consuming effort to determine effectiveness, select the best constructs, and optimize use for knockdown applications. Testing requires the use of antibodies (which may not be available), consumable qPCR probes, expensive equipment, and/or phenotypic measurements.

To solve this problem, OriGene has developed the RNAi Validation System, a construct designed to incorporate a cDNA clone and a luciferase reporter gene as a chimeric transcript. This new tool will measure the effectiveness of RNAi constructs using nothing more than a luminescence plate reader. With the RNAi Validation System, one can quickly identify the most effective knockdown construct as well as optimal transfection conditions. High throughput application of this reporter system can be used to optimize experiments involving multiple genes and cell lines.

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ORIGENE
Your Gene Company

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HOW IT WORKS

Using unique restriction sites, the gene of interest is cloned into the validation vector 3′ to the firefly luciferase gene. The construct is then transfected into the mammalian cell of choice where a chimeric transcript is produced. Cotransfection of HuSH shRNA or another RNAi construct will initiate the RNA interference process. If the RNAi is effective, the chimeric firefly luciferase-target gene transcript will be degraded, reducing the luciferase activity. If the shRNA or siRNA has a low affinity for the target, the chimeric transcript will not be targeted and luciferase activity will be high. Reduction of the luciferase activity indicates specificity for the target, and effective gene silencing by the shRNA construct or siRNA oligonucleotide.

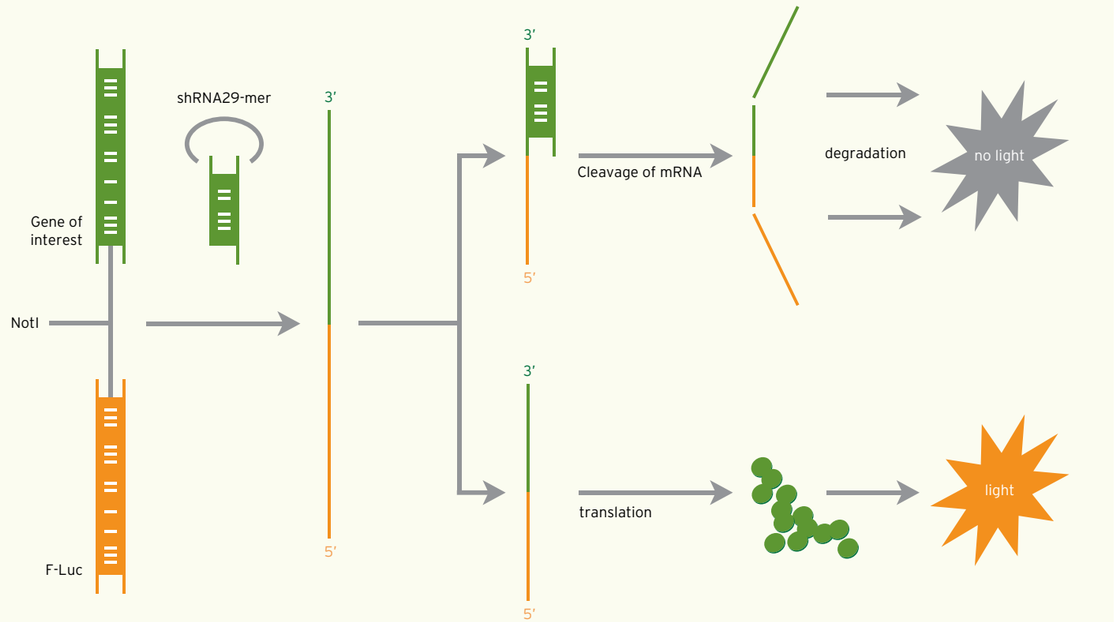
EASY TO USE

Any OriGene TrueClone can be subcloned into the RNAi Validation System with a simple Not I digestion and ligation. Any other cDNA can be subcloned into the validation vector using other restriction sites or appropriate adapter sequences. Since the RNAi Validation System uses firefly luciferase (rather than another luciferase gene) as the reporter, the substrate is affordable and commonly available.

HIGH THROUGHPUT APPLICATION

Because subcloning is so simple, this reporter system can be used to determine effective shRNA or siRNA constructs for multiple genes and cell lines in a single experiment.

RNAi VALIDATION SYSTEM



With the RNAi Validation Vector, one can quickly identify the most effective knockdown construct as well as optimal transfection conditions. High throughput application of this reporter system can be used to optimize experiments involving multiple genes and cell lines.

RELIABLE DETECTION

The initiation of RNA interference toward the gene of interest subcloned into the validation construct leads to degradation of the chimeric transcript (luciferase + gene of interest) which results in a detectable decrease in the luciferase activity, indicating a functional RNAi effect.

COMPATIBLE WITH ANY RNAi TECHNOLOGY

Currently there are three RNAi gene-silencing platforms that can trigger an RNAi response: short interfering RNA (siRNAs), short hairpin RNA (shRNA), and micro RNA (miRNA) constructs. The RNAi Validation System can measure the effectiveness of knockdown using any of these RNAi platforms.

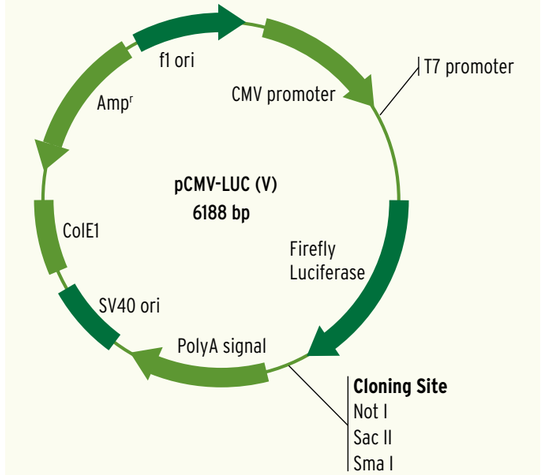
VECTOR DETAILS

Vector size: 6.2kb
Selection marker in E. coli: ampicillin
Selection marker in mammalian cells: None. For transient transfection only
Promoter for in vitro expression in mammalian cells: CMV promoter
Transcription termination and polyadenylation signals: from human growth hormone (hGH) gene.

FEATURES FOR pCMV-LUC(V) VECTOR

Start	End	Description
171	841	CMV promoter
983	988	T7 promoter
1067	2719	firefly luciferase
2729	2736	Not I
2733	2738	Sac II
2765	2770	Sma I
3409	3645	Human growth hormone polyA signal
3446	3753	SV40 promoter
3938	4610	ColE1 origin of replication
4755	5615	Beta-lactamase for ampicillin resistance
5764	6070	f1 ori

RNAi VALIDATION VECTOR



ORDERING INFORMATION

RNAi Validation System
TR30004 $200 USD

Validation vector:
1 vial of pCMV-LUC (V) vector, 5μg lyophilized DNA
Sequencing primer:
1 vial of LucVP3, 100 pmol lyophilized DNA
Controls:
1 vial of pCMV-LUC(V)-CASP1, 1 ug lyophilized DNA
1 vial HuSH-29 shRNA construct against CASP1, 1 ug lyophilized DNA
1 vial of pRS (TR20003), 1 ug lyophilized DNA

Find more information online at http://www.origene.com/rna/validation_vector.mspx

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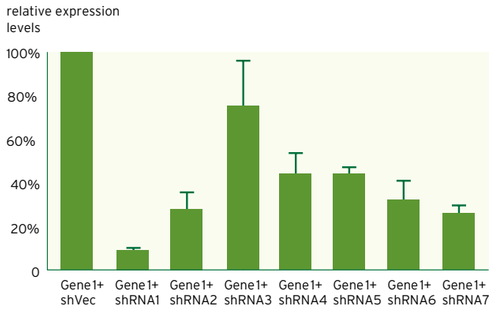
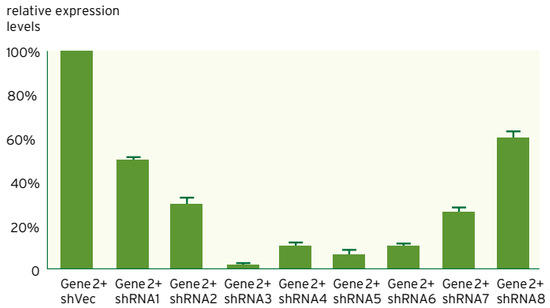
VALIDATION OF RNAi EFFECT USING THE pCMV-LUC(V) VALIDATION VECTOR

A.



B.



Target genes were cloned into the pCMV-LUC(V) vector in the sense orientation. Fifteen nanograms of these plasmids were cotransfected with 100 nanograms of a HuSH-29 shRNA construct (shRNA) or control (empty vector, shVec) into HEK293 cells using TurboFectin 8.0. Twenty-four hours post-transfection, cells were lysed using Perkin Elmer's luciferase substrate solution and the luciferase activity was measured with the PE Victor3 plate reader. Relative light units for each condition (triplicate wells) were measured, and are shown as percentage of expression when compared with cotransfection of the target gene and the empty pRS vector. A. Target gene 1 is NM_001363, B. Target gene 2 is NM_001797

Barry Westfall, B.S. and Weiqun Li, M.D.
OriGene Technologies, Inc.

Who doesn't want **something for free?**

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 TurboFectin or Magnetofection transfection reagents, HuSH-29 constructs, GFC-Transfection Arrays, or other OriGene products. If you are interested in such a collaboration, and would like to receive free reagents, arrays, or other products, 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.

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Send your email address to cDNA@origene.com, and we'll email you new issues of GeneDex as they are published. You'll receive all the updated information that GeneDex offers, with no impact on the environment.

RNAi Validation Vector Protocols

Subcloning your cDNA of interest into the validation vector

The full-length cDNA inserts in OriGene's TrueClones are flanked by two Not I sites. Not I specifically recognizes an uncommon eight base sequence; therefore, the majority of the TrueClone inserts can be released through Not I digestion without internal cutting of the insert. If an internal Not I site exists in the TrueClone insert, a complete Not I digestion will result in multiple fragments, unsuitable for subcloning directly. This situation would require either a partial digestion strategy or PCR subcloning (see instructions below).

Any cDNA clone can be used in the RNAi Validation Vector. If the restriction sites surrounding your clone aren't compatible with Not I subcloning, then a PCR subcloning strategy utilizing the Not I, Sac II, and/or Sma I restriction sites will be necessary. Perform restriction analysis of your DNA or cDNA to confirm that there are no internal restriction sites for the chosen enzymes before proceeding.

DIGESTING THE VALIDATION VECTOR, pCMV-LUC(V)

1. *Set up a digestion reaction using 1 ug DNA with Not I (or other enzymes as necessary) using the conditions recommended by the restriction enzyme provider.*
2. *Incubate at 37ºC for 90 min, or as recommended by the manufacturer.*
3. *If digesting with a single enzyme, add 0.5 uL DNase-free calf intestinal phosphatase (CIP; Promega #9PIM182) to the digestion reaction, and incubate at 37ºC for an additional 30 minutes.*
4. *Run the digestion reaction on a 1% agarose gel. Cut out the band corresponding to the 6.2 Kb linearized vector.*
5. *Use a gel purification kit to purify the linearized vector.*
6. *Estimate the recovered DNA quantity by A_{260} spectrophotometric analysis or by running 1 uL of the purified eluate on an agarose gel and comparing the band intensity to a reference such as DNA Quanti-Ladder (cat #QLD200).*
7. *Continue with the ligation and transformation protocol, below.*

NOT I DIGESTION OF cDNA CLONE (INSERT)

1. *Digest 0.5 - 1 ug donor plasmid DNA (such as an OriGene TrueClone) with Not I using the conditions recommended by the restriction enzyme provider.*
2. *Run the digestion reaction on a 1% agarose gel to separate the insert and vector fragments. Cut out the band corresponding to the cDNA insert.*
3. *Use a gel purification kit to purify the insert fragment.*
4. *Estimate the recovered DNA quantity by A_{260} spectrophotometric analysis or by running 1 uL of the purified eluate on an agarose gel and comparing the band intensity to a reference such as DNA Quanti-Ladder (cat #QLD200).*
5. *Continue with the ligation and transformation protocol, below.*

PARTIAL DIGESTION OF cDNA (INSERT)

Partial digestion uses limiting enzyme and incubation time to produce vector/clone fragments that are not completely digested. Some of the DNA will be cut at only one of the sites, some at multiple but not all sites, and some will be cut at all sites. In this case, you will be looking for digestion at two sites only—specifically, the two sites in the MCS but not internal to the insert.

To do a partial digestion, set up the following master mix:

10 uL 10X buffer
1 uL 100X BSA
1-4 ug DNA
up to 100 uL H_2O

1. *Aliquot this master mix to five tubes, containing 30 uL, 20 uL, 20 uL, 20 uL, and 10 uL, respectively.*
2. *To the first tube containing 30 uL, add 0.5 uL Not I enzyme (10U/uL). Pipet up and down to mix well.*
3. *Remove 10 uL from this tube, and add it to the next tube in the series. Pipet up and down to mix well.*
4. *Repeat steps 2 and 3 with all remaining tubes. Now you have 5 tubes of 20 uL digestion reactions with a serial dilution of enzyme.*
5. *Incubate for 15 minutes at 37ºC (instead of the usual 1-2 hours).*
6. *Run each digestion reaction separately on a 1% agarose gel, and look for the reaction in which you see only the full size of the insert (a partial digestion product which has left the internal restriction sites intact). Purify that fragment as the intact cDNA clone. Continue with the ligation and transformation protocol, below.*

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PCR SUBCLONING

Primer design

Design gene specific primers with restriction sequences at the ends. Consider the following recommendations when designing primers.

- *Use sequence complementary to your cDNA for 18-22 bases.*
- *Introduce the sequence for the 5′ restriction site at the 5′ end of the forward primer and the sequence for the 3′ restriction site at the 5′ end of the reverse primer.*
- *Include additional bases at the 5′ end of each primer to allow for efficient digestion by the restriction enzymes. Some of these extra bases can form a G/C clamp.*
- *Avoid too many Gs or Cs at the 3′ ends of either primer.*
- *Avoid long runs of any single nucleotide.*
- *Make sure that the forward and reverse primers don't form primer dimers (especially avoid complementary sequences at the 3′ ends) or significant secondary structure.*
- *Be certain to amplify the region of your gene that is targeted by your shRNA or siRNA constructs.*

Sample primers designed using this strategy for TP53 (NM_000546)

Forward oligo:

GC Clamp	Not I	Gene Specific
5′ GGCGC	AATA	GCGGCCGC	AAGTCTAGAGCCACCGTCCA 3′

Reverse oligo:

GC Clamp	Sma I	Gene Specific
5′ GGCCG	AA	GGGCCC	AAGCGAGACCCAGTCTCAAA 3′

PCR amplification

Assemble all of the following components on ice:

5 ng plasmid DNA containing your cDNA clone
5 uL 10X PCR buffer with Mg^{2+}
1 uL 10 mM dNTPs
20 pmoles forward primer
20 pmoles reverse primer
1 uL Pfu polymerase
up to 50 uL water

Annealing temperatures are dictated by the primer sequences; the temperatures given below are for the sample primers indicated above. You should determine the appropriate annealing temperature for your primers. When introducing new restriction sites at the end of the fragment, only the 3′ part of the primer matches the target so the first few annealing cycles should be done at a lower temperature than the subsequent annealing reactions. Calculate the Tm for the gene specific region only of each primer and allow the primers to anneal to the template at a temperature that is 5 degrees below the calculated Tm. For the subsequent cycles, calculate the Tm for the entire length of the primer. If it is greater than 70ºC, use an annealing temperature of 65ºC. For a Tm less than 70ºC, use an annealing temperature that is 5ºC below the calculated Tm. The length of the extension time is dictated by the length of the expected amplification product. Use 1 minute for every 1 Kb of length.

Sample cycling conditions for TP53 primers:

94ºC for 5 minutes

94ºC for 1 minutes
60ºC for 1 minute
72ºC for 2 minutes
Repeat once

94ºC for 1 minute
65ºC for 1 minute
72ºC for 2 minutes
Repeat 30-33 times

72ºC for 10 minutes

Purify the PCR product and digest with the restriction enzymes whose sites were added to the ends (Not I and Sma I in the above example). Purify the digestion product, and continue with the ligation and transformation protocol, below.

LIGATION AND TRANSFORMATION

Prepare a ligation reaction according to the following protocol:

1 uL 10x ligation buffer
10 ng purified digested pCMV-LUC(V) vector
10 ng purified digested insert from donor plasmid*
0.25 U ligase
up to 10 uL H_2O

* The optimal insert: vector molar ratio is about 2:1. Adjust the insert concentration accordingly, making sure to account for vector/insert sizes in the calculations.

The initiation of RNA interference toward the gene of interest subcloned into the validation construct leads to degradation of the chimeric transcript (luciferase + gene of interest) which results in a measurable decrease in the luciferase activity.

Incubate the tube at room temperature for two hours or 16ºC overnight (according to the manufacturer's recommendation).

Transform the mixture according to the directions of the manufacturer of the competent cells. A sample protocol for chemically competent cells is listed here.

1. *Add 3 to 5 uL of the ligation mixture to 50 uL of competent cells (efficiency rated > 10^8 cfu/ug DNA) on ice.*
2. *Mix the tube gently and incubate on ice for 20 minutes.*
3. *Heat shock the tube for 45 seconds at 42ºC.*
4. *Incubate the tube on ice for 2 minutes, then add 500 uL SOC (recovery medium).*
5. *Incubate the tube at 37ºC with agitation for 1 hour.*
6. *Spread 50 uL of the cells on an LB ampicillin- agar plate. You may also wish to plate larger amounts of this transformation mixture on separate LB-amp plates after pelleting the cells and resuspending in a smaller volume.*
7. *Incubate the plates at 37ºC for 16 hours to allow colony formation.*
8. *Pick single colonies and inoculate liquid LB-amp cultures; grow at 37ºC for 16 hours with agitation.*
9. *Perform DNA purification, and estimate the DNA quantity by A_{260} spectrophotometric analysis or by running 1 uL of the purified eluate on an agarose gel and comparing the band intensity to a reference such as DNA Quanti-Ladder (cat #QLD200).*
10. *If the ligation was not directional (e.g. used a single restriction site such as Not I to insert the cDNA), sequence several independent clones with the LucVP3 primer to confirm that the cDNA was inserted in the sense orientation with respect to the CMV promoter. If none of the clones contain cDNA in the sense orientation, screen a larger number of clones until you identify one that has been inserted correctly.*

Validating the RNAi effect: introduction of the validation construct and gene specific shRNA into mammalian cells via transfection

1. *Plate HEK293 cells at 3 x 10^3 cells/well/100 uL in a 96-well plate. Grow the cells overnight in a 37ºC, 5% CO_2 incubator to achieve 50% confluence.*
2. *In a small sterile tube, combine the following reagents in the prescribed order. The amounts given below are for triplicate wells.*

Serum-free medium (OptiMEM I)	30 uL
TurboFectin 8.0 transfection reagent	1.8 uL
Validation construct (pCMV-LUC (V) - cDNA)	45 ng in 1-3 uL
HuSH shRNA plasmid against the target gene	0.3 ug in 1-3 uL
or HuSH shRNA against luciferase, TR30002	0.3 mg in 1-3 mL

Note: Add TurboFectin 8.0 directly into the serum-free media.
Do **not** let TurboFectin 8.0 touch any plastic other than the pipette tip.

3. *Mix the tube contents gently. Do **not** vortex.*
4. *Incubate at room temperature for 15-45 minutes.*
5. *Add the DNA-TurboFectin mix to the triplicate wells of the tissue culture plate (10 uL in each of three wells) directly without removal of the culture media. Mix by gently rocking the plate.*
6. *Twenty-four to forty-eight hours post-transfection, add 15 uL of luciferase substrate to each well. The luminescent signal can be determined by a plate reader immediately and for up to 15 minutes with virtually no loss in sensitivity.*

Frequently Asked Questions...
about the RNAi validation vector

The pCMV-LUC (V) vector is appropriate for screening siRNA as well as shRNA constructs.

CAN I USE THE VALIDATION VECTOR WITH OTHER RNAi PRODUCTS LIKE siRNA OLIGOS?

Answer: The pCMV-LUC (V) vector is appropriate for screening siRNA as well as shRNA constructs. You must be certain to subclone into the validation vector the region of the cDNA targeted by your RNAi constructs; for example, if your siRNA targets the 5′ UTR, you must clone the cDNA including 5′ UTR into pCMV-LUC (V).

WHAT ADVANTAGES DOES THIS SYSTEM HAVE OVER OTHER SIMILAR REPORTER SYSTEMS?

Answer: Our vector is particularly suitable for the subcloning of OriGene's TrueClone cDNA clones, as Not I sites exist on both vectors. Moreover, our vector utilizes firefly luciferase for measurement of the effect; the substrate of firefly luciferase is readily and commercially available, unlike the substrate for other forms of luciferase.

DOES THIS REPLACE THE USE OF ANTIBODIES FOR WESTERN BLOT ANALYSIS OR qRT-PCR KNOCKDOWN DETECTION?

Answer: No. This vector is recommended as a prescreening method to choose the most effective construct with which to do your experiments. It is still advisable to perform Western blot analysis, qPCR, or functional assays to monitor the level of gene knockdown after you find the best knockdown construct using this screening method.

WHAT ADVANTAGES DOES THIS VECTOR OFFER IN THE DESIGN OF LARGE SCREENING ASSAYS?

Answer: If OriGene TrueClones are used in the design, one can subclone many genes into this vector in parallel using Not I. Bulk clone pricing is available; contact OriGene's Customer Service (custsupport@origene.com, 888-267-4436 or 301-340-3188) for more information.

DOES THIS VECTOR PRODUCE A LUCIFERASE – GENE FUSION PROTEIN?

Answer: No. Since the luciferase cDNA cloned into this vector contains its own stop codon, there will be no fusion protein of luciferase and your target gene produced. However, an intact luciferase protein will be translated if the chimeric transcript is not degraded due to RNAi against the target gene.

HOW DOES A GENE SPECIFIC shRNA OR siRNA END UP DEGRADING THE LUCIFERASE REPORTER IN THIS VECTOR?

Answer: The shRNA/siRNA forms a complex with DICER and the chimeric mRNA in the RISC in order to degrade the mRNA sequence. This RNAi effect extends in the 5′ direction, into the luciferase mRNA, leading to a decrease in luciferase enzymatic activity.

IS THE CHIMERIC TRANSCRIPT REALLY SIMILAR TO THE GENE I'M TRYING TO SILENCE?

Answer: In principal, the chimeric mRNA (containing luciferase and your target gene) should be similar to the endogenous version of your target mRNA. However, since the amounts of overexpressed mRNA may be much higher than endogenous mRNA, it may be more difficult to quantitatively knockdown your target gene as part of the chimeric mRNA compared to the endogenous target.

HAS THE VALIDATION VECTOR BEEN SHOWN BY OTHER MEANS THAT IT PROVIDES SPECIFIC QUANTIFIABLE KNOCKDOWN?

Answer: OriGene is currently testing this and we would also appreciate if you would share with us your own experimental results of this comparison. Please contact OriGene's Technical Support (techsupport@origene.com, 888-267-4436, 301-340-3188) for details about setting up such a collaboration.

CAN I SUBCLONE GENES THAT ARE NOT FROM THE ORIGENE TRUECLONE COLLECTION INTO THE VALIDATION VECTOR?

Answer: Yes, you can subclone any cDNA into this vector in order to determine the potential knockdown effect of shRNA/siRNA constructs. Please see the Protocols section in this manual for advice on subcloning cDNA into pCMV-LUC (V).

OTHER THAN NOT I, WHAT OTHER MCS RESTRICTION SITES ARE AVAILABLE FOR SUBCLONING?

Answer: Sac II and Sma I are also available at the MCS. Please confirm that these restriction sites do not exist in the internal region of the cDNA if you are using this subcloning strategy.

WHICH CLONING METHODS WORK BEST WITH THE pCMV-LUC (V) VECTOR?

Answer: Non-directional Not I subcloning or restriction and PCR-based directional subcloning are equally compatible with this vector. It is the customer's preference which to choose. Please note that non-directional cloning requires a screening step to confirm that the cDNA is cloned in the sense orientation with respect to the CMV promoter and luciferase cDNA.

HOW CAN I SUBCLONE A GATEWAY COMPATIBLE CLONE INTO THIS VECTOR?

Answer: Determine if there are any cloning sites in the Gateway vector compatible with pCMV-LUC (V). If not, use the PCR directional cloning strategy (see the Protocols section in this manual) to clone the Gateway cDNA into pCMV-LUC (V).

WHY MUST THE cDNA BE CLONED IN THE SENSE ORIENTATION TO VALIDATE MY shRNA OR siRNA?

Answer: The mechanism of RNA-induced transcript degradation depends on an enzyme complex called the RNA-induced silencing complex (RISC). The RISC complex contains an endoribonuclease that utilizes only the anti-sense strand of the trigger shRNAs (or siRNAs) to target the complementary mRNA sequence for degradation. A cDNA cloned in the anti-sense orientation will not produce a transcript that is complementary to the anti-sense sequence used by RISC and thus will not be degraded.

WHAT IS THE SEQUENCE OF THE PRIMER LUCVP3?

Answer: The nucleotide sequence of our sequencing primer is 5′ GGAAAACTCGACGCAAGAAA 3′. This primer anneals near the end of the firefly luciferase coding region, and will read through the MCS and into your subcloned cDNA so that you can confirm the orientation of the subcloned cDNA.

DO I HAVE TO COTRANSFECT THE shRNA AND THE VALIDATION VECTOR OR SHOULD THEY BE INTRODUCED TO THE CELLS SEPARATELY? WHICH WOULD BE BEST?

Answer: We recommend that you cotransfect both the pCMV-LUC (V) - cDNA construct and shRNA plasmids simultaneously. Commercially available transfection reagents, such as OriGene's Turbofectin 8.0 (catalog #TF81001), work very well for the cotransfection of cDNA and shRNA plasmids.

WHAT LUCIFERASE SUBSTRATE WOULD YOU RECOMMEND FOR USE WITH THE RNAi VALIDATION VECTOR?

Answer: We typically use Perkin Elmer's Ultra-High Sensitivity Luminescence Reporter Gene Assay System (No. 6016977).

ONCE I HAVE DETERMINED WHICH KNOCKDOWN CONSTRUCT WORKS BEST, SHOULD I KEEP OR DISCARD THE OTHERS?

Answer: One construct may work better in one cell line but be less efficient in another. If you change the cell type used in your experiment, it may be necessary to rescreen to find the best performer. The other constructs may also be useful for partial knockdown studies.

CAN THE VALIDATION VECTOR ITSELF INTERFERE WITH GENE EXPRESSION IN MY CELLS?

Answer: We do not have data to exclude this possibility. Thus, it is critical to include suitable negative controls in the cotransfection experiments.

WHAT CAN I CONCLUDE FROM SEEING POSITIVE RESULTS (REDUCTION OF LUCIFERASE ACTIVITY) USING THIS VALIDATION VECTOR?

Answer: A reduction in the activity of luciferase indicates successful targeting of the chimeric mRNA by your shRNA or siRNA construct(s). When screening a number of constructs, choose the one producing the greatest decrease in luciferase activity for use in future experiments.

DOES ORIGENE PROVIDE ANY GUARANTEE OF KNOCKDOWN IN SUBSEQUENT EXPERIMENTS WHEN AN RNAi CONSTRUCT HAS BEEN VALIDATED USING THIS METHOD?

Answer: No. The degree of knockdown of your target gene will be dependent on a number of factors, including transfection or infection method and efficiency, target cells, and transcript or protein half-life. For this reason, OriGene cannot guarantee knockdown using a pCMV-LUC (V) validated construct.

Magnetofection:
A Novel Method to Deliver Nucleic Acids into Hard-to-Transfect Cells

Delivering genetic material (DNA, RNA or oligonucleotides) into mammalian cells is an important technique used to affect cellular gene expression. The subsequently altered phenotype of and molecular interactions in the cells that harbor and express the exogenous genetic material can reveal important clues about the functions of the target gene.

Nucleic acid delivery is generally accomplished by one of the following methods:

Transfection: *Usually done by mixing nucleic acids with a cationic lipid or polymers to form liposomes, which subsequently fuse with the cells' plasma membranes and transport the nucleic acids inside.*

Electroporation or gene gun: *Uses an electric field pulse to induce microscopic pores in the plasma membrane, which allow nucleic acids to pass into the cells.*

Viral infection: *A "Trojan horse" strategy taking advantage of the natural process of viral infection of mammalian cells to deliver the nucleic acids as cargo into the target cell.*

The key issues in nucleic acid delivery are:
Delivery efficiency
Cell toxicity
Convenience
Biosafety

All the current methodologies have their intrinsic advantages and disadvantages. For many commonly studied cell lines such HeLa and Jurkat, the efficiency of transfection is still undesirably low. While more efficient, electroporation can cause high levels of cell death due to stress on the cell membrane. And while users of many primary cell lines have turned to viral infection to deliver nucleic acids, this involves additional packaging steps and requires a number of safety precautions due to the use of viral delivery systems. Despite continued efforts, the perfect delivery system still remains to be discovered.

Magnetofection is a new product in the nucleic acid delivery field that provides a novel alternative to the traditional transfection/infection methods. Magnetofection combines the advantages of nucleotide binding chemicals and physical transfection while eliminating the disadvantages of low efficiency and high toxicity. This is an attractive option for all cell lines or primary cells that are refractory to conventional transfection.

OZ Biosciences (http://www.ozbiosciences.com/home.html) is the leading source for Magnetofection reagents, and OriGene is a distributor of Magnetofection reagents to US customers. This article discusses the applications of this novel transfection method, and highlights the effectiveness of this reagent in many cell types.

Principle of Magnetofection

Magnetofection's key component is a solution of magnetic nanoparticles made of biodegradable iron oxide coated with cationic molecules specific for each application. When mixed with nucleic acids (plasmid DNA, siRNA, oligonucleotides or virus), the particles efficiently bind the material through colloidal aggregation and electrostatic interaction. This mixture of nucleic acids and magnetic particles is then added to the cells and a magnetic plate is placed under the cell culture plate. The "loaded" magnetic particles are concentrated onto cells by the magnetic force and enter the cells through endocytosis and pinocytosis. The nucleic acid cargo is released into the cytoplasm while the nanoparticles are retained in vacuoles without negative effects on cell function. Without the damaging effects of electric pulses or harsh chemicals, the cell membrane stays intact throughout the delivery process, minimizing cell toxicity.

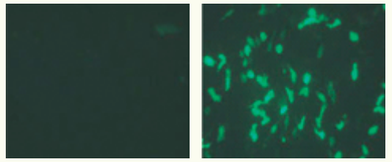
The interaction of the nucleic acid-coated magnetic particles and the magnetic field rapidly concentrates the nucleic acids at the cell surface, providing excellent opportunities for endocytic uptake.

Magnetofection combines the advantages of nucleotide binding chemicals with physical transfection, and is an attractive option for all cell lines or primary cells that are refractory to conventional transfection.

The Proven Benefits of Magnetofection

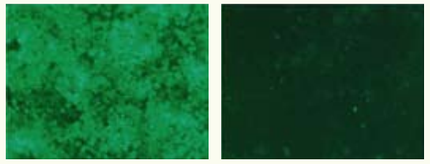
1. HIGH EFFICIENCY:
Significantly increases transfection or infection efficiency over that of standard reagents, even at very low doses of nucleic acids.

Overexpression
Confluent Primary Human Keratinocytes



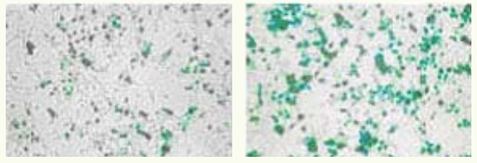
Standard Transfection | PolyMag MagnetoFection

Gene Silencing
HeLa cells, stably transfected with GFP



Control | SilenceMag 10nM siRNA

Overexpression
Confluent Primary Human Keratinocytes

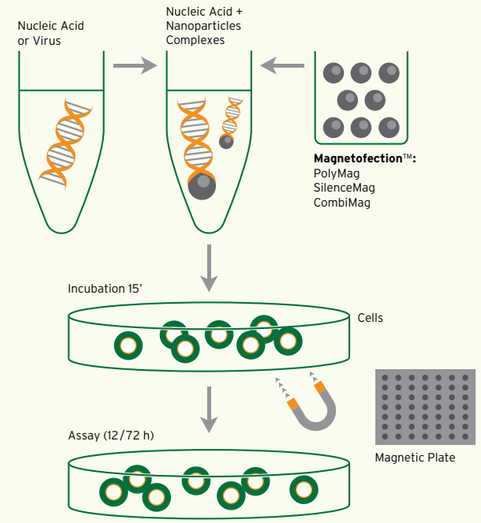


Reagent F alone | CombiMag+Reagent F

Viral infection
NIH 3T3 Cells lacking CAR receptor infected with an Adenovirus (coding for LacZ) and ViroMag

(L to R) Without magnetic field; With magnetic field

MAGNETOFECTION PROTOCOL: SIMPLE AND STRAIGHTFORWARD



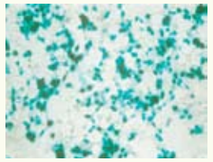
Magnetofection's key component is a solution of magnetic nanoparticles, which efficiently bind nucleic acids. The "loaded" magnetic particles are concentrated onto cells by magnetic force and enter the cells through endocytosis and pinocytosis.

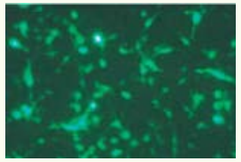
Continued on Page 12

Continued from Page 11

2. BROAD CELL RANGE:

Successfully transfects many primary cell lines and increases transfection efficiency in commonly used cell lines (see Table 1 on Page 15).





Primary Cells	Cell Lines
Chondrocytes (photo)	293
Peripheral Blood Lymphocytes	MCF-7
Aortic Endothelial Cells	HeLa
Human Fibroblasts	Hep G2
Nasal Epithelium	CHO-K1
Pulmonary Epithelium	PC12
and many more...	and many more...

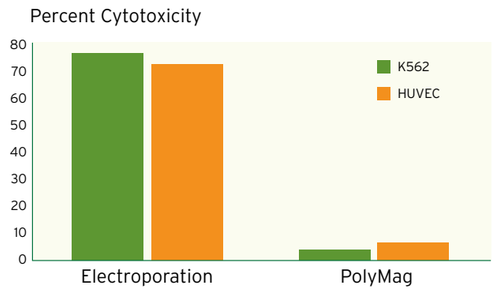
3. VERSATILITY:

Available as four types of ready-to-use Magnetofection reagents:

PolyMag, *designed for all nucleic acids*
SilenceMag, *specific for siRNA oligo delivery*
CombiMag, *designed to enhance efficiency of your transfection reagent*
ViroMag, *unique solution for viral applications*

4. LOW TOXICITY:

Application of magnetic nanoparticles and exposure to a magnetic field is significantly less damaging to the cells' membranes than electroporation, resulting in decreased cell death post-transfection.



Magnetofection is not only theoretically sound, it has been successfully tested in laboratories on a broad range of immortalized cell lines as well as primary cells.

5. SIMPLE PROCEDURE WITHOUT EXPENSIVE EQUIPMENT:

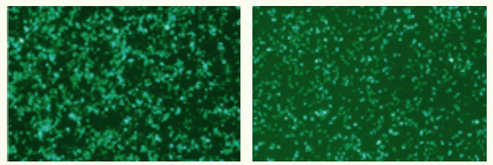
The only required accessory for Magnetofection is the magnetic plate to be placed under the cell culture vessel. The plate containing 96 individual magnets is suitable for all cell culture vehicles, including T75 flasks; 35, 60, or 100mm dishes; or 6-, 12-, 24-, 48- and 96-well plates.

A picture is worth a thousand words: a gallery of successful transfections

Magnetofection is not only theoretically sound, it has been successfully tested in laboratories on a broad range of immortalized cell lines as well as primary cells.

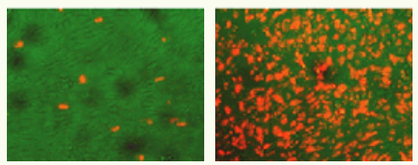
POLYMAG: HIGHLY EFFICIENT TRANSFECTION FOR VARIOUS APPLICATIONS

DNA: GFP Expression Vector



COS7 Hela

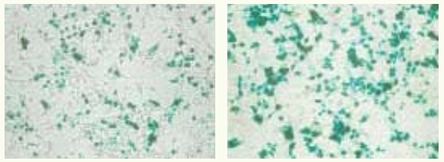
Oligonucleotides: HUVEC-C



Reagent E PolyMag

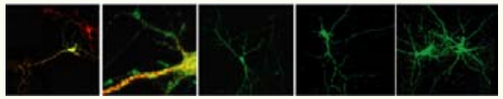
COMBIMAG: ENHANCES TRANSFECTION EFFICIENCY OF ALL TYPES OF NUCLEIC ACIDS BY COMPLEXING WITH ANY WORKING TRANSFECTION REAGENT

Primary Chondrocytes (Pig)



Standard Transfection (reagent F) MagnetoTransfection CombiMag +reagent

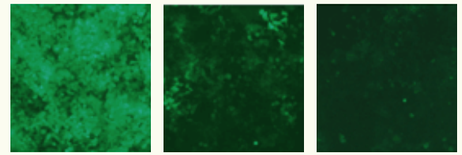
Primary Rat Hippocampal Neuron Transfection Reagent L + CombiMag



Application of magnetic nanoparticles and exposure to magnetic field is significantly less damaging to the cells' membranes than electroporation, resulting in decreased cell death post-transfection.

SILENCEMAG: THE MOST EFFICIENT siRNA DELIVERY SYSTEM FOR COTRANSFECTION OR ENDOGENOUS GENE SILENCING.

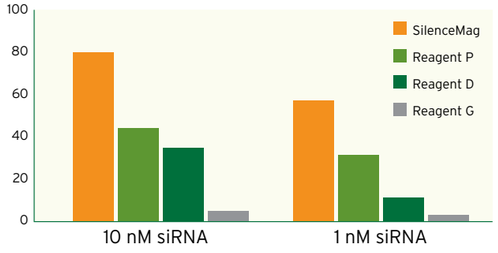
HeLa cells, stably transfected with GFP



Control SilenceMag 4nM siRna SilenceMag 10nM siRNA

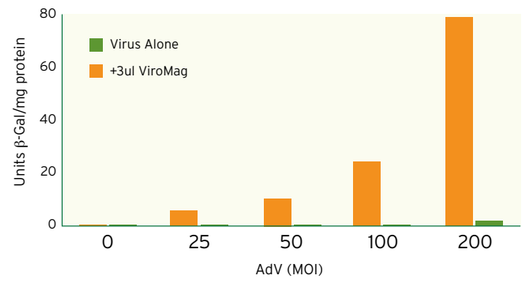
HUVEC Primary Cells Transfected with SilenceMag

Percent inhibition of GFP expression in HeLa cells transfected with siRNA against GFP



VIROMAG: PROMOTES VIRAL TRANSDUCTION EFFICIENCY, EVEN IN NON-PERMISSIVE CELLS.

Adenoviral Magnetofection on NIH3T3



Continued on Page 14

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Magnetofection reagents are a novel line of high efficiency, low toxicity delivery systems for DNA, RNA, oligonucleotides, or viruses. Whether used alone or in combination with your current transfection reagent, these products are certain to increase the transfection or infection efficiency of your target cells. No new protocols are required, and no additional safety measures are necessary. Try Magnetofection in your lab today, and see improved transfection efficiency tomorrow.

Magnetofection products available

STARTING KITS

(include the magnetic plate and small volume of the reagents)

Cat. Number *Product*	PolyMag Vol(µL)	CombiMag Vol(µL)	SilenceMag Vol(µL)	ViroMag Vol(µL)
TT100020 *Starting Kit* [1]	100	100		
TT100021 *siRNA Starting Kit* [2]			200	
TT100022 *Super Starting Kit* [3]	100	100	200	
TT100023 *ViroMag Starting Kit* [4]				200

[1] Contains 100µL of PolyMag, 100µL of CombiMag and the Magnetic Plate
[2] Contains 200µL of SilenceMag and the Magnetic Plate
[3] Contains 100µL of PolyMag, 100µL of CombiMag, 200µL of SilenceMag and the Magnetic Plate
[4] Contains 200µL of ViroMag and the Magnetic Plate

OriGene is a leading distributor of Magnetofection reagents to US customers.

REAGENTS FOR SPECIFIC APPLICATIONS

Cat. Number *Product*	Volume (µL)	Number of transfections in 24-well plate	Number of transfections in 96-well plate
TT100028 *Magnetic Plate*	-	-	-
TT100029 *PolyMag 100*	100	100	1000
TT100030 *PolyMag 200*	200	200	2000
TT100031 *PolyMag 1000*	1000	1000	10000
TT100039 *CombiMag 100*	100	100	1000
TT100040 *CombiMag 200*	200	200	2000
TT100041 *CombiMag 1000*	1000	1000	10000
TT100032 *SilenceMag 200*[5]	200	200	400
TT100033 *SilenceMag 500*[5]	500	500	1000
TT100034 *SilenceMag 1000*[5]	1000	1000	2000
TT100035 *SilenceMag 3000*[5]	3x1000	3000	6000
TT100036 *ViroMag 100*	100	10-100[6]	30-500[6]
TT100037 *ViroMag 200*	200	20-200[6]	60-1000[6]
TT100038 *ViroMag 1000*	1000	100-1000[6]	300-5000[6]

[5] Number of transfections given for a concentration of 10 nM siRNA.
[6] Number of transductions

For more information on Magnetofection products, visit our website at http://www.origene.com/cdna/transfection.mspx or contact our Technical Support specialists at techsupport@origene.com or 1-888-267-4436 (301-340-3188 outside the US).

TABLE 1: CELL LINES AND PRIMARY CELLS SUCCESSFULLY TRANSFECTED WITH POLYMAG OR COMBIMAG

Cell Line	Cell Type	Source	Notes	PolyMag	CombiMag
143B	Osteosarcoma	Human			OK
15P-1	Sertoli (Testis)	Mouse			OK
16HBE	Airway Epithelium	Human			OK
16HBE14o	Airway Epithelium	Human		OK	OK
181RDB	Pancreatic Cells	Human			OK
293	Embryonic Kidney	Human		85	90
293EBNA	Embryonic Kidney	Human		85	90
293T	Embryonic Kidney	Human		95	90
3T6	Embryonic fibroblast	Mouse		50	60
A-293	Transformed Embryonic Kidney	Human		85	90
A431	Epidermal Carcinoma	Human			50
A549	Non-small cell lung carcinoma	Human			65
AM-C6SC8	Kidney	Porcine			OK
AR42J	Exocrine pancreatic tumor	Rat		OK	
β-TC	Pancreactic Islet β cells	Mouse		OK	
B16F10	Melanoma	Mouse		50	60
B16F10 .9	Melanoma	Mouse		50	60
BEAS-2B	Bronchial epithelial cells	Human		45	60
BHK-21	Kidney	Hamster		75	80
BIU-87	Bladder cancer	Human		50	
BTK-143	Osteosarcoma	Human			OK
C2C12	Myoblast	Mouse			OK
C6	Glioma	Rat		OK	OK
CALU 3	Lung adenocarcinoma (epithelial)	Human		OK	OK
CHO	Epithelial-like (Ovary)	Hamster		85	90
CHO-K1	Epithelial-like (Ovary)	Hamster		85	90
CL7.1	Fibroblast	Mouse		20	25
Colo-205	Colon adenocarcinoma	Human			OK
COS-1	Fibroblast (Kidney)	Green Monkey		80	90
COS-7	Fibroblast (Kidney)	Green Monkey		80	90
CRFK	Kidney	Feline			OK
CT-26	Colon Carcinoma	Mouse		45	55
CV-1	Fibroblast-like (Kidney)	Monkey		OK	OK
DU-145	Prostate	Human			OK
ECV-304	Uroepithelium	Human			OK
EJ28	Bladder carcinoma	Human		OK	
F9	Embryonal teratocarcinoma	Mouse		OK	
FaDu	Pharynx carcinoma	Human		80	
GD25-β1	Embryonal fibroblast	Mouse			OK
H295R	Adrenocortical carcinoma	Human			OK
H4IIE	Hepatoma	Rat			OK
HaCaT	Immortalized Keratinocytes	Human		OK	50
HBL-100	Transformed Breast	Human			OK
HCT-116	Colon adenocarcinoma	Human			OK
HCT-15	Colon adenocarcinoma	Human			OK
HEK293	Embryonic Kidney	Human		85	90
HeLa	Cervix carcinoma	Human		50	60
HeLa-S3	Cervix carcinoma	Human			OK
Hep2	Laryngeal Epithelium	Human		60	60
Hep 3B	Liver Carcinoma	Human			OK
HepG2	Hepatoma	Human		OK	OK
HFF	Foreskin Fibroblast	Human		OK	OK
HMEC-1	Human Microvascular Endothelial Cell	Human		40	OK
hOSE	Ovarian epithelium	Human			50
HSG	Salivary gland epithelium	Human			OK
HT1080	Fibrosarcoma	Human		65	75
HT-22	Hippocampus	Mouse			70
HT-29	Colon carcinoma	Human			OK
HUVEC	Human umbilical vein endothelial cells	Human		DNA: 50 ODN: 90 siRNA: 98	90
Jurkat	Acute-T cell lymphoma	Human	1, 2		
K562	Myelogenous Leukemia	Human	1	OK	OK
KC	Embryonic	Drosophila		OK	
L6	Muscle cells	Rat		OK	
L929	Fibrosarcoma	Mouse		30	45
LLC-PK1	Kidney epithelium	Porcine			OK
LNCaP	Prostate Carcinoma	Human			OK
LoVo	Colon adenocarcinoma	Human			OK
LS174T	Colon adenocarcinoma	Human			OK
M-1	Kidney	Mouse		34	
MCF-7	Breast Adenocarcinoma	Human		40	50
MDCK	Normal -Kidney	Canine		20	25
MEF	Embryonic fibroblast	Mouse			OK
MeWo	Melanoma	Human			OK
mICc12	Intestine	Mouse			OK
MRC5	Lung Embryonic	Human		OK	OK
N2A	Neuroblastoma	Mouse		65	80
NCI-H82	Small cell lung carcinoma	Human			OK
NG108-15	Neuroblastoma-glioma fusion	Mouse-Rat			OK
NIH3T3	Fibroblasts	Mouse		60	75
NS20Y	Neuroblastoma	Mouse		70	80
OK, OK/NHE1	Kidney	Opossum			
OLN-93	Oligodendrocyte-oligodendroglia like	Rat			OK
P815	Mastocytoma	Mouse	1		OK
PC-12	Pheochromocytoma (adrenal)	Rat		OK	OK
PC3	Prostate carcinoma	Human			OK
Pre-B	Pre-B Lymphoma	Rat	1		15
PT-11	Kidney Fibroblast	Bovine			OK
RAW264.7	Monocytic-macrophage	Mouse	1		30
Rcho-1	Giant trophoblasts	Mouse			OK
SaOS	Osteosarcoma	Human			OK
SaOS-2	Osteosarcoma	Human			OK
SH-SY5Y	Neuroblastoma	Human		OK	OK
SK-MEL-28	Melanoma	Human			OK
SK-MES-1	Lung squamous carcinoma	Human		OK	
SKOV-3	Ovarian carcinoma	Human			OK
SM10	Placenta	Mouse			OK
Sp2/0	Myeloma	Mouse	1		OK
SW-480	Colon adenocarcinoma	Human			OK
THP-1	Myelomonocytic	Human	1		OK
tsA201	Embryonic kidney	Human			OK
U87	Glioma-astrocytoma	Human		70	25
U937	Pleural effusion lymphoma	Human	1	OK	OK
V79	Lung Fibroblasts	Hamster			OK
Vero	Kidney	Green Monkey		OK	OK
VSa13	Bone-derived	Fish		OK	OK
VSa16	Bone-derived	Fish		OK	OK
Y79	Retinoblastoma	Human	1		OK

Primary Cells		Source	Notes	PolyMag	CombiMag
	Airway epithelium	Human, Porcine			
	Carotid Artery Smooth Muscle	Bovine		OK	
	Chondrocytes	Porcine		65	80
	Chromaffin	Bovine			OK
	Cytotrophoblastic				OK
	Dendritic	Human		OK	
MEF	Embryonic Mouse Fibroblasts	Mouse			OK
ES	Embryonic Stem Cells	Mouse			20
EMC	Epicardial Mesothelial Cells	Rat			OK
	Fibroblasts	Human			OK
	Fibrochondrocytes	Porcine		OK	OK
	Glial	Human, Mouse			
	Hippocampal neurons	Mouse, Rat			10
HDF	Human Diploid Fibroblast	Human			OK
HFF	Human Foreskin Fibroblast	Human		OK	OK
HUVEC	Human umbilical vein endothelial cells	Human		DNA: 50 ODN: 90 siRNA: 98	90
	Keratinocytes	Human, Mouse			
	Nasal Airway epithelium	Human		OK	OK
	Neurons	Mouse, Rat			OK
PAEC	Primary Aortic Endothelial Cells	Bovine, Human			40
PBL	Peripheral Blood Lymphocytes	Human, Mouse	1		OK
RHC	Rat Hepatocyte Cells	Wistar Rats			OK
SMC	Smooth Muscle Cells	Porcine			OK
	Stroma Endotrium	Human		OK	
	Trophoblastic	Human			OK

Notes
When available, percent transfection efficiency is indicated. "OK" indicates that cells were successfully transfected but the percentage of transfected cells was not determined.
1 Suspension Cells
2 With Jurkat cells, transfection efficiency is highly variable due to differences among Jurkat cells (clone, wild-type, transformed etc...)

Genes in the News

INTEGRATIVE GENOMIC APPROACHES IDENTIFY IKBKE AS A BREAST CANCER ONCOGENE

Jesse S. Boehm, Jean J. Zhao, Jun Yao, So Young Kim, Ron Firestein, Ian F. Dunn, Sarah K. Sjostrom, Levi A. Garraway, Stanislawa Weremowicz, Andrea L. Richardson, Heidi Greulich, Carly J. Stewart, Laura A. Mulvey, Rhine R. Shen, Lauren Ambrogio, Tomoko Hirozane-Kishikawa, David E. Hill, Marc Vidal, Matthew Meyerson, Jennifer K. Grenier, Greg Hinkle, David E. Root, Thomas M. Roberts, Eric S. Lander, Kornelia Polyak, and William C. Hahn

Cell 2007;129:1065–1079.

IKBKE (IKK€) was recently identified as a novel oncogene implicated in the development of breast cancer through studies by a collaboration of scientists from the Dana-Farber Cancer Institute, Brigham and Women's Hospital, and the Broad Institute of Harvard and MIT. This discovery is significant not only because of its connection with a disease that strikes over two hundred thousand new patients yearly and commands millions of federally and privately funded research dollars, but because of the novel approach used to make this discovery.

Immortalized cells can be rendered tumorigenic by the introduction of H-RAS^{V12} (Hahn et al., 1999). A screen was established to determine what downstream elements of the Ras pathway could also trigger this transformation. Mutant alleles of three members each of the MAPK and PI3K pathways were coexpressed in all possible combinations, and two combinations of mutant alleles (BRAF^{E600} or MEK^{DD} and myr-AKT) were shown to stimulate tumor growth to the same degree as Ras hyperactivation both in vitro and in vivo. This validated screen was then used to determine what other elements of these pathways could substitute for myr-AKT and also cause tumorigenicity.

An expression library of kinases was functionally tested for the ability of each protein to effectively substitute for myr-AKT by inducing transformation in non-tumorigenic cells that contained a mutation in the MAPK pathway. This approach successfully identified four kinases that cooperate with activated MEK1 to replace H-RAS^{V12} in human cell transformation. When co-expressed with MEK^{DD}, each of these four kinases produced anchorage-independent growth in human cells and tumor formation in immunodeficient mice – the classic hallmarks of an oncogene.

To validate the involvement of these four kinases in human cancer, copy number analysis was performed to look for amplification of these genes in 179 cell lines representing many forms of cancer. While three of these kinases were not found to be amplified in cancer lines, IKBKE copy number increases were found in over 16% of all breast cancer cell lines examined. Amplification of IKBKE was also found in over 33% of the breast cancer biopsy samples studied. Forty percent of early stage biopsies showed evidence of IKBKE amplification, indicating that this aberration is an early event in transformation. IKBKE nucleotide mutations were not found in the breast cancer biopsies or cell lines used, indicating that overexpression of IKBKE (both due to gene copy number increase and independent of it) is the method of deviation. Overexpression of the wild type (unmyristoylated) form of IKBKE also produced a transforming effect on cells. This data identified IKBKE as an important kinase that is amplified or overexpressed both in breast cancer cell lines and primary surgical samples of breast cancer.

RNAi directed against IKBKE in several breast cancer cell lines reduced viability and proliferation, showing that IKBKE causes the condition of "oncogene addiction" in these cell lines. Oncogene addiction is the phenomenon in which a cell line requires increased levels of expression from a particular gene for normal growth and proliferation. IKBKE was also shown to stimulate the NFκB pathway, indicating that transformation via IKBKE is accomplished via NFκB and its downstream elements in both transformed cell lines and primary samples of breast cancer.

Gain of function screens such as the kinase library screen used in this paper to identify IKBKE as a breast cancer oncogene are important tools in the cancer researcher's arsenal. Candidates for therapeutic development can be merely identified as associated with the incidence of disease, but candidates shown to have functional consequences in the affected pathway are higher value targets. OriGene's GFC-Transfection Array for protein kinases and similar products (see http://www.origene.com/cdna/gfc-array/ for more details) stand ready to help you identify the next gene to make headlines. These arrayed cDNA libraries are ideal for functional genomic screens of a gene family, the entire genome, or a custom subset of the genome. Contact OriGene today to find out how GFC-Transfection Arrays can put you on the road to identifying the next IKBKE.

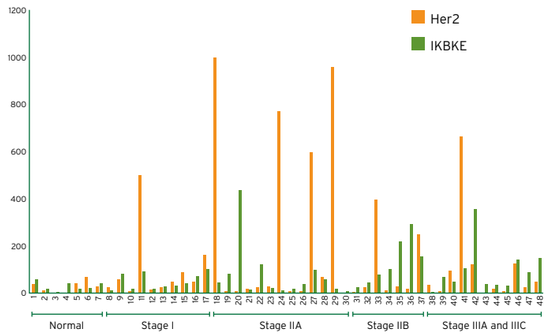
Hahn, W.C., Counter, C.M., Lundberg, A.S., Beijersbergen, R.L., Brooks, M.W., and Weinberg, R.A. Creation of human tumour cells with defined genetic elements. Nature 1999;400:464–468.

Validating a newly discovered oncogene **in no time flat**

One of the most convenient products for validating a gene identified in a microarray or library screen is the TissueScan panel. For each panel, forty-eight independent tissue samples have been processed and used to generate first strand cDNA, which is then normalized and aliquotted to individual wells of a real-time PCR plate. The contents of this plate are lyophilized and ready to use; all you need to add is a PCR master mix and your gene-specific primers. OriGene scientists read with interest the report of IKBKE as a novel oncogene, and wondered if that designation would be supported by data from our TissueScan Oncology panels. Using PCR primers designed to amplify a short region of the IKBKE gene, OriGene looked for IKBKE expression across normal tissues and samples representing stage I through stage IV breast cancer. As shown by the data in Figure 1, not only is IKBKE upregulated in human breast cancers, but the pattern of upregulation is unique from that of Her2. IKBKE could well represent a novel mechanism of breast cancer development, and a unique target for therapeutic treatment.

TissueScan Oncology qPCR experiments show that IKBKE is upregulated in human breast cancers, in a pattern unique from that of Her2.

A. Breast Cancer Panel I



B. Breast Cancer Panel II

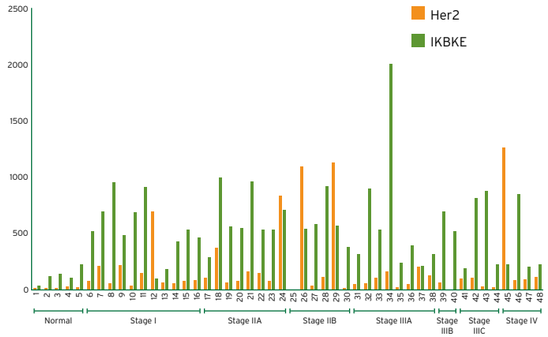


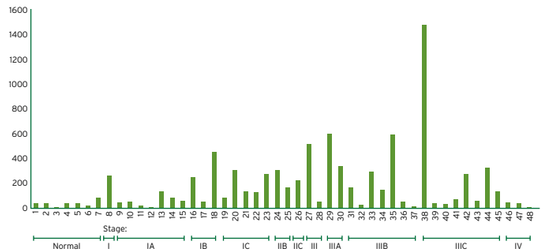
Figure 1. Relative levels of IKBKE and Her2 gene expression across forty-eight normal tissues and breast cancer samples. TissueScan Oncology Breast Cancer panels were interrogated via SYBR green real-time PCR reactions using primers designed to amplify either the Her2 transcript or IKBKE. Both genes show higher levels of expression in cancer biopsy samples than in normal tissues. (Tissue pathology information for each sample is available at http://www.origene.com/geneexpression/). A, Breast Cancer I panel (OriGene #BCRT101). B, Breast Cancer II panel (OriGene #BCRT102).

Continued on Page 18

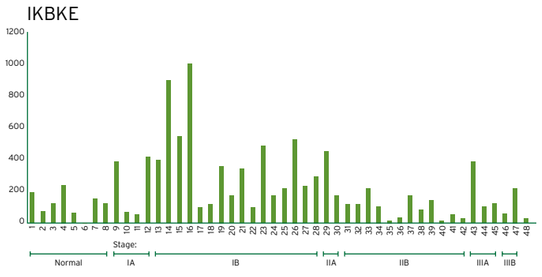
Continued from Page 17

As some oncogenes have been found to play a role in multiple types of cancer, other TissueScan Oncology panels were also examined for IKBKE expression. Using a simple real-time PCR reaction as above, OriGene scientists looked for IKBKE gene expression changes in ovarian, lung, and prostate cancer tissue samples. Figure 2 shows evidence of upregulation of IKBKE in ovarian cancer samples, upregulation of IKBKE in early stage lung cancer, but no distinct pattern of expression level changes in prostate cancer samples.

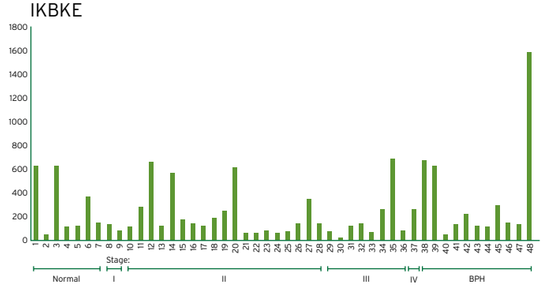
A. Ovarian Cancer Panel



B. Lung Cancer Panel



C.Prostate Cancer Panel



TissueScan Oncology experiments provided evidence of upregulation of IKBKE in ovarian cancer samples and in early stage lung cancer.

Figure 2. Relative levels of IKBKE gene expression across normal tissues and cancer samples. TissueScan Oncology panels were interrogated via SYBR green real-time PCR reactions using primers designed to amplify IKBKE. (Tissue pathology information for each sample is available at http://www.origene.com/geneexpression/). A, Ovarian Cancer panel (OriGene #HORT101). B, Lung Cancer panel (OriGene #HLRT101). C, Prostate Cancer panel (HPRT101).

Each TissueScan Oncology experiment takes about two hours (excluding primer design and synthesis). There isn't a quicker way to test your theory about gene expression changes in cancer progression, or confirm the results of your library screen. And at less than $8 per sample, there couldn't be a more cost-efficient way to obtain clinical specimens, processed for RNA and cDNA, and ready for analysis of relevant expression changes.

Want to know more about this product? Visit our website at www.origene.com/geneexpression/disease-panels/products.mspx, or call OriGene at 888-267-4436 (301-340-3188 outside the US).

What can **GFC-Transfection Arrays** do for you?

A FUNCTIONAL GENOMICS APPROACH TO THE MODE OF ACTION OF APRATOXIN A

Hendrik Luesch, Sumit K. Chanda, R. Marina Raya, Paul D. DeJesus, Anthony P. Orth, John R. Walker, Juan Carlos Izpisua Belmonte, and Peter G. Schultz.

Nature Chemical Biology 2006 2:158-167.

This paper describes the use of a custom GFC-Transfection Array from OriGene Technologies to identify the unknown targets of the anti-tumor agent apratoxin A. This bioactive cyanobacterial metabolite can be isolated from the coral reefs surrounding the Pacific islands of Guam and Palau[1,2] and has significant oncologic therapeutic potential. Functional screening of a GFC-Transfection Array is a valid and convincing technology for determining the mechanism of action of other potential therapeutic agents derived from natural sources. GFC-Transfection Arrays are provided in a convenient format for high-throughput functional screening, and can be used for a wide variety of assays and reporter platforms.

Analysis of a panel of NCI tumor cell lines indicated differential cytotoxicity of the agent apratoxin A. The characteristics of its cytotoxicity profile are not common to any other agent, indicating a potentially unique mechanism of action. Microarray studies compared the transcriptional profiles of cells treated with apratoxin A to those of untreated cells. Over one hundred genes were differentially expressed; most significantly, stress-related genes were upregulated and genes regulating cellular metabolism and cell cycle control were downregulated. This differential expression profile indicates that treatment with apratoxin A would likely disrupt cell cycle progression, causing G1 arrest. In vitro studies supported the theory that the apparent cause of apratoxin A-induced cytotoxicity is induction of an anti-proliferation response.

To identify the mechanism of apratoxin A's anti-proliferative effect, the U2OS osteosarcoma cell line was used in a reverse transfection assay on a custom GFC-Transfection Array, a panel of cDNA clones in a uniform expression vector arrayed in 96 well or 384 well plates. This assay served as a functional screen to identify which cDNAs were capable of blocking the apoptotic effects of apratoxin A. Those cDNAs showing anti-apratoxin A effects were rescreened individually, then tested to eliminate those that non-specifically block apoptosis or cause apratoxin A-specific cell cycle arrests. Of the twenty-two cDNAs resulting from these screens, five were identified as fibroblast growth factor receptors (FGFRs); others were transcription factors and ras oncogene homologs.

FGFR1 and FGFR3 were also found to be overexpressed in cell lines that demonstrate resistance to apratoxin A cytotoxicity. Treatment with apratoxin A was shown to prevent phosphorylation of STAT3, a transcription factor in the FGFR cascade. Overexpression of STAT3 blocks the antiproliferative effects of apratoxin A; therefore, inhibition of STAT3 should mimic apratoxin A action. siRNA against STAT3 did indeed decrease viability, supporting the notion that apratoxin A inhibits FGF signaling by antagonizing STAT3 activity.

In vivo studies of tube formation in HUVECs showed that apratoxin A can inhibit angiogenesis in this model system. Zebrafish embryos treated with apratoxin A showed evidence of reduced mkp3 expression and severe deformations of the fin, a developmental process known to be regulated by the FGFR cascade. This work indicates that apratoxin A is a promising candidate for antitumor therapy, especially for cancers known to have increased activity of the FGFR cascade.

High-throughput gain-of-function screening is an excellent method for identifying the genes involved in a cellular process of interest. Rather than screening individual genes or investing in expensive robotic equipment, scientists can perform such screens with GFC-Transfection Arrays, which minimize the time and effort to obtain accurate, reproducible data. To see a list of available GFC-Transfection Arrays, visit the OriGene website at http://www.origene.com/cdna/gfc-array/. To inquire about custom arrays such as those used in the apratoxin A study, contact OriGene at techsupport@origene.com or 888-267-4436 (outside the US, dial 301-340-3188).

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Citations

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