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OriGene RNAi Target Validation System

Description
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, phenotypic measurements or expensive equipment.

To solve this problem, OriGene has developed the **RNAi Target Validation System**, a vector designed to incorporate a cDNA clone and a luciferase reporter gene as a fusion construct. This new tool will measure the effectiveness of RNAi constructs using nothing more than a luminescent plate reader. With the RNAi Target 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.

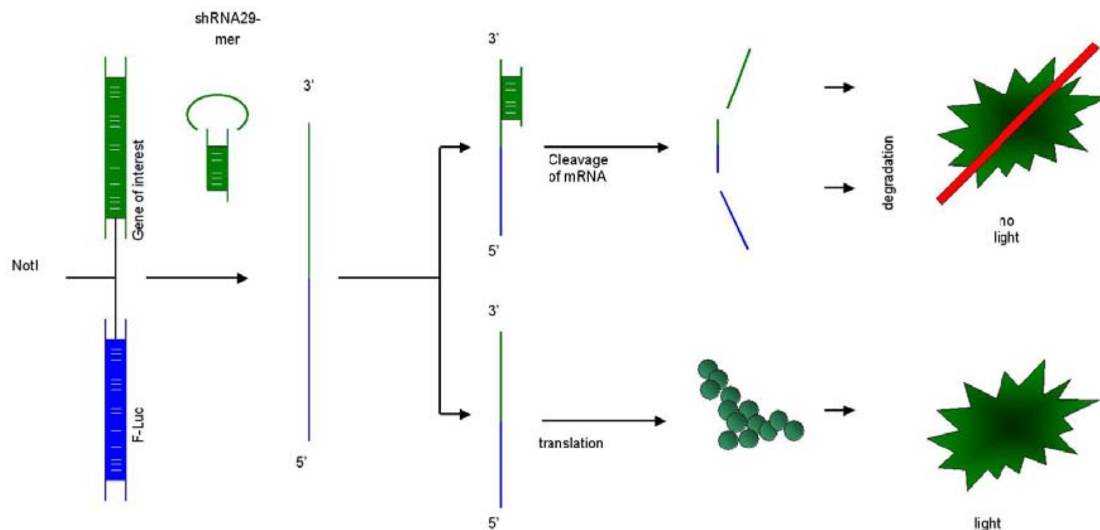
Package Contents and Storage Conditions

Each kit comes with the following five (5) components:
Validation vector:
1 vial of pCMV-LUC (V) vector (TR30004), 5 µg 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 HuSH29mer shRNA construct against CASP1, 1 ug lyophilized DNA
1 vial of pRS (TR20003), 1 ug lyophilized DNA

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 fusion transcript mRNA is transcribed. Cotransfection of HuSH shRNA or other RNAi will initiate the RNAi process. If the RNAi is effective, the fused firefly luciferase-target gene mRNA will be degraded, reducing the measurable luciferase levels. If the shRNA has a low affinity for the target, the fusion transcript will not be degraded and measurable luciferase will not be reduced. Reduction of the luciferase signal indicates specificity for the target, and effective gene silencing by the shRNA construct.



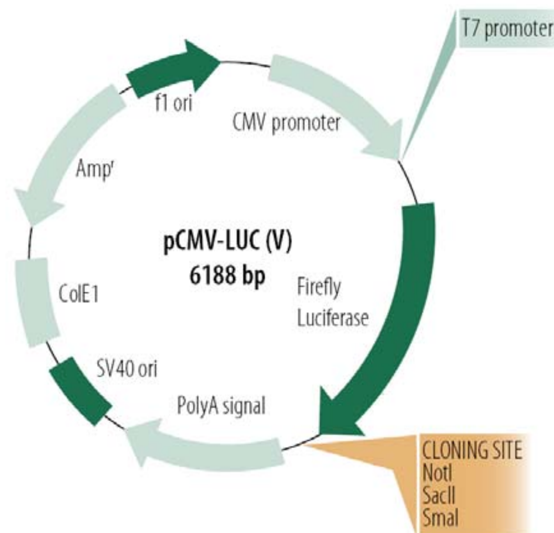
Easy to use
Any OriGene TrueClone can be subcloned into the RNAi Target Validation System with a simple not I digest and ligation. Any other cDNA can be subcloned in the validation vector using other restriction sites or appropriate adapter sequences.

High throughput application
Because subcloning is so simple, this reporter system can be used for multiple genes and cell lines in a single experiment.

Reliable detection
The initiation of RNA interference toward the gene of interest subcloned into the validation construct leads to degradation of the fusion mRNA (luciferase-gene of interest) which results in a measurable decrease in the luciferase signal, indicating a functional RNAi effect. Since the RNAi Target Validation System uses firefly luciferase as the reporter, the substrate is affordable and commonly available.

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 Target Validation System can measure the effectiveness of knockdown using any of these RNAi platforms.

RNAi Target Validation Vector



The basic backbone of the vector.

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

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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

SAMPLE DATA GENERATED USING THE RNAI TARGET VALIDATION SYSTEM:

Validation of Gene 1 shRNA Effect Using pCMV-Luc (V) Vector

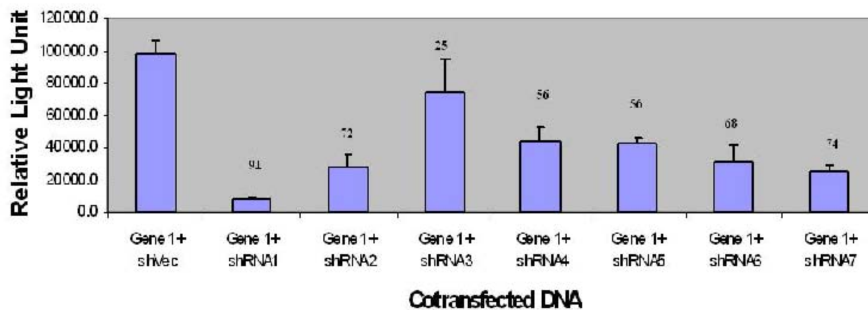

Fig 1. Target gene 1 (NM_001363) was cloned into the pCMV-Luc(V) in the sense orientation. Fifteen ng of this cDNA was co-transfected with 100 ng of each shRNA construct cloned in OriGene's pRS vector into HEK293 cells using TurboFectin 8.0. Twenty four hrs post transfection, cells were lysed using Perkin Elmer's Luciferase substrate solution and the luciferase activity was measured with PE Victor 3 Station. Relative light unit of each triplicate wells is shown for each condition together with SD bar. Numbers on the top of each bar represent the percentage of inhibition when compared with the number derived from co-transfection of target gene 1 with the pRS vector.

Validation of Gene 2 shRNA Effect Using pCMV-Luc (V) Vector

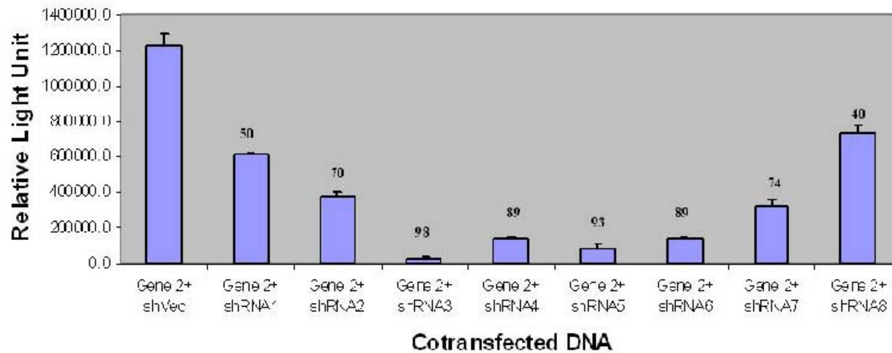

Fig 2. Target gene 2 (NM_001797) was cloned into the pCMV-Luc(V) in the sense orientation. Fifteen ng of this cDNA was co-transfected with 100 ng of each shRNA construct cloned in OriGene's pRS vector into HEK293 cells using TurboFectin 8.0. Twenty four hrs post transfection, cells were lysed using Perkin Elmer's Luciferase substrate solution and the luciferase activity was measured with PE Victor 3 Station. Relative light unit of each triplicate wells is shown for each condition together with SD bar. Numbers on the top of each bar represent the percentage of inhibition when compared with the number derived from co-transfection of target gene 2 with the pRS vector.