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1453 N. Cuyamaca St, El Cajon, CA 92020
Phone: (866)-620 4018, (619)-562-1518
Fax: (619)-562-1326 • info@targetingsystems.net
www.targetingsystems.com

Targeting Systems Introduces :

New Products

Innovative tools for studying gene expression

New Products : psiScreen system- Gaussia luciferase-based expression vectors and assay kits for rapid and quantitative evaluation of gene silencing.

The psiScreen Vectors are designed to provide a rapid, quantitative approach for evaluation and optimization RNA interference (RNAi). These vectors enable monitoring of changes in expression of a target gene fused to a novel luciferase reporter gene. In both vectors, Gaussia luciferase is used as a primary reporter gene, and the target gene of interest can be cloned into multiple cloning sites located downstream of the translational stop codon of the luciferase gene. Transfection of cells with the psiScreen vectors results in the production of an mRNA in which the mRNA encoding Gaussia luciferase is expressed as a fusion with the mRNA encoding the target gene. Initiation of the gene silencing occurs when co-transfection of siRNA towards the target gene of interest results in cleavage and subsequent degradation of fusion mRNA. Measurement of decreased Gaussia luciferase activity serves as an indicator of RNA interference. Since the Target gene is sub cloned after the stop codon of Gaussia luciferase, the activity of Gaussia luciferase is unaffected by the fusion partner.

Components of the psiScreen system

Gaussia Luciferase-based expression vector and reagents for siRNA screening

Catalog #	Capacity	Price
#S001	25 µg psiScreen, plus GAR-1 reagent (100 assays)	$420
#C001	pCMV-Fluc (25 µg)	$200

Gaussia Luciferase RNAi Assay Kit

Product cord/code	Capacity	Price
GAR-1	1000 assays	$350
GAR-2	1000 assays	$350

Advantages

An easy method to screen a wide variety of siRNAs for gene silencing :

The effectiveness of different siRNAs to silence the target gene of interest is evaluated quantitatively simply by measuring the luciferase activity.

Simplicity :

Gaussia luciferase is a secreted into the media. It is therefore necessary to only assay cell supernatants for luciferase activity without the need for lysing the cells. Considerable time is saved since time course experiments can be performed using the same group of transfected cells without lysing at each time point.

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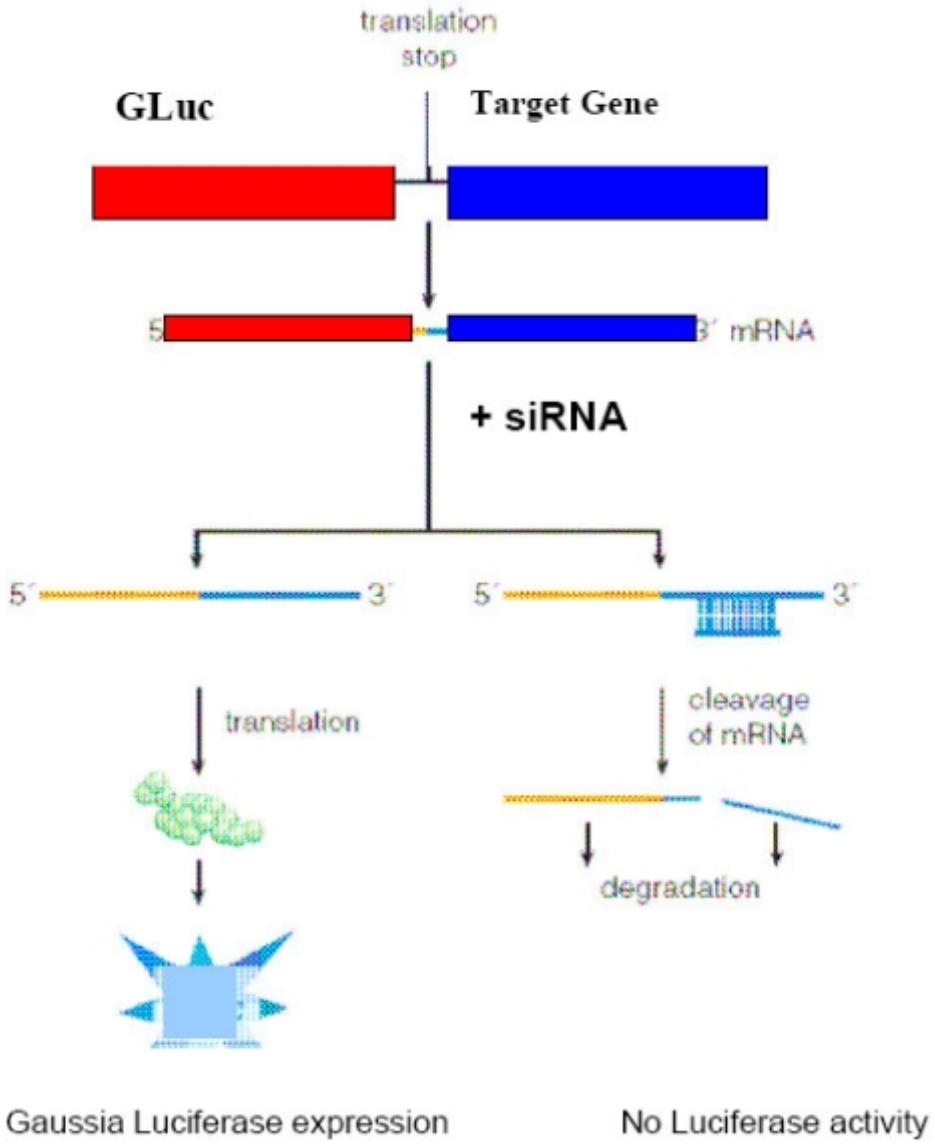
Sensitivity :

Gaussia luciferase, a thermostable enzyme, is 1000 times brighter than Renilla and firefly Luciferase thus increasing sensitivity of the assay.

Convenience of an easy-to use assay kit :

Using the GAR reagents, a simple one-step reporter assay is possible.

The GAR-2 reagent has stabilizing components which stabilize the bioluminescent signal enabling the system to be useful for high throughput screening applications



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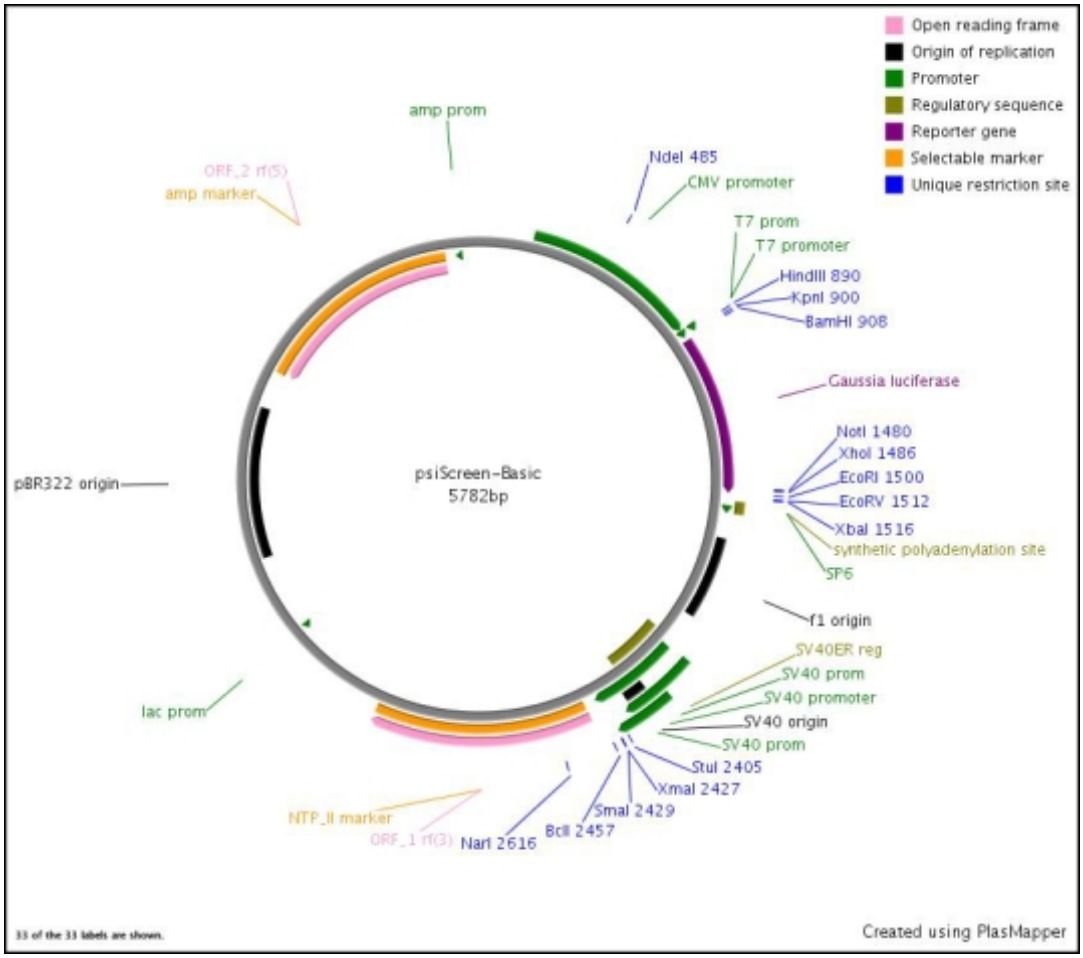
The psiScreen vector

The psiScreen vector has a multiple cloning site after the stop codon of the Gaussia luciferase gene



Features of the psiScreen-Basic vector :

- pCMV-GLUC-1 (5764 bp)
- CMV promoter bases: 209-863
- Gaussia luciferase gene: 907-1497
- T7 promoter bases: 864-882
- Polylinker bases: 889-907
- SP6 promoter: 1513-1530
- Synthetic polyadenylation site: 1497-1541
- SV40 promoter bases: 2082-2417
- SV40 origin of replication: bases 2196-2281
- Neomycin ORF : bases 2453-3247
- SV40 PolyA: bases 3302-3674
- ColE1 origin: bases 3934-4607

The sequence of the psiScreen vector can be found at our website www.targetingsystems.com
And also on the last page of this brochure

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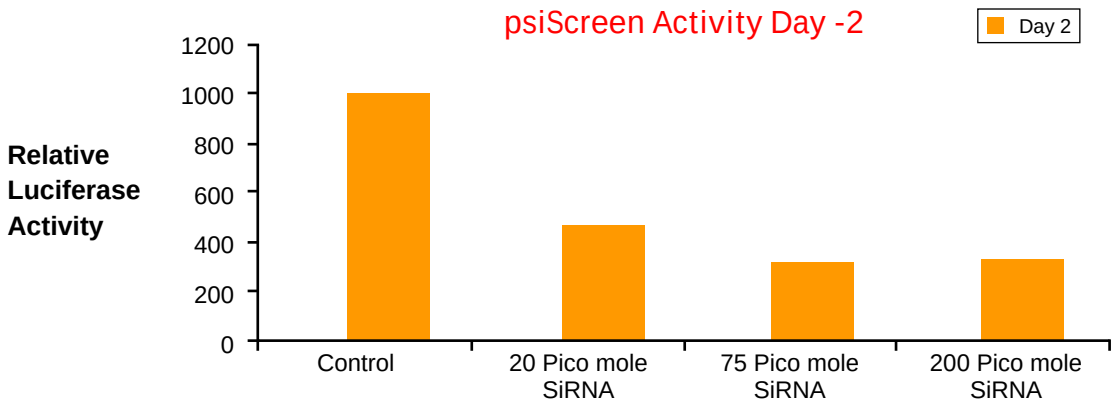

Figure 2 : This figure shows dose response of siRNA for gene silencing of the target gene (human tumor suppressor p53 gene). The data has been normalized for transfection efficiency using a firefly luciferase expression vector as a control plasmid. Unrelated siRNA was shown to be ineffective in gene silencing (data not shown).

Evaluation of the siScreen system for screening siRNAs against the human tumor suppressor p53 gene : Different siRNAs against human p53 gene were synthesized and evaluated for their ability to silence the p53 gene by co-transfection with a siScreen vector in which the p53 coding sequence was subcloned after the stop codon. The sequences and scoring of the siRNA duplexes used for evaluation are shown below. As expected siRNA sequences with a high score showed very effective gene silencing of the target genes. The data p-lotted shows results after normalization for transfection efficiency using a firefly luciferase expression vector pCMV-Fluc as a denominator plasmid.

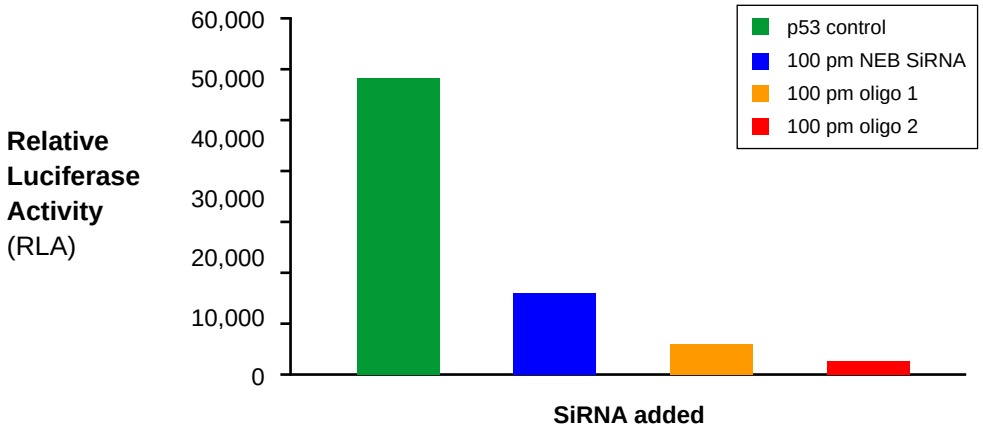

Figure 3 : This figure shows the effect effectiveness of different siRNAs in silencing the p53 gene. HEK'293 cells were cotrasnfected with the siScreenp53 expression vector and siRNA against either the p53 gene oligo 2 , and siRNA mix form NEB New England Biolabs, siRNA against gaussia luciferase oligo 1 or unrelated siRNA green bar . The cell supernatants were assayed for gaussia luciferase activity 24 hrs after transfection. The results have been normalized for transfection efficiency using a firefly luciferase expression vector HIV'Luc as a denominator plasmid. Transfection

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complexes were prepared by mixing 6 µg of pCMV''Gluc'p53 vector with 3 µg pHIVFluc and 100 pmols siRNA with 12 µl of the Targefect F2 reagent in 0.5 ml of high glucose DMEM. The contents of the tube were mixed well after each addition and incubated at 37º C for 30 min to form the trasnfection complexes. 65 µl of the trasnfection complexes were added to 0.5 ml of the supernatant cell culture media containing 5% serum to cells in 24'well dishes. The dishes were gently swirled and then incubated at 37º C overnight and assayed for luciferase activity at 24 hrs post-transfection.

Sequence of siScreen vector with MCS site

1 gacggatcgg gagatctccc gatcccctat ggtcgactct cagtacaatc tgctctgatg
61 ccgcatagtt aagccagtat ctgctccctg cttgtgtgtt ggaggtcgct gagtagtgcg
121 cgagcaaaat ttaagctaca acaaggcaag gcttgaccga caattgcatg aagaatctgc
181 ttagggttag gcgttttgcg ctgcttcgcg atgtacgggc cagatatacg cgttgacatt
241 gattattgac tagttattaa tagtaatcaa ttacggggtc attagttcat agcccatata
301 tggagttccg cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc
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541 atgcccagta catgacctta tgggactttc ctacttggca gtacatctac gtattagtca
601 tcgctattac catggtgatg cggttttggc agtacatcaa tgggcgtgga tagcggtttg
661 actcacgggg atttccaagt ctccacccca ttgacgtcaa tgggagtttg ttttggcacc
721 aaaatcaacg ggactttcca aaatgtcgta acaactccgc cccattgacg caaatgggcg
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841 ctgcttactg gcttatcgaa attaatacga ctcactatag ggagacccaa gcttggtacc
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