

GeneSilencer[®] shRNA Expression Vectors

Instruction Manual

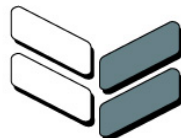
Catalog Numbers

P100100

P600100

P100300

P600300



Genlantis

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Purchaser Notification

Limited License

The purchase price paid for the siRNA Expression Vectors and Kits by end users grants them a non-transferable, non-exclusive license to use the Vectors and associated components (as listed in the Contents section). These Vectors are intended for **internal research only** by the purchaser. Such use is limited to the cloning of genes into the Vectors for sequencing, subcloning, and for *in vitro* protein expression and gene silencing in cells or *in vivo* protein expression and gene silencing in animals conducted by licensed facilities. Furthermore, **internal research only** use means that these Vector kits and all of their contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of Gene Therapy Systems, Inc (“GTS”).

Separate licenses are available from GTS for the express purpose of non-research use or applications of the siRNA Expression Vectors. To inquire about such licenses, or to obtain permission to transfer or use the enclosed material, contact the Director of Licensing at GTS.

Purchasers may terminate this License at any time by returning all siRNA Expression Vectors and Kit components to GTS, or by destroying all siRNA Expression Vectors or Kit components. Purchasers are advised to contact GTS with the notification that siRNA Expression Vectors are being returned in order to obtain a refund and/or to expressly terminate the **internal research only** license granted through the purchase of the Kit(s).

This document covers in full the terms of the siRNA Expression Vectors **internal research only** license, and does not grant any other express or implied license. The laws of the State of California shall govern the interpretation and enforcement of the terms of this License.

Product Use Limitations

The siRNA Expression Vectors and all of the Kits components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the handling of the kit components by following appropriate research lab practices.

For more information, or for any comments on the terms and conditions of this License, please contact:

Director of Licensing

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OVERVIEW

Available Kits and Contents

The following siRNA Expression vectors are currently available from Gene Therapy Systems, Inc.

Product Name	Description	Kit Contents	Catalog Number
pGSH1 siRNA Expression Vector Kit	For transcribing siRNAs using the H1 RNA Pol III promoter	1 x 25 µl linearized pGSH1 Vector, 50 ng/µl 1 x 1.4 ml Annealing Buffer 1 x 10 µl Luciferase siRNA sense oligo, 1 µg/µl 1 x 10 µl Luciferase siRNA antisense oligo, 1 µg/µl	P100100
pGSU6 siRNA Expression Vector Kit	For transcribing siRNAs using the U6 RNA Pol III promoter	1 x 25 µl linearized pGSU6 Vector, 50 ng/µl 1 x 1.4 ml Annealing Buffer 1 x 10 µl Luciferase siRNA sense oligo, 1 µg/µl 1 x 10 µl Luciferase siRNA antisense oligo, 1 µg/µl	P600100
pGSH1-GFP siRNA Expression Vector Kit	For transcribing siRNAs using the H1 RNA Pol III promoter; vector also expresses GFP gene from the hCMV promoter	1 x 25 µl linearized pGSH1-GFP Vector, 50 ng/µl 1 x 1.4 ml Annealing Buffer 1 x 10 µl Luciferase siRNA sense oligo, 1 µg/µl 1 x 10 µl Luciferase siRNA antisense oligo, 1 µg/µl	P100300
pGSU6-GFP siRNA Expression Vector Kit	For transcribing siRNAs using the U6 RNA Pol III promoter; vector also expresses GFP gene from the hCMV promoter	1 x 25 µl linearized pGSU6-GFP Vector, 50 ng/µl 1 x 1.4 ml Annealing Buffer 1 x 10 µl Luciferase siRNA sense oligo, 1 µg/µl 1 x 10 µl Luciferase siRNA antisense oligo, 1 µg/µl	P600300

Accessory Products

GTS offers the following products for use in conjunction with the siRNA Expression vectors.

For efficient transfection of DNA and high-level expression in cells:

Product Name	Catalog Number	Quantity
GenePORTER™ 2 Transfection Reagent	T202007	75 reactions (0.75 ml)
GenePORTER™ 2 Transfection Reagent	T202015	150 reactions (1.5 ml)
GenePORTER™ 2 Transfection Reagent	T202075	75 reactions (5 x 1.5 ml)
BoosterExpress™ Reagent Kit	T20100B	3 boosters (1.5 ml each)

For efficient transfection of siRNAs into cells

Product Name	Catalog Number	Quantity
GeneSilencer siRNA Transfection Reagent	T500750	200 reactions (0.75 ml)
GeneSilencer siRNA Transfection Reagent	T505750	5 x 200 reactions (5 x 0.75 ml)
GeneSilencer 96 Titration Plate	T500960	1 x 96 well plate
GeneSilencer 96 Titration Plate	T504960	4 x 96 well plates
GeneSilencer 96 Standard Plate High	T500961	1 x 96 well plate
GeneSilencer 96 Standard Plate High	T504961	4 x 96 well plate
GeneSilencer 96 Standard Plate Low	T500962	1 x 96 well plate
GeneSilencer 96 Standard Plate Low	T504962	4 x 96 well plate

For in vitro generation and transfection of heterologous pools of siRNAs

Product Name	Catalog Number	Quantity
Dicer siRNA Generation Kit	T510001	5 genes/50 transfections

Shipping and Storage

The siRNA Expression Vector Kits are shipped frozen. For maximum stability, we recommend that you store the siRNA Expression Vector Kits at -20°C upon receipt.

METHODS AND PROCEDURES

Introduction

Small interfering RNAs (siRNAs) are short double-stranded RNA molecules that facilitate potent and sequence-specific gene suppression via the mechanism of RNA interference (RNAi). When introduced into cultured mammalian cells, siRNAs facilitate the degradation of mRNA sequences to which they are homologous, thereby silencing the encoding gene.

The convenience of producing and using siRNAs has made them important tools for studying gene function. They can be synthesized *in vitro* and then introduced into cells directly, or they can be encoded in DNA expression vectors that are transfected into cells and subsequently expressed. Upon transfection or expression, the siRNAs proceed to mediate RNAi-induced target gene silencing.

The siRNA Expression Vectors from Genlantis are designed to induce siRNA mediated gene suppression via the latter route. Specifically, an siRNA coding sequence of 45-50 nucleotides is cloned into an RNA polymerase III expression cassette. After transfection, the siRNA sequence is expressed as fold-back stem-loop structure that is subsequently processed in the cell into functional siRNAs.

The Genlantis siRNA Expression Vectors contain either the U6 or the H1 RNA polymerase III promoter, allowing optimal expression in a wide variety of cell types. Additionally, the GTS siRNA Expression Vectors are available with GFP reporter genes to allow easy determination of vector transfection efficiency. All of the siRNA Expression Vectors contain the neomycin resistance gene for convenient selection of stable cell lines. In summary, the GTS siRNA Expression Vectors offer the following benefits:

- Maximized high-level expression with optimized H1 and U6 promoters.
- G418 resistance gene for the selection of stable cell lines.
- Available with GFP gene expression to track transfection efficiency.
- Simple vector design for easy siRNA cloning.
- Small vector sizes for efficient transfection

siRNA Expression Vector Features

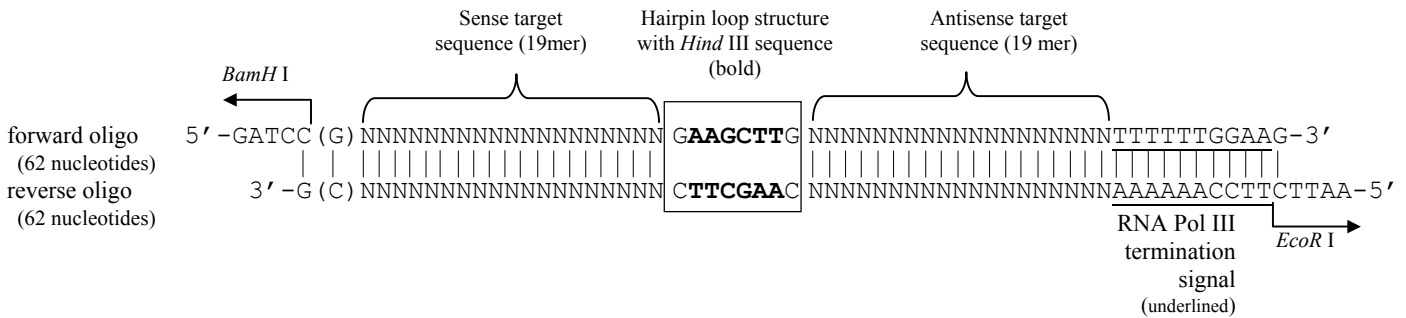
Feature	Function
Optimized H1 or U6 RNA Pol III promoters	High-level expression promoters <i>in vitro</i>
SV40 promoter	High-level expression of the neomycin resistance gene for selection of stably transfected cells
Kanamycin/G418 resistance gene	Efficient selection of vector in <i>E. coli</i> cells
	Efficient selection of stable mammalian cell lines using G418 sulfate
pUC origin	High copy number replication of vector in <i>E. coli</i> cells

1. Oligo Design for Hairpin siRNAs

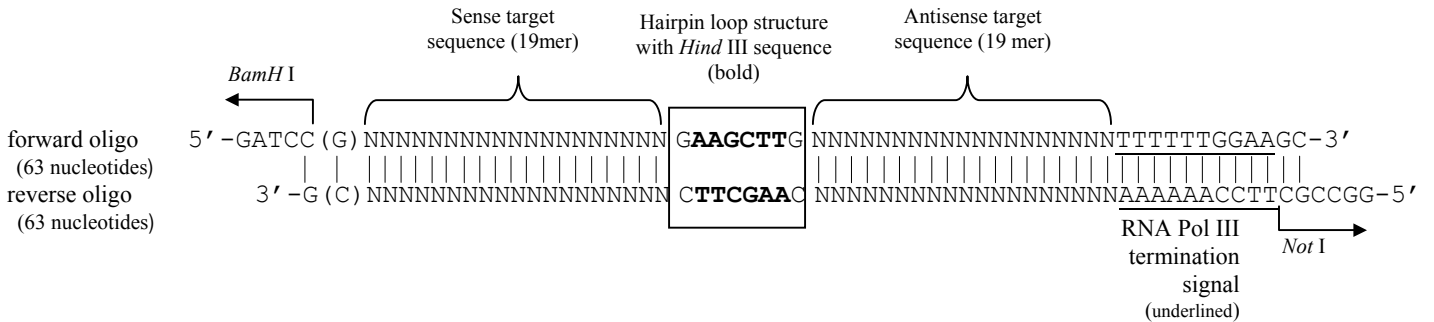
Genlantis' siRNA Expression Vectors are supplied in linear form and ready for ligation. The pGSH1 and pGSU6 vectors are linearized with *Bam*H I and *Eco*R I; the pGSH1-GFP and pGSU65-GFP vectors are linearized with *Bam*H I and *Not* I.

- 1.1. Design forward and reverse oligos using the sequences provided below. Appendix A on page 14 provides some guidelines on how to choose the Sense or Antisense target sequences for the gene of interest.

For pGSH1 and pGSU6 Vectors:



For pGSH1-GFP and pGSU6-GFP Vectors:



NOTE

1. The RNA polymerase promoter prefers to initiate transcription with a purine (A or G), so if the +1 position of the target RNA sequence (i.e. the first N position in the Forward Oligo sequence) is a T or a C, you should add a G in the Oligo sequences as indicated in parenthesis. You will not need to add the additional G if the first nucleotide in the target RNA sequence is an A or a G.
2. The Hind III site in the hairpin loop structure area is for the selection of positive clones.
3. While gene silencing via siRNAs can be generally quite effective, the relatively small size of the target sequence (i.e. 19 nucleotides) limits the chances that a chosen target sequence will produce effective gene silencing effects. Therefore, It might be necessary to choose several target sequences (we recommend 3 to 4 sequences along the stretch of the whole gene open reading frame) and try each separately or jointly through co-transfections in order to achieve the desired silencing effect. In doing so, make sure that the chosen target sequences do not contain 5 or more continuous T nucleotides so as to avoid premature RNA Polymerase III termination.

2. Oligo Annealing

- 2.1. After receiving the forward and reverse oligos, resuspend or dilute the oligos to a concentration of 1 $\mu\text{g}/\mu\text{l}$.
- 2.2. Setup the following annealing reaction mix:

2 μl	forward oligo
2 μl	reverse oligo
<u>46 μl</u>	<u>Annealing Buffer</u>
50 μl total reaction volume	
- 2.3. Vortex the annealing reaction mix and briefly spin down in a microfuge.
- 2.4. Heat the annealing reaction mix to 90°C using a heat block (or PCR thermocycler) for 3 minutes, then allow the annealing reaction mix to slowly cool down to room temperature. It takes approximately 1 hour for the annealing reaction mix to cool down from 90°C to 37°C.
- 2.5. Add 350 μl PCR grade water to the annealing reaction mix for a final volume of 400 μl and a working concentration of 10 ng/ μl .

3. Ligation of Annealed Oligos Into a Linear siRNA Expression Vector

- 3.1. Setup the following ligation reaction mix:

1 μl	siRNA Expression Vector (50 ng/ μl)
1 μl	Annealed oligos (10 ng/ μl)
5 μl	2X ligation buffer (provided by Ligase supplier)
1 μl	T4 DNA Ligase
<u>2 μl</u>	<u>H₂O</u>
10 μl total reaction volume	
- 3.2. Incubate the ligation reaction mix at room temperature overnight.
- 3.3. The ligation reaction mix may be stored at – 20°C before use, or it may be used immediately in the following transformation procedure.

4. Transformation

The following transformation protocol for the propagation of the siRNA Expression vectors is optimized for use with the SmartCells™ Chemically Competent *E. coli*

Catalog Number	Description and Genotypes	Kit Size
C101020	SmartCells™ Chemically Competent <i>E. coli</i> Genotype: F ⁻ <i>recA1 endA1 hsdR17 supE44 thi-1 gyrA96 relA1</i> <i>φ80lacZΔM15 Δ(lacZYA-argF)U169</i>	20 x 50 μl
C101120	SmartCells™ F' Chemically Competent <i>E. coli</i> Genotype: F' <i>recA1 endA1 hsdR17 supE44 thi-1 gyrA96 relA1</i> <i>φ80lacZΔM15 Δ(lacZYA-argF)U169</i>	20 x 50 μl

SmartCells™ Chemically Competent *E. coli* have been prepared by a unique procedure to warrant the highest and most robust transformation performance under diverse conditions. There is no need to dilute or purify your ligation mix before transformation. If needed, over 10 μl of full strength ligation mix can be added to 50 μl competent cells without significantly compromising transformation results.

- 4.1. Thaw one tube of the SmartCells™ competent cells on ice (10-15 minutes).
- 4.2. Add 2-10 μl of the ligation mix from Step 1.6 above to SmartCells™ *E. coli*; mix gently and incubate on ice for 15 to 30 minutes.
- 4.3. Heat the mix at 42° C for 45 seconds.
- 4.4. Add 100 μl SOC medium and incubate at 37° C for 1 hour in an air incubator. Shake tubes horizontally at 225 rpm.
- 4.5. Spread 100 μl of the transformation mix on LB/Agar plates containing 50 μg/ml kanamycin.
- 4.6. Incubate overnight at 37° C.
- 4.7. Pick colonies and analyze positive recombinant plasmids by digesting miniprep DNA and running on an agarose gel.

NOTE

You can propagate the siRNA Expression vectors in many other commercially available strains by following the supplier's protocol.

5. Growth and Selection of Positive Clones

- 5.1. Pick 3-4 colonies from the transformation plates using sterile toothpicks or pipette tips.
- 5.2. Transfer colonies into 1.0 – 1.5 ml of LB media containing 50 μg/ml kanamycin.
- 5.3. Grow overnight in a shaking incubator at 37° C.
- 5.4. Perform a miniprep DNA isolation using either a commercially available kit or a protocol available in standard laboratory manuals¹.

5.5. To test for positive plasmid DNA, setup the following restriction digest reaction:

1-17 μ l	miniprep plasmid DNA (100-200 ng)
1 μ l	<i>Hind</i> III restriction enzyme
2 μ l	Restriction enzyme buffer, 10X
0-16 μ l	PCR grade H ₂ O
<hr/>	
20 μ l	total reaction volume

5.6. Incubate restriction digestion reaction at 37°C for 2 hours.

5.7. Load 20 μ l digestion reactions onto a 1% agarose gel (TAE) and include one lane with undigested supercoiled plasmid control. Positive siRNA containing clones will have a unique *Hind* III site in the middle of the loop structure (see diagrams on Page 7), so the digest will produce a linear plasmid on the agarose gel. Negative clones will appear similar to the supercoiled plasmid control after the *Hind* III digestion.

5.8. *Optional*: Sequence the recombinant plasmids to confirm the hairpin siRNA insert identity. Use the following primer sequences for this task:

For the pGSH1 and pGSU6 vectors:	5' -CAGCAACGCGGCCTTTTTACG-3'
For the pGSH1-GFP and pGSU6-GFP vectors:	5' -AATGAGGAAATTGCATCGCATTGTCTGAGTAG-3'

5.9. After identifying positive clones, prepare sufficient quantities of plasmid DNA to continue to the next step by using commercially available plasmid DNA preparation kits or standard laboratory manuals.

6. Transfection

Plasmid DNA for transfection into mammalian cells must be clean and free of phenol and sodium chloride. Methods for transfection include calcium phosphate, cationic lipid, and electroporation. We recommend the use of GenePORTER™ 2 Transfection Reagent for maximized transfection efficiency and high expression levels in a wide variety of adherent and suspension cells.

Product Name	Cat. No.	Quantity
GenePORTER™ 2 Transfection Reagent	T202007	75 reactions (0.75 ml)
GenePORTER™ 2 Transfection Reagent	T202015	150 reactions (1.5 ml)
GenePORTER™ 2 Transfection Reagent	T202075	750 reactions (5 x 1.5 ml)

7. Stable Cell Line Selection

The siRNA Expression vectors contain the neomycin resistance gene for selection of stable cell lines using neomycin (G418). Neomycin (G418) is an aminoglycoside that blocks protein synthesis in mammalian cells by interfering with ribosomal function. Expression of the bacterial aminoglycoside phosphotransferase gene (APH) in mammalian cells results in the detoxification of neomycin (G418).

7.1. Determining G418 Sensitivity

In order to generate a stable cell line expressing your siRNA of interest from a cloned siRNA Expression Vector, you need to determine the minimum concentration of G418 required to kill your non transfected host cell line. Because natural resistance varies among cell lines, we recommend that you test a range of concentrations using the following protocol:

- 7.1.1. Split a confluent plate so the cells will be approximately 25% confluent. Prepare a set of 7 plates. Allow cells to adhere overnight.
- 7.1.2. The next day, substitute culture medium with medium containing varying concentrations of G418 (0, 50, 100, 200, 400, 600, 800 µg/ml G418).
- 7.1.3. Replenish the selective media every 3-4 days, and observe the percentage of surviving cells.
- 7.1.4. Count the number of viable cells at regular intervals to determine the appropriate concentration of G418 that prevents growth within 2-3 weeks after addition of G418.

7.2. Creating Stable Integrants.

Once you have determined the appropriate G418 concentration to use for selection in the host cell line, you can generate a stable cell line expressing your siRNA of interest.

- 7.2.1. Transfect your mammalian host cell line with linearized siRNA Expression construct. We recommend that you select a unique restriction enzyme site in the vector backbone to linearize. Include a plate of untransfected cells as a negative control.
- 7.2.2. 24 hours post transfection, wash the cells and add fresh medium.
- 7.2.3. 48 hours post transfection, split the cells into fresh medium containing G418 at the pre-determined concentration. Split the cells so that they are no more than 25% confluent.
- 7.2.4. Feed the cells with selective medium every 3-4 days until G418-resistant foci can be identified.
- 7.2.5. Pick and expand colonies in 96- or 48-well plates as needed.

Appendix

Quality Control

The pGSH1, pGSU6, pGSH1-GFP, and pGSU6-GFP siRNA Expression Vectors are verified for identity and quality by restriction enzyme digestion analysis. Further, the polylinker regions of each vector have been verified by sequencing. The siRNA Expression Vectors can also be verified for identity by users according to the restriction digest patterns listed below.

Vector	Restriction Enzyme	Number of Cuts	Expected Fragments (size in base pairs)
pGSH1	<i>Nde</i> I	0	2921 (supercoiled)
	<i>Bsa</i> I	2	1013, 1908
	<i>Sph</i> I	3	72, 763, 2086
	<i>Eae</i> I	3	210, 877, 1834
pGSU6	<i>Ase</i> I	0	3087 (supercoiled)
	<i>Nde</i> I	1	3087 (linear)
	<i>Pvu</i> II	2	608, 2479
	<i>Eae</i> I	4	210, 622, 877, 1378
pGSH1-GFP	<i>BssH</i> I	0	5318 (supercoiled)
	<i>Dra</i> III	1	5318 (linear)
	<i>Nde</i> I	2	841, 4477
pGSU6-GFP	<i>Hind</i> III	0	5552 (supercoiled)
	<i>Dra</i> III	2	789, 4763
	<i>BssH</i> II	3	9, 2, 5541
	<i>Pvu</i> II	3	608, 1553, 3391

Appendix

Troubleshooting

The troubleshooting table below is not all-inclusive and is meant to provide initial assistance to some of the most common and anticipated problems that may occur with the use of this type of kit. For additional troubleshooting assistance or for any product related questions, please contact our Technical Services Department at any of the numbers listed in the Contact Information Section below.

Problem Description	Possible Causes	Recommended Solutions
Low or no colonies after ligation of siRNA hairpin with linearized siRNA Expression vector.	Wrong plasmid used.	Check for correct plasmid usage. Use Quality Control table to verify plasmid identity.
	siRNA hairpin oligo quality.	Check that the sequence used is correct and provides the appropriate overhangs.
		Anneal or re-anneal primers if not done properly or not done at all.
	Wrong agar plates used.	Make sure to use LB agar plates with the correct antibiotic for appropriate selection.
Inefficient ligation	Make sure that correct and fresh ligation buffer was used; use fresh ATP if not provided in the ligation buffer already.	
Low number of positive recombinants	Degraded primers	Run a small sample of the annealed primers on a 3% agarose gel to check for primer integrity.
	High uncut vector background	Run a vector only transformation control by transforming 1 μ l (i.e. 50 ng) of the linear siRNA Expression Vector into Smart Cells™. The difference between the vector only control and the positive reaction should be at least two to three fold more on the positive reaction plate. If you obtain an excessive number of colonies on the vector only control plate, please contact our Technical Services Department at the numbers listed below.
	Vector amount	If the linear vector transformation above yields very few colonies, for example 10 or less, then use 100 ng of vector (or 2 μ l) per ligation reaction.

Contact Information

Telephone: 858-457-1919 OR 888-428-0558 (US toll free)	Fax: 858-623-9494
E-mail: tech1@gelantis.com	Web: http://www.genlantis.com

For a complete list of Genlantis international distributors, visit our web site at <http://www.genlantis.com>

Guide For Designing siRNAs (Small Interfering RNAs)

The sequence of the target siRNA can be selected as follows:

1. Start 75-100 bases downstream from the start codon “ATG” of your gene of interest.
2. Locate the first “AA” dimer.
3. Record the next 19 nucleotides following the AA dimer.
4. Calculate the percentage of G/C content of the AA-N19 21-base sequence. It must be between 30% and 70% with 50% being ideal. If the sequence does not meet the criteria, the search continues downstream to the next “AA” dimer until this condition is met.
5. The 21-base sequence is subjected to a BLAST-search (NCBI database) against EST libraries of your organism to ensure that no other gene(s) is targeted. (The complement is automatically searched as well.)
6. If the conditions in either step 4 or 5 are not met, repeat steps 2 - 5.

The sequence selection process has no other constraints. It is important to note that structure within the targeted mRNA appears to have minimal effect on the availability of the mRNA target and efficacy of the siRNA silencing approach. To date, successful silencing has been achieved using the above method to select the target sequence, although the method is essentially random with respect to accounting for mRNA structure.

Although siRNA silencing appears to be extremely effective by selecting a single target in the mRNA, it may be desirable to design and employ two independent siRNA duplexes to control for specificity of the silencing effect. This recommendation is only for specificity for it is yet unknown if the targeting of a gene by two different siRNA duplexes would be more effective than using a single siRNA duplex. It is believed that the rate-limiting component of the siRNA effect is the availability of cellular nuclease components and not mRNA target availability. Therefore, doubling the number of siRNA duplexes is not expected to double the rate or efficiency of silencing.

If the selected siRNA duplex(es) do not function for silencing, the following steps are recommended. First, a search is conducted for sequencing errors in the gene and possible polymorphisms. Initial studies on the specificity of target recognition by siRNA duplexes indicates that a single point mutation located in the paired region of an siRNA duplex is sufficient to abolish target mRNA degradation. Second, a reexamination is performed to confirm whether the cell line is from the expected species. Third, a second and/or third target are selected and the corresponding siRNA duplexes are prepared.

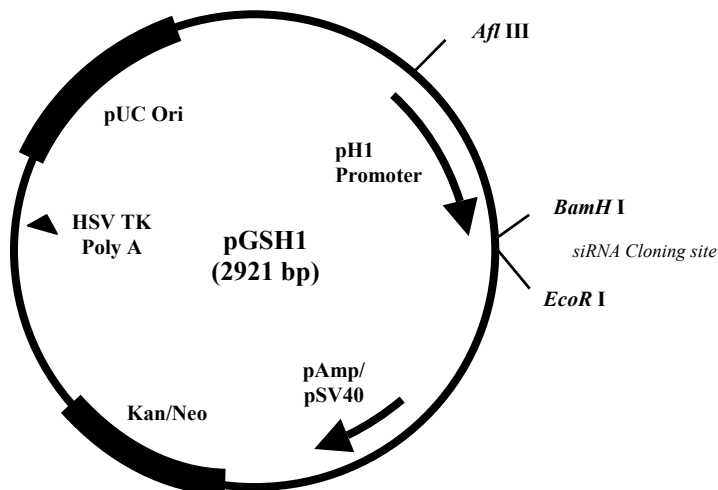
References:

1. Elbashir, S. M, *et al.* (2001a) *Nature* **411**: 494-498.
2. Elbashir, S. M, *et al.* (2001b) *Genes & Dev.* **15**: 188-200.

Appendix

Vector Information

Map of pGSH1: 2921 bps, Circular DNA



Vector Elements

Element	Start-End	Description
pH1 promoter	1-94	H1 RNA Polymerase III promoter
pAmp/pSV40	407-748	Ampicillin and SV40 promoters (in tandem)
Kan/Neo	870-1664	Kanamycin and Neomycin resistance gene sequence
HSV TK Poly A	1900-1918	HSV Thymidine Kinase polyadenylation signal sequence.
pUC Ori	2249-2892	pUC origin of replication sequence.

MCS sequence of linearized pGSH1 (spanning nucleotides 71-132)

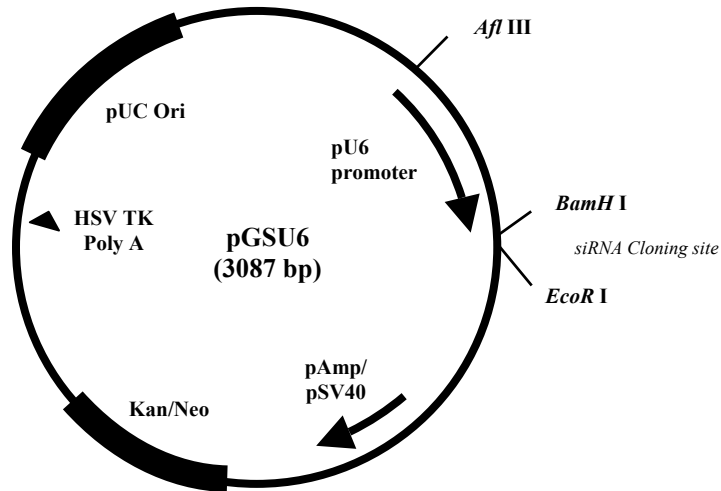


For complete vector sequence and a comprehensive list of enzyme cut sites, visit our web site at <http://www.genlantis.com>

Appendix

Vector Information (continued)

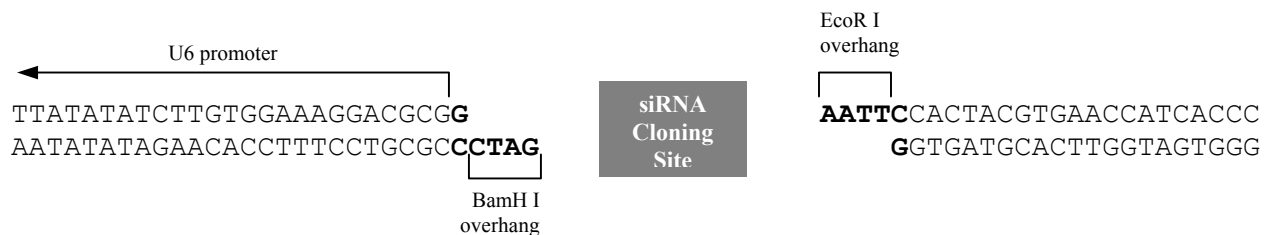
Map of pGSU6: 3087 bps, Circular DNA



Vector Elements

Element	Start-End	Description
pU6 promoter	1-260	U6 RNA Polymerase III promoter
pAmp/pSV40	573-914	Ampicillin and SV40 promoters (in tandem)
Kan/Neo	1036-1830	Kanamycin and Neomycin resistance gene sequence
HSV TK Poly A	2066-2084	HSV Thymidine Kinase polyadenylation signal sequence.
pUC Ori	2415-3058	pUC origin of replication sequence.

MCS sequence of linearized pGSU6 (spanning nucleotides 236-294)

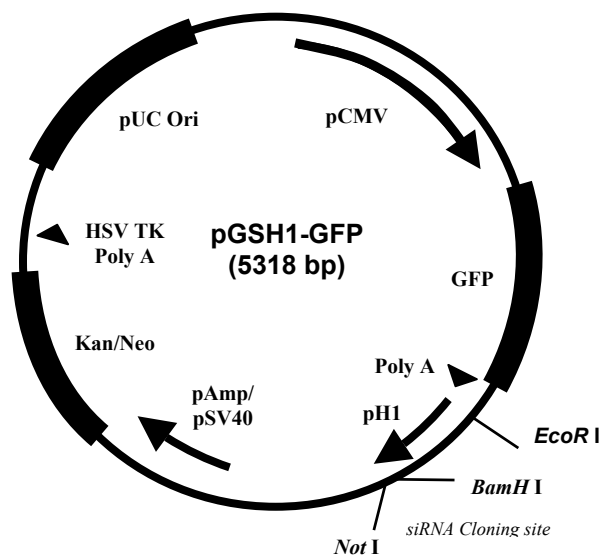


For complete vector sequence and a comprehensive list of enzyme cut sites, visit our web site at <http://www.genlantis.com>

Appendix

Vector Information (continued)

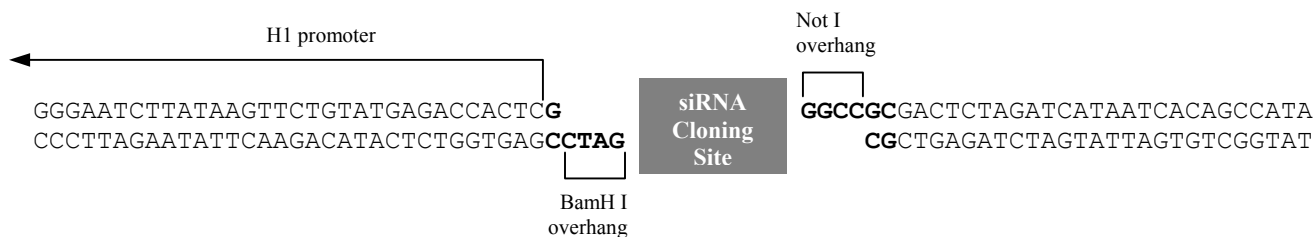
Map of pGSH1-GFP: 5318 bps, Circular DNA



Vector Elements

Element	Start-End	Description
pCMV	59-808	human CMV promoter sequence
GFP	843-1500	Green Fluorescent Protein gene sequence
Poly A	1572-1801	Transcription stop and polyadenylation sequence
pH1 promoter	1808-1900	H1 RNA Polymerase III promoter
pAmp/pSV40	2689-3030	Ampicillin and SV40 promoters (in tandem)
Kan/Neo	3152-3942	Kanamycin and Neomycin resistance gene sequence
HSV TK Poly A	4182-4200	HSV Thymidine Kinase polyadenylation signal sequence.
pUC Ori	4531-5174	pUC origin of replication sequence.

MCS sequence of linearized pGSH1-GFP (spanning nucleotides 1931-2016)

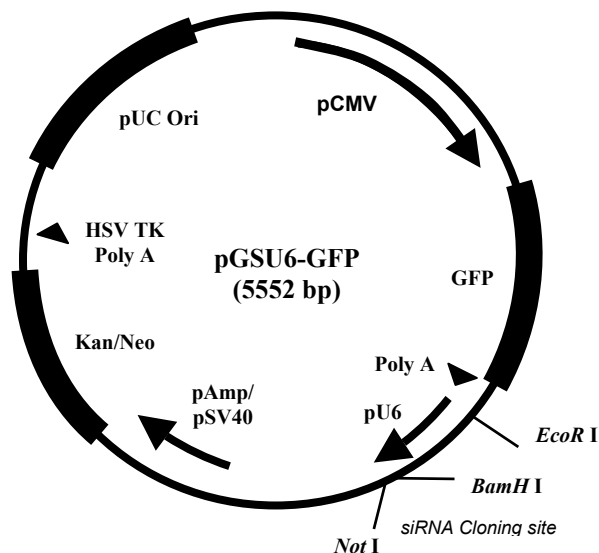


For complete vector sequence and a comprehensive list of enzyme cut sites, visit our web site at <http://www.genlantis.com>

Appendix

Vector Information (continued)

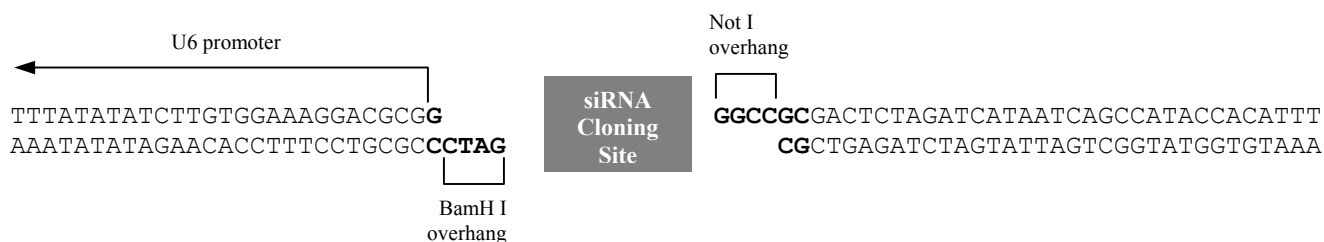
Map of pGSU6-GFP: 5552 bps, Circular DNA



Vector Elements

Element	Start-End	Description
pCMV	59-808	human CMV promoter sequence
GFP	843-1500	Green Fluorescent Protein gene sequence
Poly A	1572-1801	Transcription stop and polyadenylation sequence
PU6 promoter	1808-2134	U6 RNA Polymerase III promoter
pAmp/pSV40	2923-3264	Ampicillin and SV40 promoters (in tandem)
Kan/Neo	3386-4180	Kanamycin and Neomycin resistance gene sequence
HSV TK Poly A	4416-4434	HSV Thymidine Kinase polyadenylation signal sequence.
pUC Ori	4765-5408	pUC origin of replication sequence.

MCS sequence of linearized pGSU6-GFP (spanning nucleotides 2171-2258)



For complete vector sequence and a comprehensive list of enzyme cut sites, visit our web site at <http://www.genlantis.com>

Appendix

References

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