

STOP

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If the purchaser is unwilling to accept the limitations of this limited use statement, InvivoGen is willing to accept return of the product with a full refund. The product must be returned in resaleable condition. For information on purchasing a license to this product for purposes other than research, contact InvivoGen, 3950 Sorrento Valley Blvd. Suite 100, San Diego, CA 92121. Tel: 858-457-5873 Fax: 858-457-5843.

pCpGfree-siRNA

A CpG-free plasmid specifically designed for the production of siRNAs *in vivo*

Catalog # kcpgf-sirna

For research use only

Version # 09G01-MM

PRODUCT INFORMATION

Content:

- 50 µg pCpGfree-siRNA plasmid provided as lyophilized DNA
- 20 µg pCpGfree-siRNA-Scramble, a control plasmid expressing a scramble sequence. si-Scr sequence: GCATATGTGCGTACCTAGCAT
- 1 vial of **LyoComp GT115**, *E. coli* lyophilized competent cells
- 10 µg of lyophilized **OL559 sequencing primer** (forward)
OL559 sequence: 5' GATAAGTAACTTGACCTAAGTG 3' (22 mer)
MW: 7072 g/mol. Tm: 57.1°C
- 10 µg of lyophilized **OLSI3' sequencing primer** (reverse)
OLSI3' sequence: 5' GCATTCTAGTTGTGGTTTGTCC 3' (22 mer)
MW: 6738 g/mol. Tm: 58.4°C
- 4 pouches of **Fast-Media® Zeo XGal**

Storage and Stability:

- Products are shipped at room temperature.
- Upon receipt, resuspend lyophilized DNA and store at -20°C. Avoid repeated freeze-thaw cycles. Resuspended DNA is stable more than one year at -20°C.
- Store LyoComp cells at -20°C. LyoComp cells are stable for 6 months when stored at -20°C.
- Store sequencing primers at -20°C.
- Store Fast-Media® Zeo XGal at room temperature. Fast-Media® pouches are stable 18 months when stored properly.

Quality control:

- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Plasmid DNA was purified by ion exchange chromatography and lyophilized.
- The transformation efficiency of LyoComp cells are evaluated periodically and are guaranteed to be stable for 6 months when properly stored (-20°C).

GENERAL PRODUCT USE

InvivoGen has developed a new family of plasmids that are completely devoid of CpG dinucleotides. These plasmids yield high levels of transgene or shRNA expression both *in vitro* and *in vivo*, and in contrast to CMV-based plasmids allow sustained expression *in vivo*. pCpGfree plasmids contain elements that naturally lack CpG dinucleotides, were modified to remove all CpGs, or entirely synthesized such as genes encoding selectable markers or reporters. pCpGfree-siRNA is a plasmid that combines the backbone of the pCpGfree plasmids with the siRNA expression cassette of the psiRNA plasmids. It is designed for long lasting expression of siRNAs *in vivo* as the plasmid does not induce inflammatory responses and gene silencing by methylation in vertebrate hosts.

PLASMID FEATURES

All the elements required for replication and selection of the plasmid in *E. coli* and gene expression in mammalian cells are completely devoid of CpG dinucleotides.

Elements for expression in *E. coli*

- Origin of replication: The *E. coli* R6K gamma ori has been modified to remove all CpGs. This origin is activated by the R6K specific initiator protein π , encoded by the *pir* gene¹.
- Bacterial promoter: E2MK is a CpG-free version of the bacterial EM7 promoter that drives the expression of the resistance gene.
- Selectable marker: The Zeocin™ resistance gene is a small gene (<400 bp) that contains numerous CpG dinucleotides. A synthetic new allele was created that contains no CpGs.

Elements for expression in mammalian cells

- 7SK prom: 7SK is an abundant and evolutionarily conserved small nuclear RNA discovered in the mid-70s. It is transcribed by RNA polymerase III from one or more genes belonging to a family of interspersed repeats in the mammalian genome. The high conservation and abundance of 7SK suggest an important physiological function of this RNA. Recent studies suggest that 7SK acts as a negative regulator of the RNA polymerase II elongation factor P-TEFb, and that this activity is influenced by stress response pathways. The human 7SK promoter presents a strong permissivity for the nucleotide in the +1 position and recognizes a cluster of 4 or more T residues as a termination signal. This promoter is ideal for the production of shRNAs as it can generate high amounts of shRNAs. The human 7SK promoter has been modified to remove all CpGs and coupled with the mouse CMV enhancer, also modified to remove all CpGs, to increase its activity².
- EM7-alpha-peptide: This bacterial expression cassette enables white/blue selection. Transformation of permissive bacterial strains, such as strains harboring the *lacZAM15* allele, leads to alpha-complementation which results in the production of active β -galactosidase (LacZ). In such transformants, LacZ catalyses the hydrolysis of X-Gal to produce a blue precipitate.
- MAR: Matrix attached regions (MARs) are sequences typically AT-rich that are able to form barriers between independently regulated domains³. pCpG plasmids contains two MARs, from the 5' region of the human IFN- β gene or β -globin gene that were chosen because they are naturally CpG-free. The MARs are placed between the bacterial and mammalian transcription units.

References:

1. Wu F. *et al.*, 1995. A DNA segment conferring stable maintenance on R6K gamma-origin core replicons. *J Bacteriol.* 177(22):6338-45.
2. Xia XG. *et al.* 2003. An enhanced U6 promoter for synthesis of short hairpin RNA. *Nucleic Acids Res.* 31(17):e100.
3. Bode J. *et al.*, 1996. Scaffold/matrix-attached regions: topological switches with multiple regulatory functions. *Crit Rev Eukaryot Gene Expr.* 6(2-3):115-38.

TECHNICAL SUPPORT

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METHODS

Plasmid resuspension

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. Resuspend DNA in the appropriate amount of sterile H₂O to obtain a plasmid solution at 1 µg/µl. Store resuspended plasmid at -20°C.

Plasmid amplification

pCpGfree-siRNA is a low yield plasmid DNA. In order to enhance the yield, we suggest to increase the quantity of DNA of your culture before using a column. To do so, prepare a larger culture than recommended in the protocol provided by the manufacturer (for example instead of preparing 250 ml, prepare 2 liters). Lyse the culture and precipitate the DNA with isopropanol. Resuspend the pellet in the volume indicated by the protocol and treat with RNase before using the column. Then follow the manufacturer's protocol.

Plasmid preparation

- Cloning with Bbs I

Digest pCpGfree-siRNA plasmid with Bbs I (NEB, 2 units enzyme/µg plasmid DNA). Elute the large fragment (2926 bp) using a 0.7% low-melting agarose gel, and if necessary dilute the purified DNA fragment to obtain a solution at 0.1 µg/µl.

siRNA sequence design

- With the help of the **siRNA Wizard** (www.sirnawizard.com), choose a sequence of 19-21 nucleotides within the coding region of the gene of interest. The **siRNA Wizard** offers two search options: the "Standard Search" uses default criteria to analyze your gene of interest whereas the "Advanced Search" lets you manually set the selection criteria.

The first nucleotide of the siRNA sequence can either be an A or a G. Sequences that contain 4 or 5 consecutive As or Ts should be avoided, as they might act as termination signals for polymerase III.

- Using the **siRNA Wizard**, design two complementary oligonucleotides compatible Bbs I /Bbs I.

Cloning into pCpGfree-siRNA

1- Annealing of siRNA insert

- Dissolve the oligonucleotides (forward and reverse) at a concentration of 100 µM.

- Prepare the annealing solution by mixing the following components:

Forward oligonucleotide (100 µM)	2 µl
Reverse oligonucleotide (100 µM)	2 µl
0.5 M NaCl	6 µl
H ₂ O to a final volume of	30 µl

- Incubate 2 minutes at 80°C then stop the heating and maintain in water bath until the temperature reaches 35°C.

- Use annealed siRNA insert immediately or store at -20°C for further use.

Note: Purification of the annealed oligonucleotide solution is not necessary for efficient ligation of the siRNA insert.

2- Ligation of siRNA insert into pCpGfree-siRNA

- Prepare the ligation solution by mixing the following components:

Digested pCpGfree-siRNA	1 µl (100 ng)
Annealed siRNA insert	1 µl
T4 DNA Ligase	1 µl (1 unit)
10X ligation buffer	2 µl
H ₂ O to a final volume of	20 µl

- Incubate the mixture at 16°C overnight (or at 27°C for 2 hours).

3- Transformation of LyoComp GT115

E. coli GT115 strain is specifically designed for the cloning of short hairpin RNAs in the pCpGfree-siRNA plasmid. This engineered strain is deficient in Dem methylation and expresses a *pir* mutant gene necessary for the replication and amplification of pCpG plasmids. In addition, GT115 contains a *sbcd* deletion (SbcCD is a protein complex that recognizes and cleaves hairpins) which makes it more compatible with hairpin harboring plasmids than standard laboratory strains.

E. coli GT115 is provided as lyophilized competent cells, called LyoComp GT115.

- Place the LyoComp GT115 vial in ice for 5 minutes.

- Add 200 µl sterile water and store in ice for 5 minutes.

- Gently homogenize and allow the cells to completely rehydrate in ice for 25-30 minutes.

- Introduce 10 µl of ligation product (or 1 µg supercoiled plasmid DNA) in pre-chilled 1.5 ml tubes and return tubes to ice.

- Gently flick the cells twice to homogenize and add 100 µl of cells to each DNA-containing tubes.

- Mix by tapping gently and place in ice immediately.

- Incubate the tubes in ice for 30 minutes.

- Incubate the tubes in a 42°C water bath for exactly 30 seconds, then place the tubes back in ice for 1-2 minutes.

- Add 900 µl of room temperature SOC (or LB) medium to each reaction. (Practice sterile techniques to avoid contamination.)

- Incubate tubes at 37°C for 1h30 with shaking at 250 rpm.

- Spread each transformation reaction (150 µl if using a ligation product or 100 µl of 10⁻¹ and 10⁻² dilutions if using a supercoiled plasmid) onto agar plate prepared with Fast-Media® Zeo XGal, to take advantage of the white/blue selection.

Notes: The white/blue selection will allow you to discriminate between blue parental clones and white recombinant clones.

To increase the number of transformants, spread the remaining transformed cells first concentrated by low speed centrifugation.

- Incubate plates at 37°C overnight.

Note: The blue/white selection is more visible after 24h incubation.

4- DNA extraction and sequencing of the siRNA insert

- Extract plasmid DNA from 5 white clones.

- Confirm the presence of the siRNA insert on a 3% agarose gel (NuSieve GTG FMC type) after digestion with Spe I and Nhe I (2h at 37°C).

pCpGfree-siRNA containing a siRNA insert will generate two bands: 2619 bp and 363 bp (for an siRNA insert consisting of a 21 bp stem and 7 bp loop).

- Verify the sequence of the siRNA insert of 3 positive clones using the sequencing primers provided: OL559 (forward primer) and OLSI3' (reverse primer).

TECHNICAL SUPPORT

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Transfection of mammalian cells

- Transfect your cell line with a recombinant plasmid containing the expected siRNA insert using standard transfection procedures.

Note: We recommend using LyoVec™, a transfection reagent optimized for pCpGfree-siRNA.

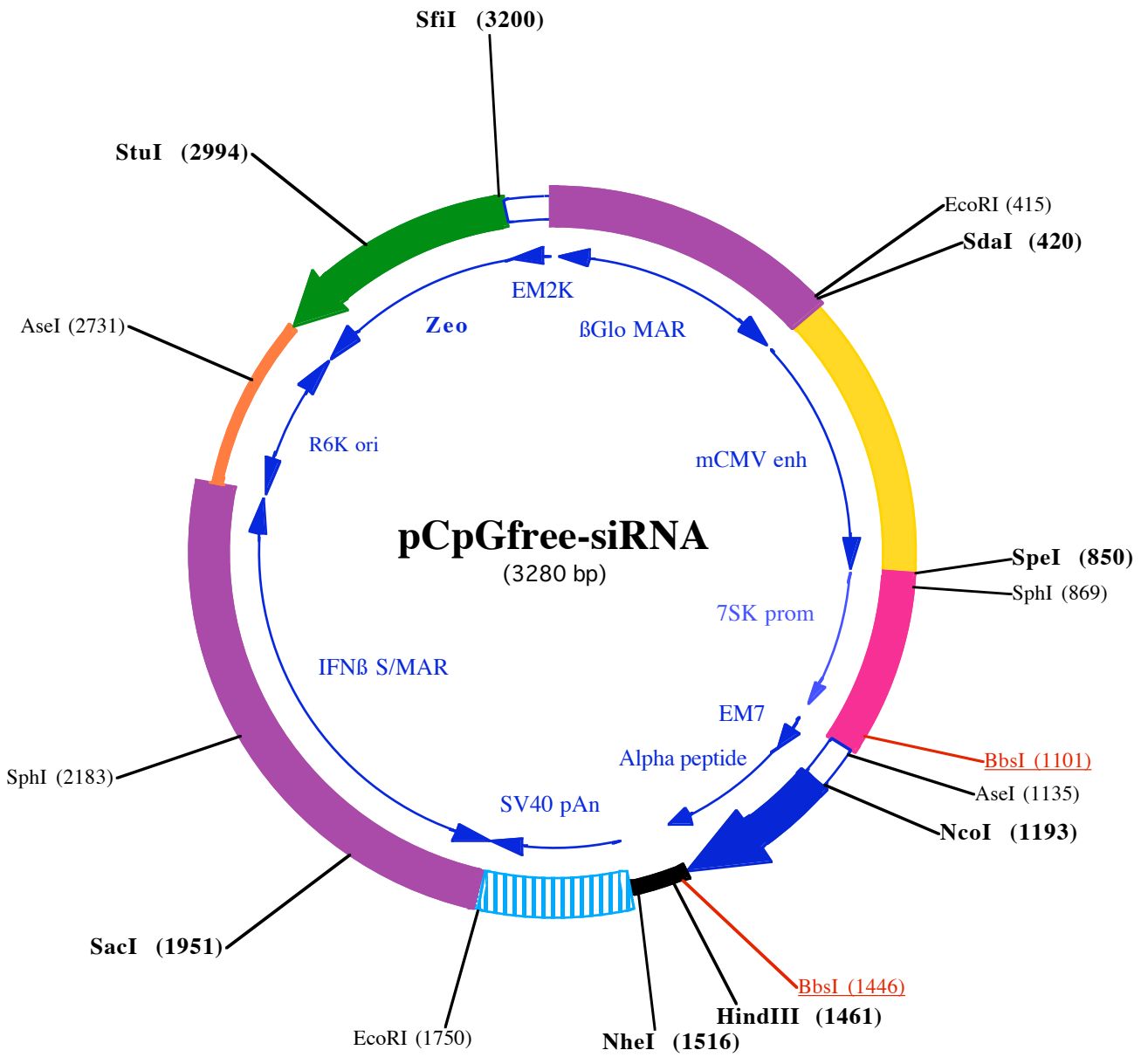
Analysis of siRNA-induced silencing

siRNA-induced silencing of your gene of interest can be determined by using different techniques, such as Northern analysis or quantitative RT-PCR at the RNA level, or Western Blot at the protein level.

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1 TTAATTAATAATTATCTCTAAGGCATGTGAACTGGCTGTCTTGGTTTTTCATCTGTA
 CCTCTGTGACCTGAAACATATTTATAATTCCATTAAGCTGTGCATATGATAGATTTATCATATGTAT
 135 TTTCTTAAAGGATTTTTGTAAGAACTAATTGAATTGATACCTGTAAAGTCTTTATCACACTACCCA
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 336 TTTTATGAATAAAAAATTATTAGCAATCAATATTGAAAACCACTGATTTTTGTTTATGTGAGCAAAC

 SdaI (420) EcoRI (415)
 403 AGCAGATTAAGGAATTCCTGCAGGAGTCAATGGGAAAAACCCATTGGAGCCAAGTACTGACTC
 470 AATAGGGACTTTCCATTGGGTTTTGCCAGTACATAAGGTCAATAGGGGGTGAAGTCAACAGGAAAGT
 537 CCCATTGGAGCCAAGTACTGAGTCAATAGGGACTTTCCAATGGGTTTTGCCAGTACATAAGGT
 604 AATGGGAGGTAAGCCAATGGGTTTTCCATTACTGACATGTATACTGAGTCAATAGGGACTTTCCA
 671 ATGGGTTTTGCCAGTACATAAGGTCAATAGGGGTGAATCAACAGGAAAGTCCCATTGGAGCCAAGT
 738 AACTGAGTCAATAGGGACTTTCCATTGGGTTTTGCCAGTACAAAAGGTCAATAGGGGGTGAAGTCA

 SpeI (850) SphI (869)
 805 ATGGGTTTTCCATTATTGGCACATACATAAGGTCAATAGGGGTGACTAGTGCTGCAGTATTTAGC
 872 ATGCCCCACCCATCTGCAAGGCATTCTGGATAGTGTCAAACAGCTGGAAATCAAGTCTGTTTATCT
 939 CAACTTTAGCATTTTGGGAATAAATGATATTTGCTATGCTGGTTAAATTAGATTTTAGTTAAATTT
 1006 CCTGCTGAAGCTCTAGTATGATAAGTAACCTGACCTAAGTGTAAAGTTGAGATTTCTTCAGGTTTA

 BbsI (1101) AseI (1135)
 1073 TATAGTCCCTATCAGTGATAGAGACCTCGGTCTTACCTGAGGTTTTTCAAAGTAGTTGACAATTA

 NcoI (1193)
 1140 ATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAGGAGGGCCACCATGGACCCTGT
 1207 TGTGCTGCAAAGGAGAGACTGGGAGAACCCTGGAGTGACCCAGCTCAACAGACTGGCTGCCACCCCT
 41 Val Leu Gl nArg Arg Asp Trp Gl uAsn Pro Gl yVal Thr Gl nLeu Asn Arg Leu Al aAl aHi sPro
 1274 CCCTTGCCTCTGGAGGAACCTGAGGAAGCCAGGACAGACAGGCCAGCCAGCAGCTCAGGTCTC
 27 Pro Phe Al aSer Trp Arg Asn Ser Gl uGl uAl aArg Thr Asp Arg Pro Ser Gl nGl nLeu Arg Ser L
 1341 TCAATGGAGAGTGGAGGTTTGCCTGGTTCCTGCCCTGAAGCTGTGCCTGAGTCTTGCTGGAGTG
 49 euAsn Gl yGl uTrp Arg Phe Al aTrp Phe Pro Al aPro Gl uAl aVal Pro Gl uSer Trp Leu Gl uCy

 BbsI (1446) HindIII (1461)
 1408 TGACCTCCAGAGGCTGACACTGTGTAACCCTGAGCTAGGAAGACTTTTTGGAAAAGCTTAATTAAG
 71 sAsp Leu Pro Gl uAl aAsp Thr Val ●●●

 NheI (1516)
 1475 CTGTACCACTATCAGCTGGTGTGGTGCCAGAAGTAAACCTGAGCTAGCTGGCCAGACATGATAAGAT
 1542 ACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTG
 1609 TGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATT
 1676 CTTTTATGTTTCAGGTT CAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAAT

 EcoRI (1750)
 1743 GTGGTATGGAATTCAGTCAATATGTTCAACCCAAAAAAGCTGTTTGTTAACTTGCCAACCTCATTCT

1810 AAAATGTATATAGAAGCCCAAAGACAATAACAAAAATATTCTTGTAGAACAAAATGGGAAAGAATG

1877 TTCCACTAAATATCAAGATTTAGAGCAAAGCATGAGATGTGTGGGGATAGACAGTGAGGCTGATAAA

SacI (1951)

1944 ATAGAGTAGAGCTCAGAAACAGACCCATTGATATATGTAAGTGACCTATGAAAAAATATGGCATT

2011 TACAATGGGAAAATGATGGTCTTTTTCTTTTTAGAAAAACAGGGAAATATATTTATATGTAAAAA

2078 TAAAAGGGAACCCATATGTCATACCATACACACAAAAAATTCCAGTGAATTATAAGTCTAAATGGA

SphI (2183)

2145 GAAGGCAAAACTTTAAATCTTTTAGAAAATAATATAGAAGCATGCCATCAA GACTTCAGTGTAGAGA

2212 AAAATTTCTTATGACTCAAAGTCCTAACCAAAAGAAAAGATTGTTAATTAGATTGCATGAATATTA

2279 AGACTTATTTTTAAATTA AAAAACCATTAAGAAAAGTCAGGCCATAGAATGACAGAAAATATTTGC

2346 AACACCCCAGTAAAGAGAATTGTAATATGCAGATTATAAAAAGAAGTCTTACAAATCAGTAAAAAAT

2413 AAAACTAGACAAAAATTTGAACAGATGAAAGAGAAACTCTAAATAATCATTACACATGAGAAACTCA

2480 ATCTCAGAAATCAGAGAACTATCATTGCATATACACTAAATTAGAGAAATATTA AAAAGGCTAAGTAA

2547 CATCTGTGGCTTAATTA AAATCAGCAGTTCAACCTGTTGATAGTATG TACTAAGCTCTCATGTTTAA

2614 TGTACTAAGCTCTCATGTTTAAATGAACTAAACCCTCATGGCTAATGTACTAAGCTCTCATGGCTAAT

AseI (2731)

2681 GTACTAAGCTCTCATGTTTCATGTACTAAGCTCTCATGTTTGAACAATAAAATTAATATAAATCAGC

2748 AACTTAAATAGCCTCTAAGGTTTTAAGTTTTATAAGAAAAAAAAGAATATATAAGGCTTTTTAAAGGT

2815 TTTAAGGTTTCCTAGCTTTAGTCCTGTTCTCAGCTACAAAATGGACACAATTTCCAGCAGGGTCTC

2882 TGAGGGCAAATTCCTTCCCAAGGTTGTTCAACCAATTTCTGTCATGGCTGGGCCAGAGGCATCCCT

StuI (2994)

2949 GAAATTTGTGCTGACTACTTCTGACCATTCTGCATAAAGCTCATCTAGGCCTCTGACCCAGACCCAA

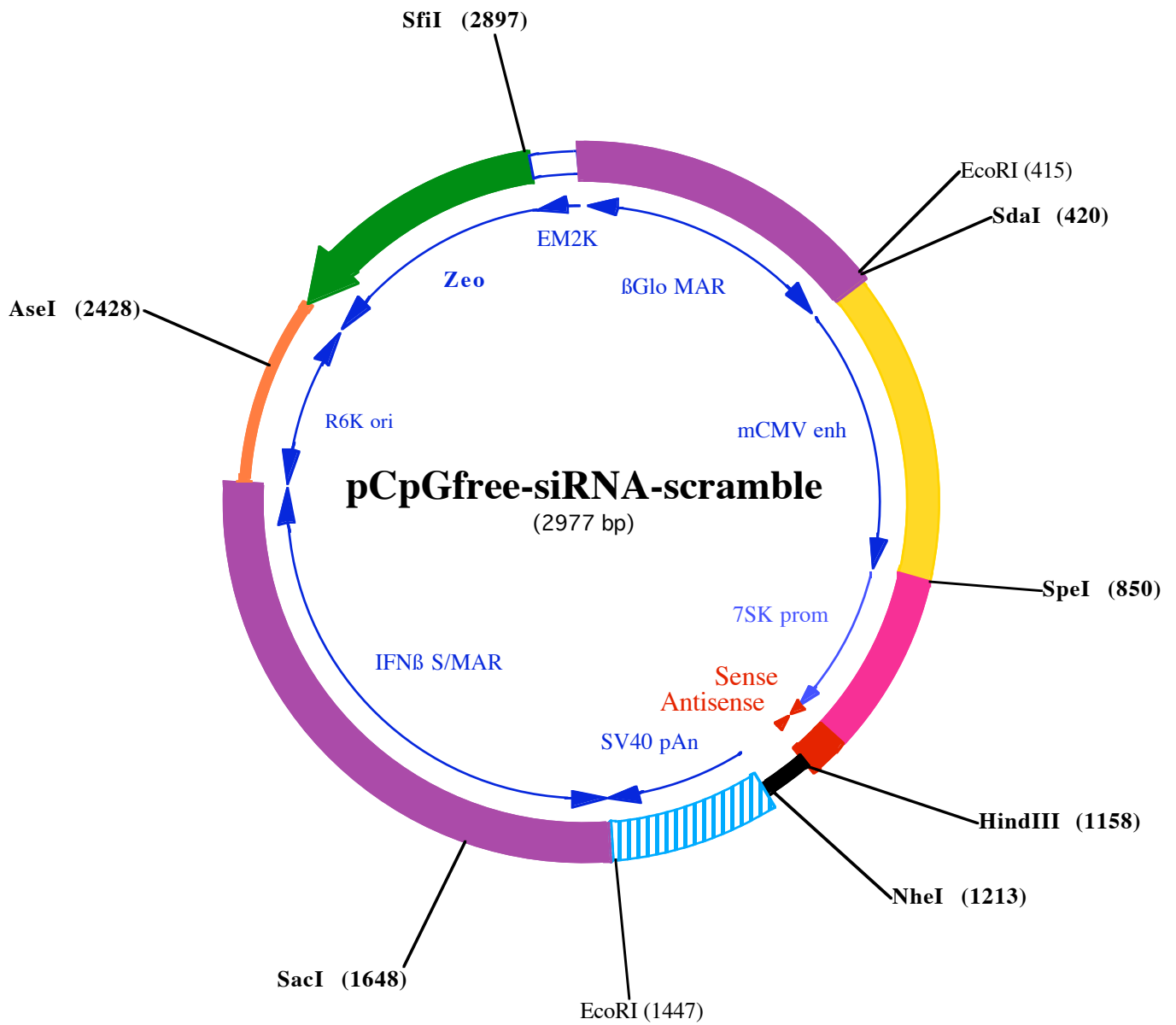
3016 GCAAGGGTGTGTCAGGGACA ACTTGGTCTGAACTGCTGAGATGAAGAGGGTGACATCATCTCTGA

3083 CAACACCAGCAAATCATCTTCAACAAAGTCTCTGGAGAATCCTAATCTGTCAGTCCAGAACTCTAC

SfiI (3200)

3150 AGCCCCTGCAACATCCCTTGCTGTGAGGACTGGGACTGCAGAAGTGAGTTTGGCCATGATGGCCCTC

3217 CTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGTTTAATTGTCAACTACTGTT



1 TTAATTAATAATTATCTCTAAGGCATGTGAACTGGCTGTCTTGGTTTTTCATCTGACTTCATCTGCTA
68 CCTCTGTGACCTGAAACATATTTATAATTCCATTAAGCTGTGCATATGATAGATTTATCATATGTAT
135 TTTCTTAAAGGATTTTTGTAAGAATAATTGAATTGATACCTGTAAAGTCTTTATCACACTACCCA
202 ATAAATAATAAATCTCTTTGTTTCAGCTCTCTGTTTCTATAAATATGTACCAGTTTTATTGTTTTTAG
269 TGGTAGTGATTTTATTCTCTTTCTATATATATACACACACATGTGTGCATTATAAATATATACAAT
336 TTTTATGAATAAAAAATTATTAGCAATCAATATTGAAAACCACTGATTTTTGTTTATGTGAGCAAAC

SdaI (420)
EcoRI (415)

403 AGCAGATTAAGGAATTCCTGCAGGAGTCAATGGGAAAAACCCATTGGAGCCAAGTACACTGACTC
470 AATAGGGACTTTCCATTGGGTTTTGCCAGTACATAAGGTCAATAGGGGGTGGAGTCAACAGGAAAAGT
537 CCCATTGGAGCCAAGTACATTGAGTCAATAGGGACTTTCCAATGGGTTTTGCCAGTACATAAGGTC
604 AATGGGAGGTAAGCCAATGGGTTTTCCATTACTGCA[↓]GTATACTGAGTCATTAGGGACTTTCCA
671 ATGGGTTTTGCCAGTACATAAGGTCAATAGGGGTGAATCAACAGGAAAAGTCCCATTGGAGCCAAGT
738 ACACTGAGTCAATAGGGACTTTCCATTGGGTTTTGCCAGTACAAAAGGTCAATAGGGGGTGGAGTCA

SpeI (850)

805 ATGGGTTTTTCCATTATTGGCACATACATAAGGTCAATAGGGGTGACTAGTGTGCGAGTATTTAGC
872 ATGCCCCACCCATCTGCAAGGCATTCTGGATAGTGTCAAAACAGCTGGAAATCAAGTCTGTTTATCT
939 CAACTTTAGCATTTTGGGAATAAATGATATTTGCTATGCTGGTTAAATTAGATTTTAGTTAAATTT
1006 CCTGCTGAAGCTCTAGTATGATAAGTAACTTGACCTAAGTGTAAAGTTGAGATTTCTTCAGGTTTA
1073 TATAGTCCCTATCAGTGATAGAGACCTCGCATATGTGCGTACCTAGCATTCAAGAGATGCTAGGTAC

HindIII (1158)

1140 GCACATATGCTTTTTGGAAAAGCTTAATTAAGCTGTACCACTATCAGCTGGTGTGGTGCCAGAAGTA

NheI (1213)

1207 AACCTGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAAGTGAATG
1274 CAGTAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCT
1341 GCAATAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAAGGGGAGGTGTGGGA

EcoRI (1447)

1408 GGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTCAGTCAATATGTTACCCCCAAA
1475 AAAGCTGTTTGTAACTTGCCAACCTCATTCTAAAATGTATATAGAAGCCAAAAGACAATAACAAA
1542 AATATTCTGTAGAACAAAATGGGAAAGAATGTTCCACTAAATATCAAGATTTAGAGCAAAGCATGA

SacI (1648)

1609 GATGTGTGGGGATAGACAGTGAGGCTGATAAATAGAGTAGAGCTCAGAAACAGACCCATTGATATA
1676 TGTAAGTGACCTATGAAAAAATATGGCATTTTACAATGGGAAAATGATGGTCTTTTTCTTTTTTAG
1743 AAAAAACAGGGAATATATTTATATGTAAAAAATAAAGGGAACCCATATGTCATACCATACACACAA
1810 AAAAATCCAGTGAATTATAAGTCTAAATGGAGAAGGCAAACTTTAAATCTTTTAGAAAAATAATAT

1877 AGAAGCATGCCATCAA GACTTCAGTGTAGAGAAAAATTTCTTATGACTCAAAGTCCTAACCACAAAG
1944 AAAAGATTGTTAATTAGATTGCATGAATATTAAGACTTATTTTTAAAATTAAAAAACCATTAAGAAA
2011 AGTCAGGCCATAGAATGACAGAAAATATTTGCAACACCCCAGTAAAGAGAATTGTAATATGCAGATT
2078 ATAAAAAGAAGTCTTACAAATCAGTAAAAAATAAACTAGACAAAAATTTGAACAGATGAAAGAGAA
2145 ACTCTAAATAATCATTACACATGAGAACTCAATCTCAGAAATCAGAGA ACTATCATTGCATATACA
2212 CTAAATTAGAGAAATATTTAAAGGCTAAGTAACATCTGTGGCTTAATTA AAATCAGCAGTTCAACCT
2279 GTTGATAGTATGTACTAAGCTCTCATGTTTAATGTACTAAGCTCTCATGTTTAATGAACTAAACCT
2346 CATGGCTAATGTACTAAGCTCTCATGGCTAATGTACTAAGCTCTCATGTTTCATGTACTAAGCTCTC

AseI (2428)

2413 ATGTTTGAACAATAAAATTAATAAATCAGCAACTTAAATAGCCTCTAAGGTTTTAAGTTTTATAA
2480 GAAAAAAAAAGAATATATAAGGCTTTTAAAGGTTTTAAGGTTTCCTAGCTTTAGTCCTGTTCCCTCAGC
2547 TACAAAATGGACACAATTTCCAGCAGGGTCTCTGAGGGCAAATTCCTTCCCCAAGGTTGTTACCA
2614 ATTTCTGTCATGGCTGGGCCAGAGGCATCCCTGAAATTTGTGCTGACTACTTCTGACCATTCTGCAT
2681 AAAGCTCATCTAGGCCTCTGACCCAGACCCAAGCAAGGGTGTGTCAGGGACA ACTTGGTCCTGAAC
2748 TGCTGAGATGAAGAGGGTGACATCATCTCTGACAACACCAGCAAAATCATCTTCAACAAAGTCTCTG
2815 GAGAATCCTAATCTGTCAGTCCAGA ACTCTACAGCCCCTGCAACATCCCTTGCTGTGAGGACTGGGA

SfiI (2897)

2882 CTGCAGAAGTGAGTTTGGCCATGATGGCCCTCCTATAGTGAGTTGTATTATACTATGCAGATATACT
2949 ATGCCAATGTTTAATTGTCAACTACTGTT