STOP

Before using this product, please read the Limited Use License statement below:

Important Limited Use License information for pCpGfree-promoter-Lucia

The purchase of the pCpGfree-promoter-Lucia vector conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes.

Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic, or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research.

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, 10515 Vista Sorrento Parkway San Diego, CA 92121 USA. Tel: 858-457-5873 Fax: 858-457-5843.

pCpGfree-promoter-Lucia

A Lucia luciferase reporter plasmid without an enhancer and devoid of CpG dinucleotides

Catalog # pcpgf-promlc

For research use only

Version 21F04-MMv02

PRODUCT INFORMATION

Content:

- 20 µg of pCpGfree-promoter-Lucia plasmid provided as lyophilized DNA
- *E. coli* GT115 strain provided lyophilized on a paper disk
- 1 ml of Zeocin™ (100 mg/ml)

Storage and Stability:

- Products are shipped at room temperature.
- Resuspended DNA is stable for 6 months when stored at -20 °C.
- Bacteria should be stored at -20 °C and are stable for at least 1 year.
- Store Zeocin™ at 4 °C or at -20 °C. The expiry date is specified on the product label.

Quality control:

Plasmid construct has been confirmed by restriction analysis and sequencing. Plasmid DNA was purified by ion exchange chromatography and lyophilized. Viability of the lyophilized bacteria upon resuspension has been verified.

GENERAL PRODUCT USE

Methylation of CpG dinucleotides within the promoter/enhancer region of genes is often associated with transcriptional silencing. This epigenetic event plays an important role in the regulation of gene activity in normal and cancer cells. Recently, it has been confirmed that the activity of enhancers is correlated with DNA methylation[1].

InvivoGen provides pCpGfree-basic and pCpGfree-promoter, two Lucia luciferase and two SEAP reporter plasmids completely devoid of CpG dinucleotides, that allow to study the effect of promoter and enhancer CpG methylation in transfection assays. The lack of CpGs within the plasmid backbone limits *in vitro* CpG methylation to the CpG dinucleotides present in the inserted promoter or enhancer fragment. pCpGfree-promoter is designed to enable to study the effect of DNA methylation in enhancer elements.

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. Furthermore, all Dam methylation sites (GATC) have been removed to prevent prokaryotic methylation.

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* gene[2].

• Bacterial promoter: EM2K is a CpG-free version of the bacterial EM7 promoter.

• 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

• The pCpGfree-promoter plasmid contains the CpG-free version of the human EF-1α promoter and a multiple cloning site.

• **Lucia luciferase** is a synthetic CpG-free gene that codes for a secreted coelenterazine-utilizing luciferase.

ORF size (from the ATG to the stop codon): 634 bp

• Polyadenylation signal: The polyadenylation signal is a CpG-free form of the late SV40 polyadenylation signal.

• MAR: Matrix attached regions (MARs) are sequences typically AT-rich that are able to form barriers between independently regulated domains[3]. pCpGfree 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.

• MCS: The multiple cloning site contains several commonly used restriction sites for convenient cloning of a gene of interest.

5' Sda I, Bsp 120I, Avr II, Nsi I, Ppu 10I, Sca I, Bam HI, Spe I 3'

Due to the presence of the R6Kγ origin of replication, pCpG plasmids can only be amplified in *E. coli* mutant strain expressing a *pir* mutant gene. They will not replicate in standard *E. coli* strains. Therefore, pCpG plasmids are provided with the *E. coli* GT115 strain, a pir mutant also deficient in *Dcm* methylation.

1. Hoivik EA. et al., 2011. DNA Methylation of Intronic Enhancers Directs Tissue-Specific Expression of Steroidogenic Factor 1/Adrenal 4 Binding Protein (SF-1/Ad4BP). Endocrinology. 152(5):2100-12. 22. **2. Wu F. *et al.* 1995.** A DNA segment conferring stable maintenance on R6K gamma-origin core replicons. J Bacteriol. 177(22):6338-45. **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.

METHODS

Plasmid resuspension

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile H_2O. Store resuspended plasmid at -20 °C.

Reconstitution of *E. coli* GT115 strain

Use sterile conditions to do the following:

1. Reconstitute *E. coli* GT115 by adding 1 ml of Luria-Bertani (LB) medium in the tube containing the paper disk. Let sit for 5 minutes.
2. Mix gently by vortexing for 1-2 minutes.
3. Streak bacteria taken from this suspension on a LB agar plate.
4. Place the plate in an incubator at 37˚C overnight.
5. Isolate a single colony and grow the bacteria in LB or terrific broth (TB) medium.
6. Prepare competent cells utilizing protocol of choice.

Plasmid amplification and cloning

Plasmid amplification and cloning can be performed in *E. coli* GT115.

Zeocin™ usage

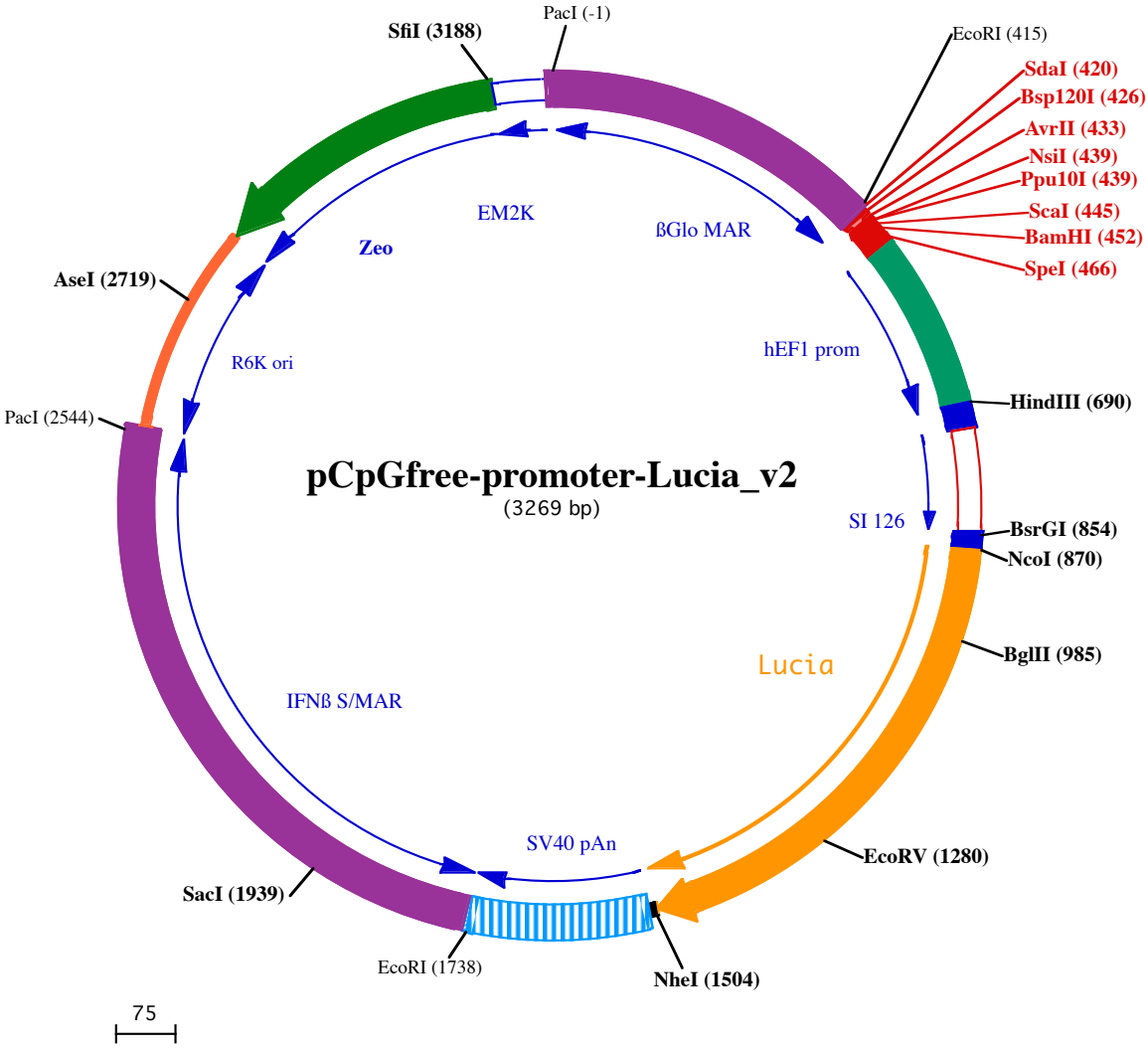
This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media.

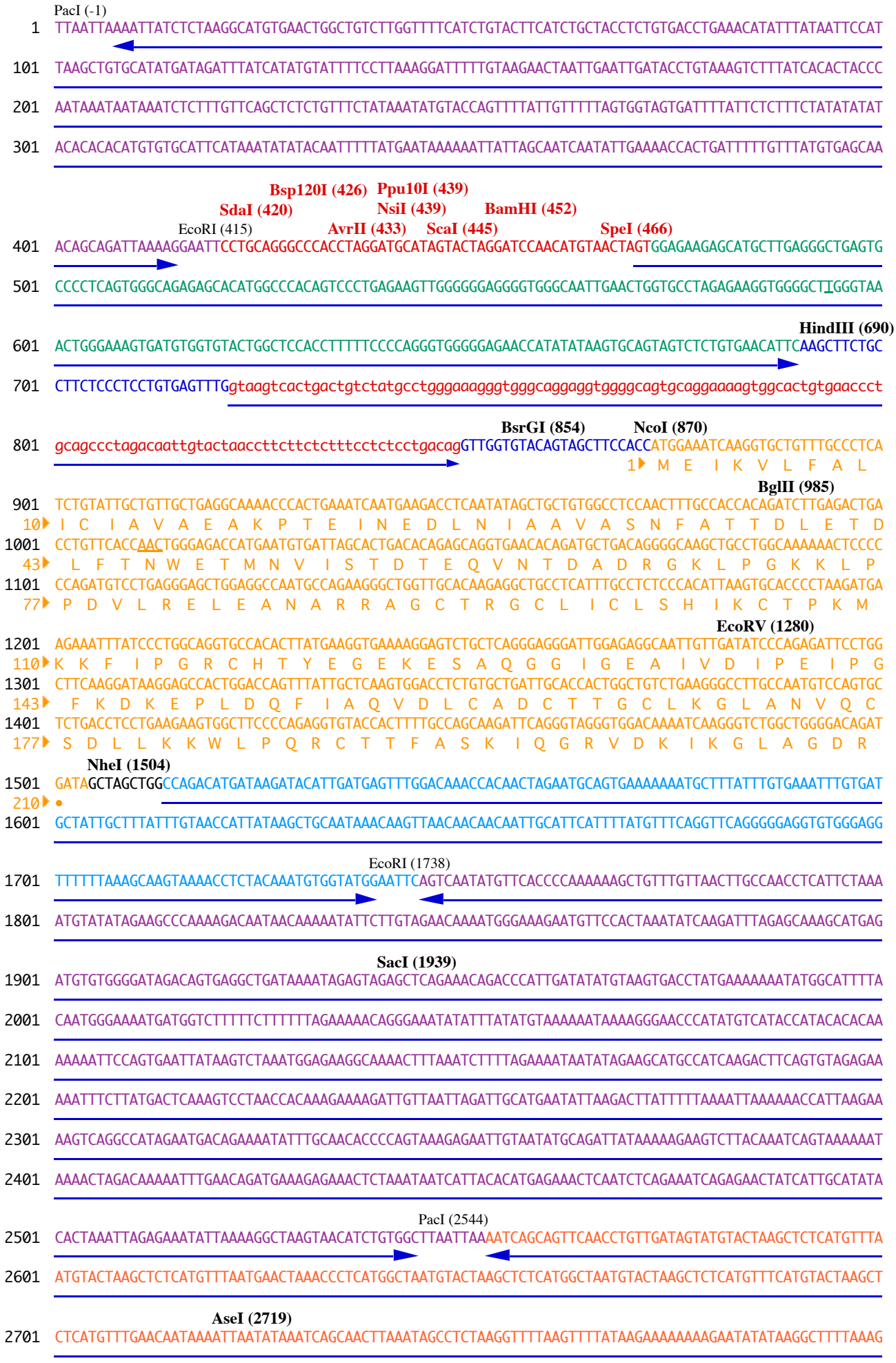
TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com

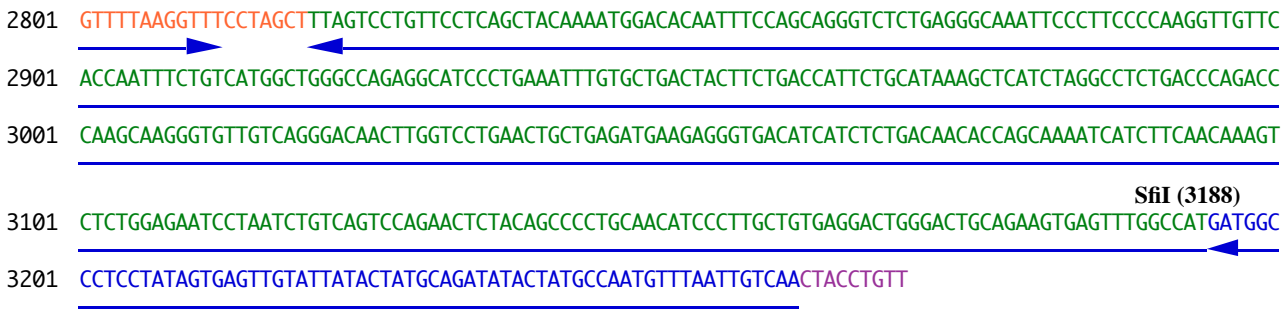
RELATED PRODUCTS

Product	Catalog Code
QUANTI-Luc™	rep-qlc1
ChemiComp GT115	gt115-11
pCpGfree-promoter (mSEAP)	pcpgf-prom
pCpGfree-basic (mSEAP)	pcpgf-bas
pCpGfree-basic-Lucia	pcpgf-baslc

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com


pCpGfree-promoter-Lucia_v2
(3269 bp)
SfiI (3188)
PacI (-1)
EcoRI (415)
SdaI (420)
Bsp120I (426)
AvrII (433)
NsiI (439)
Ppu10I (439)
ScaI (445)
BamHI (452)
SpeI (466)
HindIII (690)
BsrGI (854)
NcoI (870)
BglII (985)
EcoRV (1280)
NheI (1504)
EcoRI (1738)
SacI (1939)
AseI (2719)
PacI (2544)
EM2K
Zeo
ßGlo MAR
hEF1 prom
SI 126
Lucia
SV40 pAn
IFNß S/MAR
R6K ori
75


PacI (-1)
1 TTAATTAAAATTATCTCTAAGGCATGTGAACTGGCTGTCTTGGTTTTCATCTGTACTTCATCTGCTACCTCTGTGACCTGAAACATATTTATAATTCCAT
101 TAAGCTGTGCATATGATAGATTTATCATATGTATTTTCCTTAAAGGATTTTTGTAAGAACTAATTGAATTGATACCTGTAAAGTCTTTATCACACTACCC
201 AATAAATAATAAATCTCTTTGTTCAGCTCTCTGTTTCTATAAATATGTACCAGTTTTATTGTTTTTAGTGGTAGTGATTTTATTCTCTTTCTATATATAT
301 ACACACACATGTGTGCATTCATAAATATATACAATTTTTATGAATAAAAAATTATTAGCAATCAATATTGAAAACCACTGATTTTTGTTTATGTGAGCAA
Bsp120I (426)
Ppu10I (439)
SdaI (420)
NsiI (439)
BamHI (452)
EcoRI (415)
AvrII (433)
ScaI (445)
SpeI (466)
401 ACAGCAGATTAAAAGGAATTCCTGCAGGGCCCACCTAGGATGCATAGTACTAGGATCCAACATGTAACTAGTGGAGAAGAGCATGCTTGAGGGCTGAGTG
501 CCCCTCAGTGGGCAGAGAGCACATGGCCCACAGTCCCTGAGAAGTTGGGGGGAGGGGTGGGCAATTGAACTGGTGCCTAGAGAAGGTGGGGCTIGGGTAA
HindIII (690)
601 ACTGGGAAAGTGATGTGGTGTACTGGCTCCACCTTTTTCCCCAGGGTGGGGGAGAACCATATATAAGTGCAGTAGTCTCTGTGAACATTCAAGCTTCTGC
701 CTTCTCCCTCCTGTGAGTTTGgtaagtcactgactgtctatgcctgggaaagggtgggcaggaggtggggcagtgcaggaaaagtggcactgtgaaccct
BsrGI (854)
NcoI (870)
801 gcagccctagacaattgtactaaccttcttctctttcctctcctgacagGTTGGTGTACAGTAGCTTCCACCATGGAAATCAAGGTGCTGTTTGCCCTCA
1 M E I K V L F A L
BglII (985)
901 TCTGTATTGCTGTTGCTGAGGCAAAACCCACTGAAATCAATGAAGACCTCAATATAGCTGCTGTGGCCTCCAACTTTGCCACCACAGATCTTGAGACTGA
10 I C I A V A E A K P T E I N E D L N I A A V A S N F A T T D L E T D
1001 CCTGTTCACCAACTGGGAGACCATGAATGTGATTAGCACTGACACAGAGCAGGTGAACACAGATGCTGACAGGGGCAAGCTGCCTGGCAAAAAACTCCCC
43 L F T N W E T M N V I S T D T E Q V N T D A D R G K L P G K K L P
1101 CCAGATGTCCTGAGGGAGCTGGAGGCCAATGCCAGAAGGGCTGGTTGCACAAGAGGCTGCCTCATTTGCCTCTCCCACATTAAGTGCACCCCTAAGATGA
77 P D V L R E L E A N A R R A G C T R G C L I C L S H I K C T P K M
EcoRV (1280)
1201 AGAAATTTATCCCTGGCAGGTGCCACACTTATGAAGGTGAAAAGGAGTCTGCTCAGGGAGGGATTGGAGAGGCAATTGTTGATATCCCAGAGATTCCTGG
110 K K F I P G R C H T Y E G E K E S A Q G G I G E A I V D I P E I P G
1301 CTTCAAGGATAAGGAGCCACTGGACCAGTTTATTGCTCAAGTGGACCTCTGTGCTGATTGCACCACTGGCTGTCTGAAGGGCCTTGCCAATGTCCAGTGC
143 F K D K E P L D Q F I A Q V D L C A D C T T G C L K G L A N V Q C
1401 TCTGACCTCCTGAAGAAGTGGCTTCCCCAGAGGTGTACCACTTTTGCCAGCAAGATTCAGGGTAGGGTGGACAAAATCAAGGGTCTGGCTGGGGACAGAT
177 S D L L K K W L P Q R C T T F A S K I Q G R V D K I K G L A G D R
NheI (1504)
1501 GATAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGAT
210 •
1601 GCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGG
EcoRI (1738)
1701 TTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTCAGTCAATATGTTCACCCCAAAAAAGCTGTTTGTTAACTTGCCAACCTCATTCTAAA
1801 ATGTATATAGAAGCCCAAAAGACAATAACAAAAATATTCTTGTAGAACAAAATGGGAAAGAATGTTCCACTAAATATCAAGATTTAGAGCAAAGCATGAG
SacI (1939)
1901 ATGTGTGGGGATAGACAGTGAGGCTGATAAAATAGAGTAGAGCTCAGAAACAGACCCATTGATATATGTAAGTGACCTATGAAAAAAATATGGCATTTTA
2001 CAATGGGAAAATGATGGTCTTTTTCTTTTTTAGAAAAACAGGGAAATATATTTATATGTAAAAAATAAAAGGGAACCCATATGTCATACCATACACACAA
2101 AAAAATTCCAGTGAATTATAAGTCTAAATGGAGAAGGCAAAACTTTAAATCTTTTAGAAAATAATATAGAAGCATGCCATCAAGACTTCAGTGTAGAGAA
2201 AAATTTCTTATGACTCAAAGTCCTAACCACAAAGAAAAGATTGTTAATTAGATTGCATGAATATTAAGACTTATTTTTAAAATTAAAAAACCATTAAGAA
2301 AAGTCAGGCCATAGAATGACAGAAAATATTTGCAACACCCCAGTAAAGAGAATTGTAATATGCAGATTATAAAAAGAAGTCTTACAAATCAGTAAAAAAT
2401 AAAACTAGACAAAAATTTGAACAGATGAAAGAGAAACTCTAAATAATCATTACACATGAGAAACTCAATCTCAGAAATCAGAGAACTATCATTGCATATA
PacI (2544)
2501 CACTAAATTAGAGAAATATTAAAAGGCTAAGTAACATCTGTGGCTTAATTAAAATCAGCAGTTCAACCTGTTGATAGTATGTACTAAGCTCTCATGTTTA
2601 ATGTACTAAGCTCTCATGTTTAATGAACTAAACCCTCATGGCTAATGTACTAAGCTCTCATGGCTAATGTACTAAGCTCTCATGTTTCATGTACTAAGCT
AseI (2719)
2701 CTCATGTTTGAACAATAAAATTAATATAAATCAGCAACTTAAATAGCCTCTAAGGTTTTAAGTTTTATAAGAAAAAAAAGAATATATAAGGCTTTTAAAG


2801 GTTTTAAGGTTTCCTAGCTTTAGTCCTGTTCCTCAGCTACAAAATGGACACAATTTCCAGCAGGGTCTCTGAGGGCAAATTCCCTTCCCCAAGGTTGTTC
2901 ACCAATTTCTGTCATGGCTGGGCCAGAGGCATCCCTGAAATTTGTGCTGACTACTTCTGACCATTCTGCATAAAGCTCATCTAGGCCTCTGACCCAGACC
3001 CAAGCAAGGGTGTTGTCAGGGACAACTTGGTCCTGAACTGCTGAGATGAAGAGGGTGACATCATCTCTGACAACACCAGCAAAATCATCTTCAACAAAGT
SfiI (3188)
3101 CTCTGGAGAATCCTAATCTGTCAGTCCAGAACTCTACAGCCCCTGCAACATCCCTTGCTGTGAGGACTGGGACTGCAGAAGTGAGTTTGGCCATGATGGC
3201 CCTCCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGTTTAATTGTCAACTACCTGTT