

# STOP

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### TECHNICAL SUPPORT

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# pCpGfree-promoter

A mSEAP reporter plasmid without an enhancer and devoid of CpG dinucleotides

Catalog # pcpgf-prom

For research use only

Version 21F04-MMv02

## PRODUCT INFORMATION

### Content:

- 20 µg of pCpGfree-promoter 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.
- Lyophilized DNA is stable for 1 year when stored at -20 °C.
- 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<sup>1</sup>.

InvivoGen provides pCpGfree-basic and pCpGfree-promoter, 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  $\pi$ , encoded by the *pir* gene<sup>2</sup>.
- 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-1 $\alpha$  promoter and a multiple cloning site.
- The synthetic mSEAP $\Delta$ CpG gene: a CpG-free allele of the murine SEAP gene constructed by chemical synthesis.
- 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<sup>3</sup>. pCpGfree plasmids contains two MARs, from the 5' region of the human IFN- $\beta$  gene or  $\beta$ -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 R6K 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<sub>2</sub>O. 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

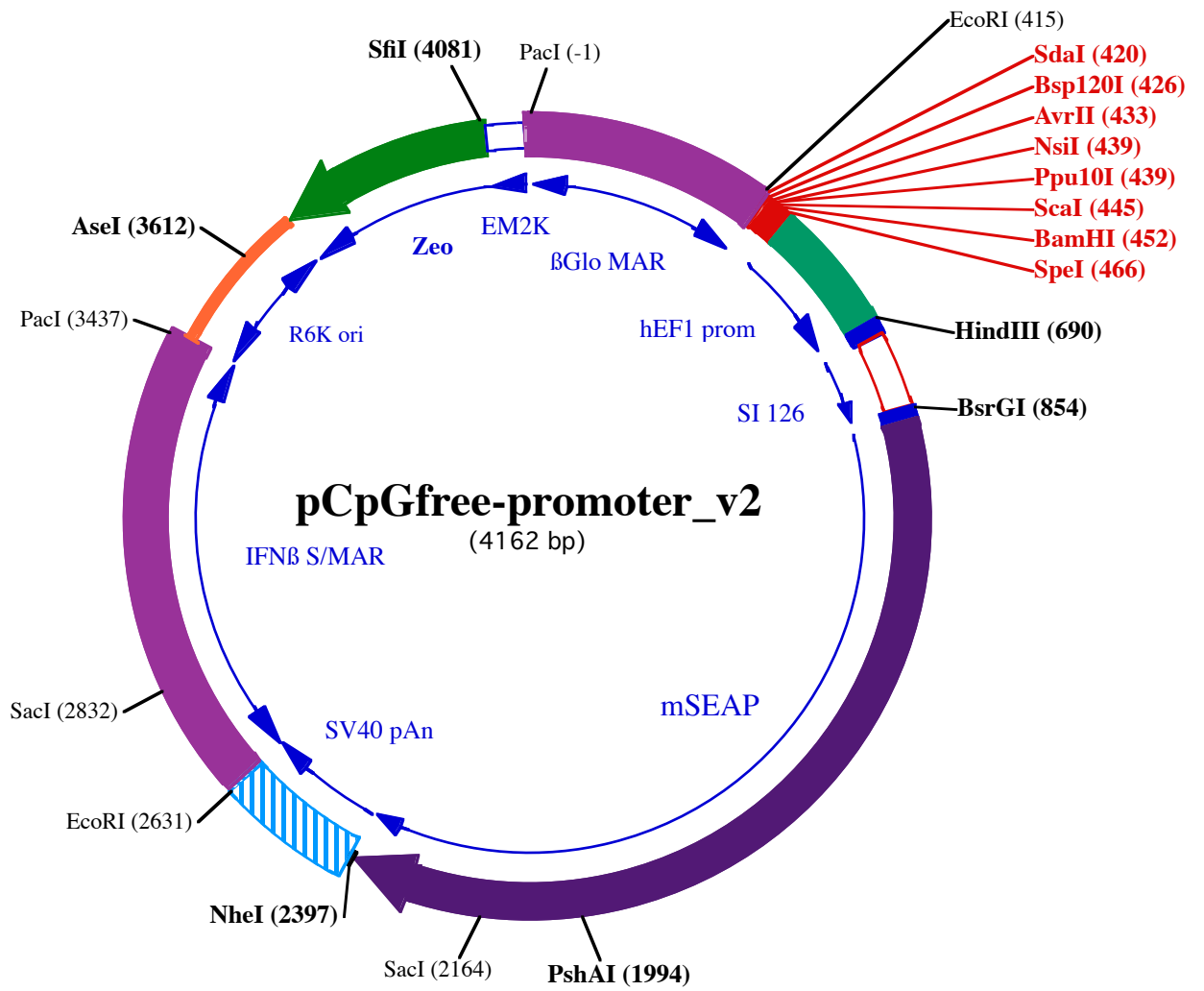
Plasmid amplification and cloning can be performed in *E. coli* GT115.

### 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  
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PacI (-1)  
 1 TTAATTAATAATTATCTCTAAGGCATGTGAAGCTGGCTGCTTGGTTTTTCATCTGTACTTCTATCTGCTACCTCTGTGACCTGAAACATATTTATAATTCCAT  
 101 TAAGCTGTGCATATGATAGATTTATCATATGATTTTTCTTAAAGGATTTTTGTAAGAACTAATTGAATTGATACCTGTAAGTCTTTATCACACTACCC  
 201 AATAAATAATAAATCTTTTGTTCAGCTCTCTGTTTCTATAAATATGTACCAGTTTTATTGTTTTAGTGGTAGTGATTTTATTCTCTTTCTATATATAT  
 301 ACACACACATGTGTGCATTCATAAATATATACAATTTTTATGAATAAAAAAATTATTAGCAATCAATATTGAAAACCACTGATTTTTGTTTTATGTGAGCAA  
  
 Bsp120I (426) Ppu10I (439)  
 SdaI (420) NsiI (439) BamHI (452)  
 EcoRI (415) AvrII (433) ScaI (445) SpeI (466)  
 401 ACAGCAGATTAAGGAATTCTCTGACGGGCCACCTAGGATGCATAGTACTAGGATCCAACATGTAAGTGTGGAGAAGAGCATGCTTGAGGGCTGAGTG  
 501 CCCCTCAGTGGGCAGAGACATGGCCACAGTCCCTGAGAAGTTGGGGGAGGGGTGGCAATTGAACTGGTGCCTAGAGAAGTGGGGCTGGGTAA  
  
 HindIII (690)  
 601 ACTGGAAAGTGATGTGGTGTACTGGCTCCACCTTTTTCCCAAGGTGGGGAGAACCATATATAAGTGCAGTAGTCTCTGTGAACATTCAAGCTTCTGC  
 701 CTCTCCCTCTGTGAGTTGgtaagtcactgactgtctatgctctgggaaaggggtgggcaggaggtggggcagtcaggaaaagtggcactgtgaacct  
  
 BsrGI (854)  
 801 gcagccctagacaattgtactaaccttcttctcttctctctctgacagGTTGGTGTACAGTAGCTCCACCATGTGGGTGCCTGTCTGCTATTGCTGG  
 901 GCTTAAGTCTTCAAGTTTCCCCAGTGTCTTCTGTGGAGGAGAAATCCTGCTTTTTGGAATAGGAAGCAGCTGAAGCCTTGGATGCAGCCAAGAA  
 101 lyLeuSe rLeuG lNva lCysP roSe rVa l l leP roVa lG lUG lUG luAsnP roA laPheT rpAsnA rgLysA laA laG luA laLeuAspA laA laLysLy  
 101 10 lyLeuSe rLeuG lNva lCysP roSe rVa l l leP roVa lG lUG lUG luAsnP roA laPheT rpAsnA rgLysA laA laG luA laLeuAspA laA laLysLy  
 43 sLeuLysP ro l leG lNth rSe rA laLysAsnLeuVa l l leLeuMe tG lyAspG lyMe tG lyVa lSe rTh rVa lTh rA laTh rA rg l leLeuLysG lyG lN  
 1101 CAACAAGTCTACTAGGCCAGAGACCCAGTTGGCAATGGACAGGTTCCCTCACATGGCCCTTTTCAAGACTTACAACACTGACAAGCAGATTCTGACT  
 77 G lNg lNg lyH isLeuG lyP roG lNth rG lNleuA laMe tAspA rgPheP roH isMe tA laLeuSe rLysTh rTy rAsnTh rAspLysG lN l leP roAspS  
 1201 CTGCTGGGACAGGCACAGCATTCTTGTGTGGAGTAAAAACCAACATGAAAGTCAATGGTCTTTCAGCTGCTGCCAGATTCAACCAGTGCACACCCACATG  
 110 110 e rA laG lyTh rG lyTh rA laPheLeuCysG lyVa lLysTh rAsnMe tLysVa l l leG lyLeuSe rA laA laA la rgPheAsnG lNcysAsnTh rTh rT r  
 1301 GGGCAATGAAGTGGTCTCTGTAATGCACAGGGCCAAAAAGCTGGGAAAAGTGTGGGTGTGGTGAACAACCTCTGTCCAGCATGCCTCTCTCTGTGGGA  
 143 pG lyAsnG luVa lVa lSe rVa lMe tH isA rgA laLysLysA laG lyLysSe rVa lG lyVa lVa l lTh rTh rTh rSe rVa lG lNth isA lSe rP roA laG ly  
 1401 ACTTATGCCACACAGTGAACAGAGGTTGGTACTCTGATGCTCAGATGCCTGCCTCAGCTTTACAAGATGGCTGCAAGGACATCAGCACCCAGCTCATCT  
 177 Th rTy rA laH isTh rVa lAsnA rgG lyT rpTy rSe rAspA laG lNMe tP roA laSe rA laLeuG lNAspG lyCysLysAsp l leSe rTh rG lNleu l leS  
 1501 CAAACATGGACATAGATGTCTTAGGGGTGGGAGAAGTTTCATGTTCCAAAGGGGACTCCTGACCAGGAGTACCCACAGACACAAAGCAGCTGG  
 210 210 e rAsnMe tAsp l leAspVa l l leLeuG lyG lyG lyA rgLysPheMe tPheP roLysG lyTh rP roAspG lNg lUty rP roTh rAspTh rLysG lN laG l  
 1601 CACAAGATTAGTGGTAGGAACCTTGTGCAAGAGTGGCTTGCAAGCATCAGGGAGCAAGGTATGTCTGGAACAGGAGTGAAGTAAATCCAGGCCTCTTTG  
 243 yTh rA rgLeuAspG lyA rgAsnLeuVa lG lNg lUty rPheA laLysH isG lNg lyA laA rgTy rVa l lTh rAsnA rgSe rG luLeu l leG lN laSe rLeu  
 1701 AACAGTCTGTCACTCACCTAATGGGTTATTTGAGCCAATGACATGAAGTATGAGATACACAGGGACCCTGCCAGGACCCCTCTTAGCAGAAATGA  
 277 AsnA rgSe rVa l lTh rH isLeuMe tG lyLeuPheG luP roAsnAspMe tLysTy rG lu l leH isA rgAspP roA laG lNAspP roSe rLeuA laG luMe tT  
 1801 CTGAAGTGTGTGAGGATGTTGCCAGAAATCCAAAAGGGTCTACCTCTTGTGGAGGGGGAAGGATTGATCATGGTCACCATGAGACAGTTGCTTA  
 310 h rG luVa lA laVa lA rgMe tLeuSe rA rgAsnP roLysG lyPheTy rLeuPheVa lG lUG lyG lyA rg l leAspH isG lyH isH isG luTh rVa lA laTy  
  
 PshAI (1994)  
 1901 CAGAGCCTTAACTGAGGCTGTGATGTTTCTGCTGTGGACAAGCTGACAACTGACCTCTGAGCAGGACACAATGATTCTAGTACTGCTGACCCAC  
 343 rA rgA laLeuTh rG luA laVa lMe tPheAspSe rA laVa lAspLysA laAspLysLeuTh rSe rG luG lNAspTh rMe t l leLeuVa l lTh rA laAspH is  
 2001 AGTCATGTTTTCTCTTTGGGGCTACACCAGAGGGGTGCTTCAATCTTTGGCCTGGCCCTTTCAAGGCAGAAGATGGGAAGAGTTTACCTCCATCC  
 377 Se rH isVa l lPheSe rPheG lyG lyTy rTh rG lN a rgG lyA laSe r l lePheG lyLeuA laP roPheLysA laG luAspG lyLysSe rPheTh rSe r l leL  
  
 SacI (2164)  
 2101 TCTATGGAAATGGTCTGGGTACAAGCTGCAAAATGGGGCCAGAGCTGATGTGACAGAAGAGGAGCTCCAACCAACCTACCAGCAGCAAGCAGCAGT  
 410 euTy rG lyAsnG lyP roG lyTy rLysLeuH isAsnG lyA laA rgA laAspVa l lTh rG luG luG luSe rSe rAsnP roTh rTy rG lNg lNg lN laA laVa  
 2201 CCCTCTTTCTCAGAAACCCACTCTGGGGAAGATGTGGCCATATTTGCCAGAGGCCCAAGCCCACTTGGTGCATGGAGTTTCCAGGAGCAGAATTACATA  
 443 lP roLeuSe rSe rG luTh rH isSe rG lyG luAspVa lA la l lePheA laA rgG lyP roG lN laH isLeuVa lH isG lyVa lG lNg lUG lNAsnTy r l le  
  
 NheI (2397)  
 2301 GCTCATGTAATGGCTTTTGTCTGCTTGGAGCCCTACACAGACTGTGGCTAGCCAGCCAGCAGCCAGTCCCTCTGCAAGTAAAGCCAGGCTAGAGCT  
 477 A laH isVa lMe tA laPheA laA laCysLeuG luP roTy rTh rAspCysG lyLeuA laSe rP roA laG lyG lNse rSe rA laVa lSe rP roG ly●●●  
 2401 AGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACAGTAAATGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTG  
  
 2501 CTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTTcAGGGGAGGTGTGGAGGTTTTTTA  
  
 EcoRI (2631)  
 2601 AAGCAAGTAAACCTCTACAAATGTGGTATGGAATTCAGTCAATATGTTACCCCAAAAAGCTGTTTGTAACTTGCCAACCTCATTCTAAAATGTATA  
  
 2701 TAGAAGCCAAAAGACAATAACAAAATATTCTTGTAGAACAAAATGGGAAGAATGTTCCACTAAATATCAAGATTTAGAGCAAAGCATGAGATGTGTG  
  
 SacI (2832)  
 2801 GGGATAGACAGTGAAGGCTGATAAAATAGAGTAGAGCTCAGAAACAGACCCATTGATATATGTAAGTGACCTATGAAAAAATATGGCATTTTACAATGGG

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3501 AAGCTCTCATGTTAATGAACTAAACCCTCATGGCTAATGTACTAAGCTCTCATGGCTAATGTACTAAGCTCTCATGTTTCATGTACTAAGCTCTCATGT  
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3701 GGTTCCTAGCTTAGTCCTGTTCTCAGCTACAAAATGGACACAATTTCCAGCAGGGTCTCTGAGGGCAAATCCCTTCCCAAGTTGTTACCAATT  
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