

STOP

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TECHNICAL SUPPORT

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pCpGfree-basic

A mSEAP reporter plasmid without a promoter and devoid of CpG dinucleotides

Catalog # pcpgf-bas

For research use only

Version 21F04-MMv02

PRODUCT INFORMATION

Content:

- 20 µg of pCpGfree-basic 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 1 year when stored at -20 °C.
- Resuspended DNA is stable 6 months when stored at -20 °C.
- Bacteria should be stored at -20 °C and are stable up to 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 methylation¹.

InvivoGen provides pCpGfree-basic a murine secreted embryonic alkaline phosphatase (mSEAP) reporter plasmid that is completely devoid of CpG dinucleotides and lacks the entire promoter region. It contains a multiple cloning site upstream of the mSEAP reporter gene. Expression of mSEAP in cells transfected with this plasmid depends on the insertion of a functional promoter or enhancer/promoter cassette upstream from the mSEAP gene. Thus, pCpGfree-basic allows to study the effect of CpG methylation on a promoter, alone or combined with 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* gene².
- 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 synthetic mSEAPΔCpG 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 domains³. 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, Hind III 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 H₂O. 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

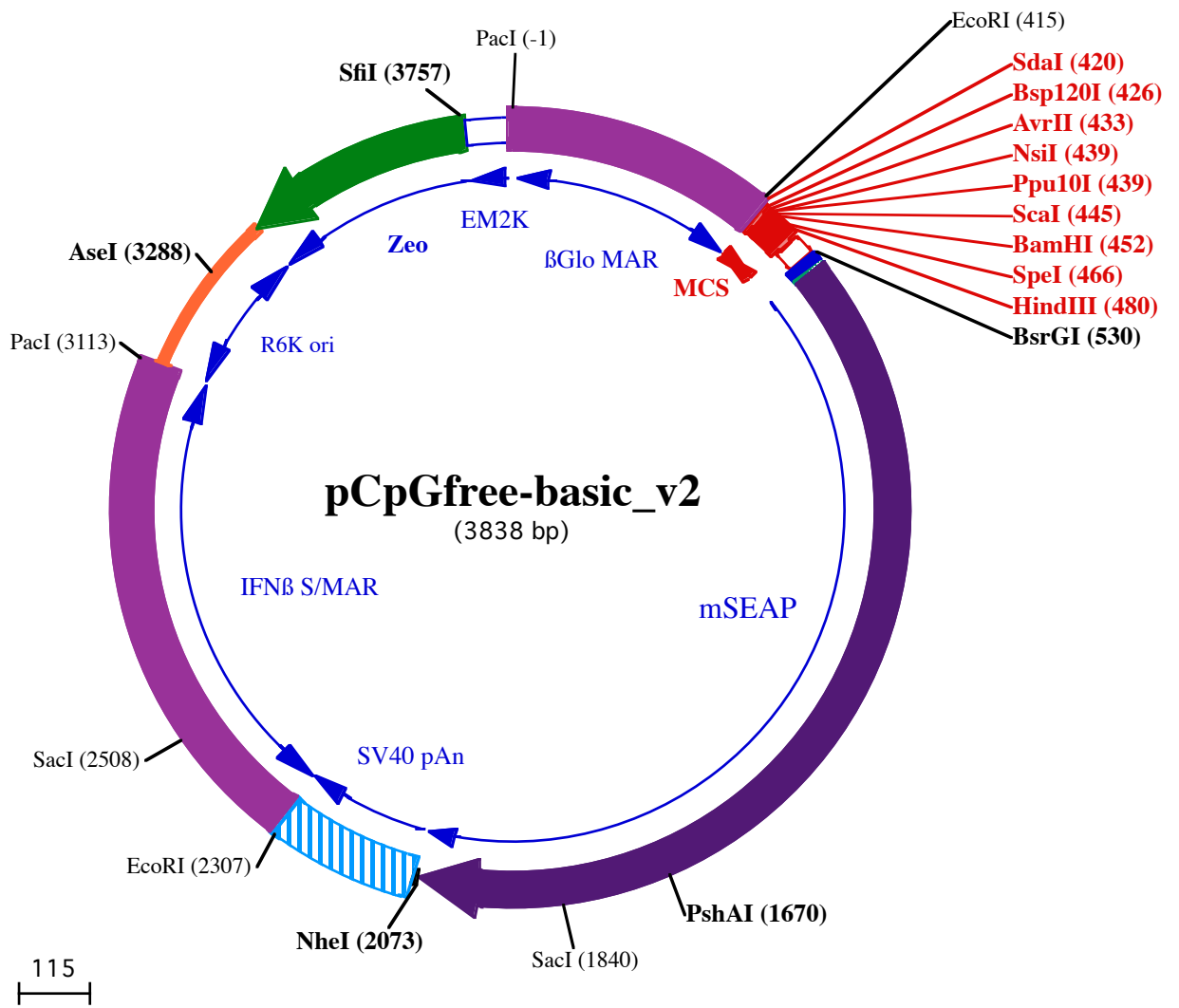
Plasmid amplification and cloning can be performed in *E. coli* GT115.

Zeocin™ usage

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

TECHNICAL SUPPORT

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PacI (-1)
 1 TTAATTAATAATTATCTCTAAGGCATGTGAAGCTGGCTGCTTGGTTTTTCATCTGTACTTCTATCTGCTACCTCTGTGACCTGAAACATATTTATAATTCCAT
 101 TAAGCTGTGCATATGATAGATTTATCATATGTATTTTCTTAAAGGATTTTTGTAAGAACTAATTGAATTGATACCTGTAAGTCTTTATCACACTACCC
 201 AATAAATAATAAATCTTTTGTTCAGCTCTCTGTTTCTATAAATATGTACCAGTTTTATTGTTTTAGTGGTAGTGATTTTATTCTCTTTCTATATATAT
 301 ACACACACATGTGTGCATTCATAAATATATACAATTTTTATGAATAAAAAATTATTAGCAATCAATATTGAAAACCACTGATTTTTGTTTTATGTGAGCAA

Bsp120I (426) Ppu10I (439)
SdaI (420) NsiI (439) BamHI (452)
EcoRI (415) AvrII (433) ScaI (445) SpeI (466) HindIII (480)
BsrGI (530)

401 ACAGCAGATTAAGGAATTCTCTGCAGGGCCACCTAGGATGCATAGTACTAGGATCCAACATGTAAGTAGTAGCATGCAAAGCTTAGAAttgtactaac
 501 cttcttctcttctctctctgacagGTTGGTGTACAGTAGCTCCACCATGTGGGTGCCTGTCTGCTATTGCTGGCTTAAGTCTTCAAGTTTGCCCA
 601 GTGTCATTCTGTGGAGGAGGAATCCTGCTTTTTGGAATAGGAAGGCAGCTGAAGCCTTGATGCAGCCAAGAAGCTCAAGCCATTTCAGACATCTGC
 18▶ e rVa I l l eP roVa IG luG luG luAsnP roA laPheT rpAsnA rglYsA laA laG luA laLeuAspA laA laLysLysLeuLysP ro I leG lnTh rSe rA I
 701 AAAGAATCTTGTATCCTCATGGGTGATGGAATGGGTGTCTCCACTGTAACAGCCACCAGGATTCTGAAGGGCCAGCAACAAGTGCATCTAGGCCAGAG
 51▶ aLysAsnLeuVa I l leLeuMe tG lyAspG lyMe tG lyVa lSe rTh rVa lTh rA laTh rARg I leLeuLysG lyG lnG lnG lnG lyH isLeuG lyP roG lu
 801 ACCCAGTTGGCAATGGACAGGTTCCCTCACATGGCCCTTCCAAGACTTACAACACTGACAAGCAGATTCTGACTCTGCTGGACAGGCACAGCATTCT
 85▶ Th rG lnLeuA laMe tAspARgPheP roH isMe tA laLeuSe rLysTh rTy rAsnTh rAspLysG ln I leP roAspSe rA laG lyTh rG lyTh rA laPheL
 901 TGTGTGGAGTAAAAACCAACATGAAAGTCATTGGTCTTTTCAGCTGTGCCAGATTCAACCAAGTGAACACCACATGGGGCAATGAAGTGGTCTCTGTAAT
 118▶ euCysG lyVa lLysTh rAsnMe tLysVa I l leG lyLeuSe rA laA laA laArgPheAsnG lnCysAsnTh rTh rT rpG lyAsnG luVa lVa lSe rVa Me
 1001 GCACAGGGCCAAAAAGCTGGGAAAAGTGTGGTGTGGTGAACACCCTCTGTCCAGCATGCCTCTCTGCTGGAACCTTATGCCACACAGTGAACAGA
 151▶ tH isArgA laLysLysA laG lyLysSe rVa IG lyVa Na lTh rTh rTh rSe rVa IG lnH isA laSe rP roA laG lyTh rTy rA laH isTh rVa lAsnArg
 1101 GGTGGTACTCTGATGCTCAGATGCCTGCCTCAGCTTTACAAGATGGCTGCAAGGACATCAGCACCCAGCTCATCTCAACATGGACATAGATGTCTATCT
 185▶ G lyT rpTy rSe rAspA laG lnMe tP roA laSe rA laLeuG lnAspG lyCysLysAsp I leSe rTh rG lnLeu I leSe rAsnMe tAsp I leAspVa I l leL
 1201 TAGGGGTGGGAGAAAGTTCATGTTCCAAAGGGGACTCCTGACCAGGAGTACCCACAGACACAAAGCAGGCTGGCACAAGATTAGATGGTAGGAACT
 218▶ euG lyG lyG lyArgLysPheMe tPheP roLysG lyTh rP roAspG lnG luTy rP roTh rAspTh rLysG lnA laG lyTh rArgLeuAspG lyArgAsnLe
 1301 TGTGCAAGAGTGGCTTCCAAGCATCAGGAGCAAGGTATGTCTGGAACAGGAGTGAAGTAAATCCAGGCCCTTTGAAACAGGTCTGTCACTCACCTAATG
 251▶ uVa IG lnG luT rpLeuA laLysH isG lnG lyA laArgTy rVa lT rpAsnA rglSe rG luLeu I leG lnA laSe rLeuAsnArgSe rVa lTh rH isLeuMe t
 1401 GGGTATTGAGCCCAATGACATGAAGTATGAGATACACAGGACCTGCCAGGACCCCTCTTAGCAGAAATGACTGAAGTTGCTGTGAGGATGTTGT
 285▶ G lyLeuPheG luP roAsnAspMe tLysTy rG lu I leH isArgAspP roA laG lnAspP roSe rLeuA laG luMe tTh rG luVa lA laVa lA rglMe tLeuS
 1501 CCAGAAATCCAAAAGGTTCTACCTCTTTGTTGAGGGGGGAAGGATTGATCATGGTACCATGAGACAGTTGCTTACAGAGCCCTTAAGTGAAGGCTGTGAT
 318▶ e rArgAsnP roLysG lyPheTy rLeuPheVa IG luG lyG lyArg I leAspH isG lyH isH isG luTh rVa lA laTy rArgA laLeuTh rG luA laVa Me

PshAI (1670)

1601 GTTTATTCTGCTGTGGACAAGGCTGACAACTGACCTCTGAGCAGGACACAATGATTCTAGTACTGCTGACCACAGTCATGTTTTCTCTTTGGGGG
 351▶ tPheAspSe rA laVa lAspLysA laAspLysLeuTh rSe rG luG lnAspTh rMe t I leLeuVa lTh rA laAspH isSe rH isVa lPheSe rPheG lyG ly
 1701 TACACCAGAGGGGTGCTTCAATCTTTGGCTGGCCCTTTCAAGGCAGAAGATGGGAAGAGTTTACCTCCATCTCTATGGGAATGGTCTGGGTACA
 385▶ Ty rTh rG lnArgG lyA laSe r I lePheG lyLeuA laP roPheLysA laG luAspG lyLysSe rPheTh rSe r I leLeuTy rG lyAsnG lyP roG lyTy rL

SacI (1840)

1801 AGCTGCAATGGGGCCAGAGCTGATGTGACAGAAGAGGAGCTCCAACCACTACCAGCAGCAAGCAGCAGTCCCTCTTTCTCAGAAACCACTC
 418▶ ysLeuH isAsnG lyA laArgA laAspVa lTh rG luG luG luSe rSe rAsnP roTh rTy rG lnG lnG lnA laVa lP roLeuSe rSe rG luTh rH isSe
 1901 TGGGGAAGATGTGGCCATATTTGCCAGAGGCCCAAGCCACTTGGTGCATGGAGTTCAGGAGCAGAATTACATAGCTCATGTAATGGCTTTTGTCTGCT
 451▶ rG lyG luAspVa lA la I lePheA laArgG lyP roG lnA laH isLeuVa lH isG lyVa lG lnG luG lnAsnTy r I leA laH isVa Me tA laPheA laA la

NheI (2073)

2001 TGCTTGGAGCCCTACACAGACTGTGGCTAGCCAGCCAGCAGGCCAGTCTCTGCAGTAAGCCAGGCTAGAGCTAGCTGGCCAGACATGATAAGATAC
 485▶ CysLeuG luP roTy rTh rAspCysG lyLeuA laSe rP roA laG lyG lnSe rSe rA laVa lSe rP roG ly●●●
 2101 ATTGATGAGTTTGGACAAACCACAACCTAGAATGCAAGTAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCT

2201 GCAATAAACAAGTTAACAACAACAATTGCATTCAATTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGTTTTTTAAAGCAAGTAAAACTCTACAATG

EcoRI (2307)

2301 TGGTATGGAATTCAGTCAATATGTTACCCCAAAAAAGCTGTTTGTAACTTGCCAACCTCATTCTAAAATGTATATAGAAGCCAAAAAGACAATAACAA
 2401 AAATATTCTGTAGAACAAAATGGGAAAGATGTTCCACTAAATATCAAGATTTAGAGCAAAGCATGAGATGTGTGGGATAGACAGTGAGGCTGATAAA

SacI (2508)

2501 ATAGAGTAGAGCTCAGAAACAGACCCATTGATATATGTAAGTGACCTATGAAAAAATATGGCATTTTACAATGGGAAAATGATGGTCTTTTTCTTTTT
 2601 AGAAAAACAGGGAATATATTTATATGTAATAAATAAAGGGAACCCATATGTCATACCATACACAAAAAATTCAGTGAATTATAAGTCTAAATGG
 2701 AGAAGGCAAACTTTAAATCTTTTAGAAAAATATAGAAGCATGCCATCAAGACTTCAGTGTAGAGAAAAATTTCTTATGACTCAAAGTCTAACCACA
 2801 AAGAAAAGATTGTAATTAGATTGCATGAATATTAAGACTTATTTTTAAATTAATAAACCATTAAGAAAAGTCAAGCCATAGAATGACAGAAAATATTT
 2901 GCAACACCCAGTAAAGAGAATTGTAATATGCAGATTATAAAAAAGAGTCTTACAATCAGTAAAAATAAACTAGACAAAAATTTGAACAGATGAAAG

3001 AGAAACTCTAAATAATCATTACACATGAGAACTCAATCTCAGAAATCAGAGAACTATCATTGCATATACACTAAATTAGAGAAATATTTAAAGGCTAAG

3101 TAACATCTGTGGCTTAATTA^{PacI (3113)}AATCAGCAGTTCAACCTGTTGATAGTATGTACTAAGCTCTCATGTTAATGTACTAAGCTCTCATGTTAATGAACTAA

3201 ACCCTCATGGCTAATGTACTAAGCTCTCATGGCTAATGTACTAAGCTCTCATGTTTCATGTACTAAGCTCTCATGTTTGAAACAATAAAATTAATAAAT^{AseI (3288)}

3301 CAGCAACTTAAATAGCCTCTAAGGTTTTAAGTTTTATAAGAAAAAAGAATATATAAGGCTTTAAAGGTTTTAAGGTTTCCTAGCTTTAGTCCTGTTC

3401 CTCAGCTACAAAATGGACACAATTTCCAGCAGGGTCTGAGGGCAAATCCCTTCCCAAGTTGTTACCAATTTCTGTCATGGCTGGCCAGAGGCA

3501 TCCCTGAAATTTGTGCTGACTACTTCTGACCATTCTGCATAAAGCTCATCTAGGCCTCTGACCCAGACCCAAGCAAGGGTGTGTCAGGGACAACCTGGT

3601 CCTGAACTGCTGAGATGAAGAGGGTGACATCATCTCTGACAACACCAGCAAATCATCTTCAACAAAGTCTCTGGAGAATCCTAATCTGTCAGTCCAGAA

3701 CTCTACAGCCCTGCAACATCCCTTGCTGTGAGGACTGGGACTGCAGAAGTGAGTTTGGCCAT^{SfiI (3757)}GATGGCCCTCTATAGTGAGTTGTATTATACTATGCA

3801 GATATACTATGCCAATGTTAATTGTCAACTACCTGTT
