

STOP

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TECHNICAL SUPPORT

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pCpGfree-mSEAP

A mSEAP expression plasmid completely devoid of CpG dinucleotides

Catalog # pcpgf-mseap

For research use only

Version 21F04-MMv02

PRODUCT INFORMATION

Content:

- 20 µg of pCpGfree-mSEAP 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

InvivoGen has developed a new family of plasmids that are completely devoid of CpG dinucleotides. These plasmids yield high levels of transgene expression both *in vitro* and *in vivo*, and in contrast to CMV-based plasmids allow sustained expression *in vivo*.

pCpG 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.

pCpG plasmids represent valuable tools to study the effects of CpGs on gene expression *in vivo* and *in vitro*, using cell lines expressing TLR9, as well as their effects on the innate and acquired immune systems. Furthermore, for RNAi applications, InvivoGen has designed pCpG-siRNA a plasmid that allows long term production of siRNAs *in vivo*.

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: 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

- Mammalian promoter: The CpG-free promoter combines the mouse CMV enhancer, the human elongation factor 1 alpha core promoter and 5'UTR containing a synthetic intron.
- 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 domains². 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.

- pCpG-mSEAP expresses the synthetic mSEAPCpG gene, a CpG-free allele of the mSEAP gene constructed by chemical synthesis.

Due to the presence of the R6K γ 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. Wu F. *et al.* 1995. A DNA segment conferring stable maintenance on R6K gamma-origin core replicons. J Bacteriol. 177(22):6338-45. 2. 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.

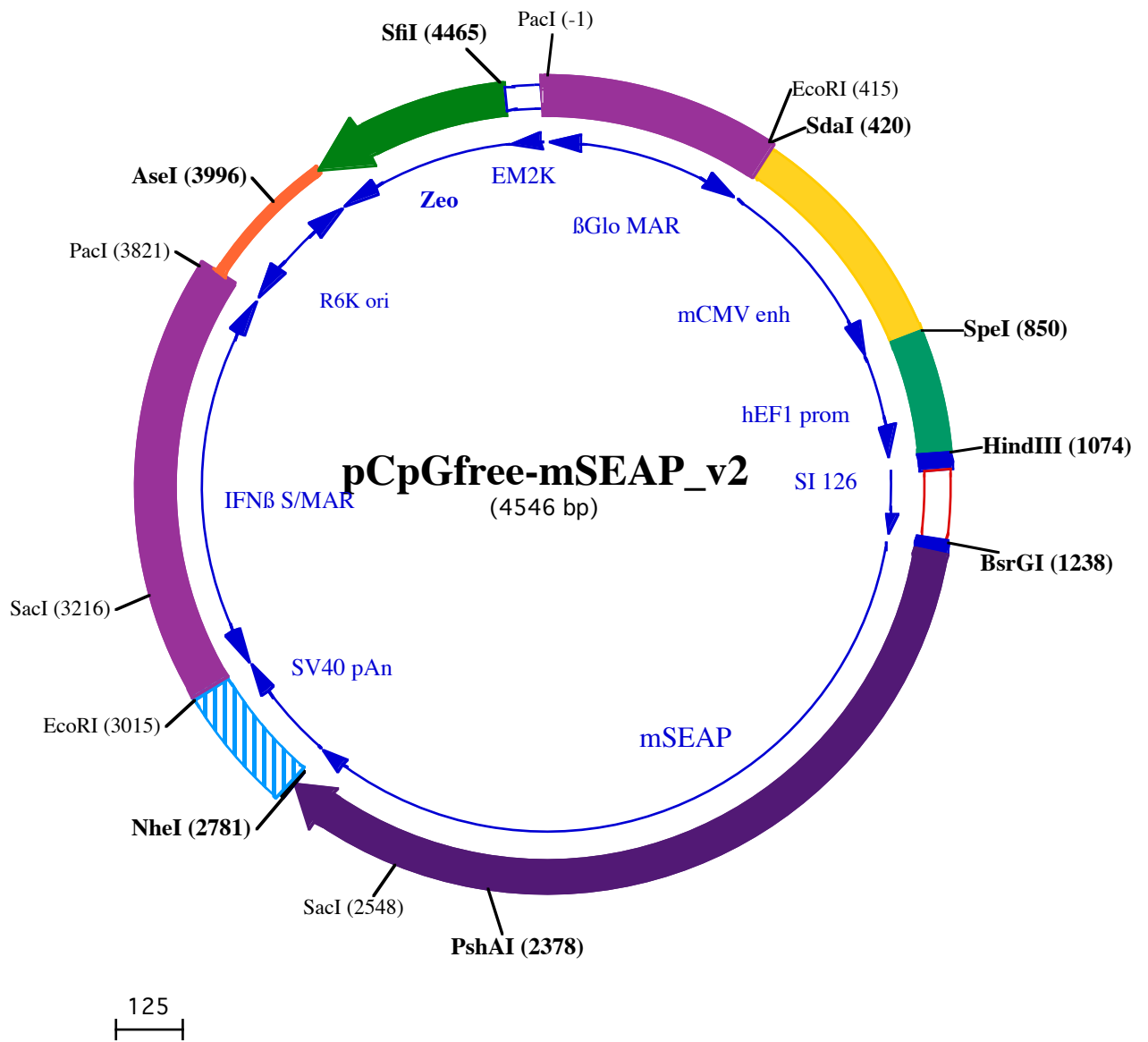
Zeocin™ usage

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

TECHNICAL SUPPORT

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1 Pacl (-1)
 1 TTAATTAATAATTATCTCTAAGGCATGTGAAGCTGGCTGCTTGGTTTTTCATCTGTACTTCTCTGCTACCTCTGTGACCTGAAACATATTTATAATTCCAT
 101 TAAGCTGTGCATATGATAGATTATCATATGATTTTTCTTAAAGGATTTTTGTAAGAACTAATTGAATTGATACCTGTAAGTCTTTATCACACTACCC
 201 AATAAATAATAAATCTTTTGTTCAGCTCTCTGTTCTATAAATATGTACCAGTTTTATTGTTTTAGTGGTAGTGATTTTATTCTCTTTCTATATATAT
 301 ACACACACATGTGTGCATTCATAAATATATACAATTTTTATGAATAAAAAAATTATTAGCAATCAATATTGAAAACCACTGATTTTTGTTTTATGTGAGCAA

SdaI (420)
 EcoRI (415)
 401 ACAGCAGATTAAGGAATTCTCTGCAGGAGTCAATGGGAAAAACCCATTGGAGCCAAGTACACTGACTCAATAGGGACTTTCCATTGGGTTTTGCCCAGT
 501 ACATAAGGTCAATAGGGGGTGAAGTCAACAGGAAAGTCCCATTGGAGCCAAGTACATTGAGTCAATAGGGACTTTCCAATGGGTTTTGCCCAGTACATAAG
 601 GTCAATGGGAGTAAAGCCAATGGGTTTTTCCATTACTGACATGATACTGAGTCAATAGGGACTTTCCAATGGGTTTTGCCCAGTACATAAGGTCAATA
 701 GGGGTGAATCAACAGGAAAGTCCCATTGGAGCCAAGTACACTGAGTCAATAGGGACTTTCCATTGGGTTTTGCCCAGTACAAAAGGTCAATAGGGGGTGA

SpeI (850)
 801 GTCAATGGGTTTTTCCATTATTGGCACATACATAAGGTCAATAGGGGTGACTAGTGGAGAAGAGCATGCTTGAGGGCTGAGTGCCCTCAGTGGGCAGA
 901 GAGCAGTGGCCACAGTCCCTGAGAAGTTGGGGGAGGGGTGGCAATTGAACTGGTGCCTAGAGAAGGTGGGCTGGGTAAGTGGGAAAGTGGTGT

HindIII (1074)
 1001 GGTGACTGGCTCCACCTTTTTCCAGGGTGGGGGAGAACCATATATAAGTGCAGTAGTCTCTGTGAACATTCAAGCTTCTGCCTTCTCCCTCCTGTGA
 1101 GTTTGtaagtcactgactgtctatgctgggaaaggggtgggcaggaggtggggcagtcaggaaaagtggcactgtgaacctgcagccctagacaatt

BsrGI (1238)
 1201 gtactaaccttcttctcttctctctctgacagGTTGGTGTACAGTAGTCCACCATGTGGGGTGCCTGTCTGCTATTGCTGGCTTAAGTCTTCAAGT
 1301 TTGCCCCAGTGTCTCTGTGGAGGAGGAGAATCTGCTTTTTGGAATAGGAAGGCAGCTGAAGCCTTGGATGCGCCAAGAAGCTCAAGCCATTGAG
 1501 GCCCAGAGACCCAGTTGCAATGGACAGGTTCCCTCACATGGCCCTTCCAAGACTTACAACACTGACAAGCAGATTCTGACTCTGCTGGACAGGCAC
 1701 TCTGTAATGCACAGGGCCAAAAAGCTGGGAAAAGTGTGGGTGTGGTGCACACCACTCTGTCAGCATGCCTCTCTGCTGGAACCTTATGCCACACAG
 1901 TGTCATCTTAGGGGTGGGAGAAAGTTCATGTTCCAAAGGGGACTCCTGACCAGGATACCCACAGACACAAAGCAGGCTGGCACAAGATTAGATGGT
 2101 ACCTAATGGGTTATTTGAGCCCAATGACATGAAGTATGAGATACACAGGACCTGCCAGGACCCCTCTTAGCAGAAATGACTGAAGTTGCTGTGAG
 2301 GCTGTGATGTTTATTCTGCTGTGGACAAGGCTGACAACTGACCTCTGAGCAGGACACAATGATTCTAGTACTGCTGACCACAGTCTTTTCTCCT
 2501 TGGGTACAAGCTGCACAATGGGGCCAGAGCTGATGTGACAGAAGAGGAGCTCCAACCAACCTACCAGCAGCAAGCAGCAGTCCCTCTTTCTCAGAA
 2701 TTGCTGCTTGGTGGAGCCCTACACAGACTGTGGCTAGCCAGCCAGCAGGCCAGTCCCTCTGCAAGTAAAGCCAGGCTAGAGCTAGGCGCAGACATGA
 2901 TATAAGCTGCAATAACAAGTAAACAACAACATTCATTCTTTATGTTTCAGGTTTCAGGGGGAGGTGGGAGGTTTTTAAAGCAAGTAAACCTC

PshAI (2378)
 2301 GCTGTGATGTTTATTCTGCTGTGGACAAGGCTGACAACTGACCTCTGAGCAGGACACAATGATTCTAGTACTGCTGACCACAGTCTTTTCTCCT
 2401 TGGGGGCTACACCCAGAGGGGTGCTTCAATCTTTGGCTGGCCCTTCAAGGCAGAAAGATGGGAAGAGTTTACCTCCATCCTCTATGGGAATGGTCC
 2501 TGGGTACAAGCTGCACAATGGGGCCAGAGCTGATGTGACAGAAGAGGAGCTCCAACCAACCTACCAGCAGCAAGCAGCAGTCCCTCTTTCTCAGAA
 2601 ACCCACTCTGGGGAAGATGTGGCCATATTTGCCAGAGGCCCAAGCCACTTGGTGCATGGAGTTACAGGAGCAGAATTACATAGCTCATGTAATGGCTT
 2701 TTGCTGCTTGGTGGAGCCCTACACAGACTGTGGCTAGCCAGCCAGCAGGCCAGTCCCTCTGCAAGTAAAGCCAGGCTAGAGCTAGGCGCAGACATGA
 2801 TAAGATACATTGATGAGTTTGGACAAACCAACTAGAATGCAGTAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCAT
 2901 TATAAGCTGCAATAACAAGTAAACAACAACATTCATTCTTTATGTTTCAGGTTTCAGGGGGAGGTGGGAGGTTTTTAAAGCAAGTAAACCTC

SacI (2548)
 2501 TGGGTACAAGCTGCACAATGGGGCCAGAGCTGATGTGACAGAAGAGGAGCTCCAACCAACCTACCAGCAGCAAGCAGCAGTCCCTCTTTCTCAGAA
 2601 ACCCACTCTGGGGAAGATGTGGCCATATTTGCCAGAGGCCCAAGCCACTTGGTGCATGGAGTTACAGGAGCAGAATTACATAGCTCATGTAATGGCTT
 2701 TTGCTGCTTGGTGGAGCCCTACACAGACTGTGGCTAGCCAGCCAGCAGGCCAGTCCCTCTGCAAGTAAAGCCAGGCTAGAGCTAGGCGCAGACATGA
 2801 TAAGATACATTGATGAGTTTGGACAAACCAACTAGAATGCAGTAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCAT
 2901 TATAAGCTGCAATAACAAGTAAACAACAACATTCATTCTTTATGTTTCAGGTTTCAGGGGGAGGTGGGAGGTTTTTAAAGCAAGTAAACCTC

NheI (2781)
 2701 TTGCTGCTTGGTGGAGCCCTACACAGACTGTGGCTAGCCAGCCAGCAGGCCAGTCCCTCTGCAAGTAAAGCCAGGCTAGAGCTAGGCGCAGACATGA
 2801 TAAGATACATTGATGAGTTTGGACAAACCAACTAGAATGCAGTAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCAT
 2901 TATAAGCTGCAATAACAAGTAAACAACAACATTCATTCTTTATGTTTCAGGTTTCAGGGGGAGGTGGGAGGTTTTTAAAGCAAGTAAACCTC

EcoRI (3015)
3001 TACAAATGTGGTATGGAATTCAGTCAATATGTTACCCCAAAAAAGCTGTTTGTAACTTGCCAACCTCATTCTAAAATGTATATAGAAGCCCAAAAGAC
3101 AATAACAAAATATTCTTGTAGAACAAAATGGGAAAGAATGTTCCACTAAATATCAAGATTTAGAGCAAAGCATGAGATGTGTGGGATAGACAGTGAGG

SacI (3216)
3201 CTGATAAAATAGAGTAGAGCTCAGAAACAGACCCATTGATATATGTAAGTGACCTATGAAAAAATATGGCATTTTACAATGGGAAATGATGGTCTTTT
3301 TCTTTTTTAGAAAAACAGGGAATATATTTATATGTAATAAATAAGGGAAACCATATGTCATACCATACACAAAAAATTCAGTGAATTATAAGT
3401 CTAATGGAGAAGCAAACTTTAAATCTTTAGAAAATAATATAGAAGCATGCCATCAAGACTTCAGTGTAGAGAAAAATTTCTTATGACTCAAAGTCC
3501 TAACCACAAAGAAAAGATTGTTAATTAGATTGCATGAATATTAAGACTTATTTTTAAAATTA AAAACCATTAAGAAAAGTCAGGCCATAGAATGACAGA
3601 AAATATTTGCAACACCCAGTAAAGAGAATTGTAATATGCAGATTATAAAAAGAAGTCTTACAAATCAGTAAAAATAAACTAGACAAAAATTTGAACA
3701 GATGAAAGAGAACTCTAAATAATCATTACACATGAGAACTCAATCTCAGAAATCAGAGAACTATCATTGCATATACACTAAATTAGAGAAATATTA

PacI (3821)
3801 AGGCTAAGTAACATCTGTGGCTTAATTAATAATCAGCAGTTCACCTGTTGATAGTATGACTAAGCTCTCATGTTAATGACTAAGCTCTCATGTTAA

AseI (3996)
3901 TGAACATAACCTCATGGCTAATGTACTAAGCTCTCATGGCTAATGTACTAAGCTCTCATGTTTCATGTACTAAGCTCTCATGTTTGAACAATAAAATTA
4001 ATATAATCAGCAACTTAAATAGCCTCTAAGGTTTTAAGTTTTATAAGAAAAAAGAATATATAAGGCTTTTAAAGGTTTTAAGGTTTCCTAGCTTTAG
4101 TCCTGTTCTCAGCTACAAAATGGACACAATTTCCAGCAGGGTCTCTGAGGGCAAATTCCTTCCCAAGGTTGTTACCAATTTCTGTCATGGCTGGGC
4201 CAGAGGCATCCCTGAAATTTGTGCTGACTACTTCTGACCATTCTGCATAAAGCTCATCTAGGCCTCTGACCAGACCAAGCAAGGGTGTGTCAGGGAC
4301 AACTTGGTCTGAACTGCTGAGATGAAGAGGGTGACATCATCTCTGACAACACCAGCAAAATCATCTTCAACAAAGTCTCTGGAGAATCCTAATCTGTCA

SfiI (4465)
4401 GTCCAGAACTCTACAGCCCTGCAACATCCCTTGCTGTGAGGACTGGGACTGCAGAAGTGAAGTTGGCCATGATGGCCCTCTATAGTGAGTTGATTAT
4501 ACTATGCAGATATACTATGCCAATGTTAATTGCAACTACCTGTT