STOP

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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[1].

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 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.

• 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 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
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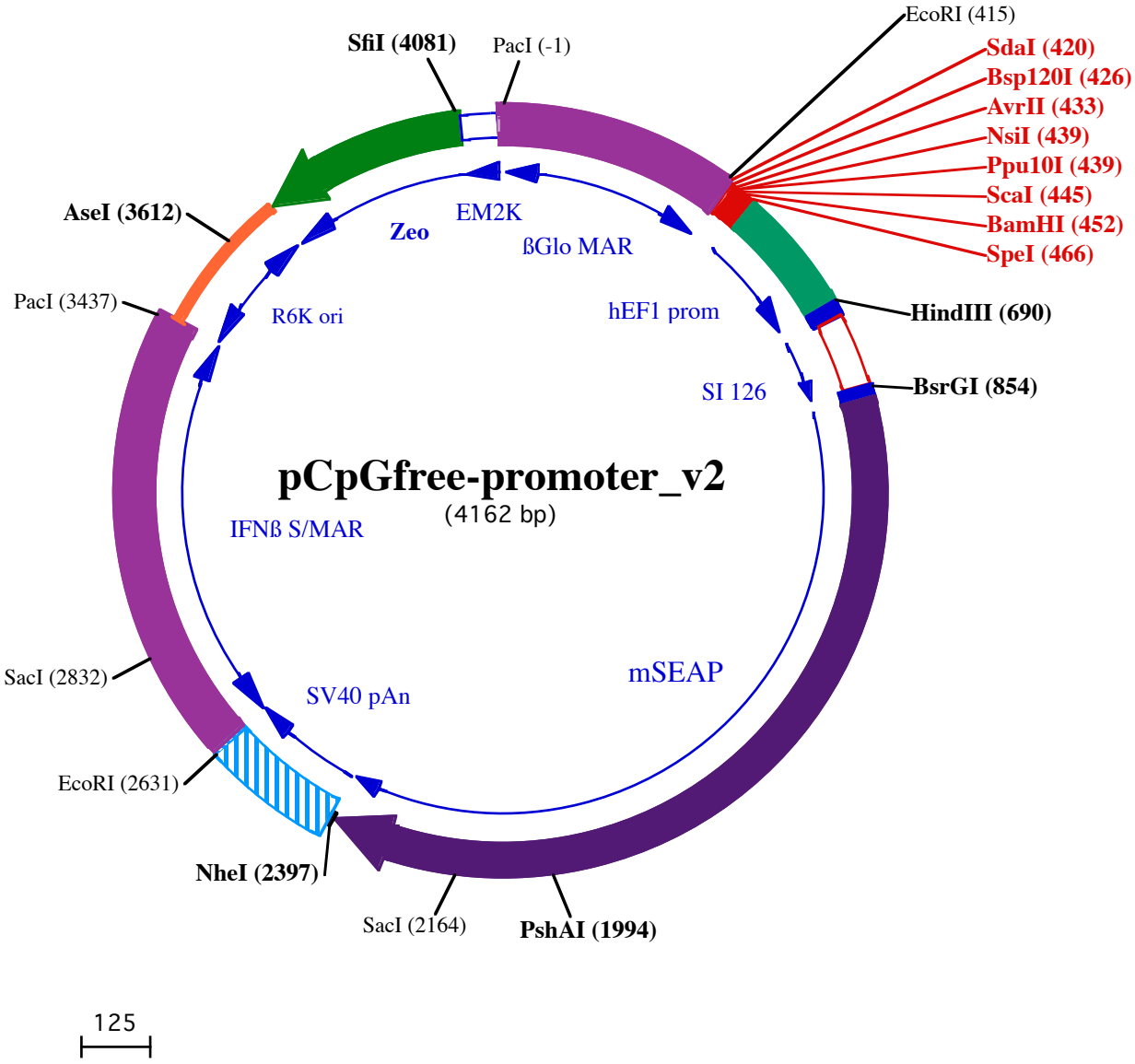
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PacI (-1)
EcoRI (415)
SdaI (420)
Bsp120I (426)
AvrII (433)
NsiI (439)
Ppu10I (439)
ScaI (445)
BamHI (452)
SpeI (466)
AseI (3612)
PacI (3437)
Zeo
EM2K
ßGlo MAR
R6K ori
hEF1 prom
HindIII (690)
SI 126
BsrGI (854)
IFNß S/MAR
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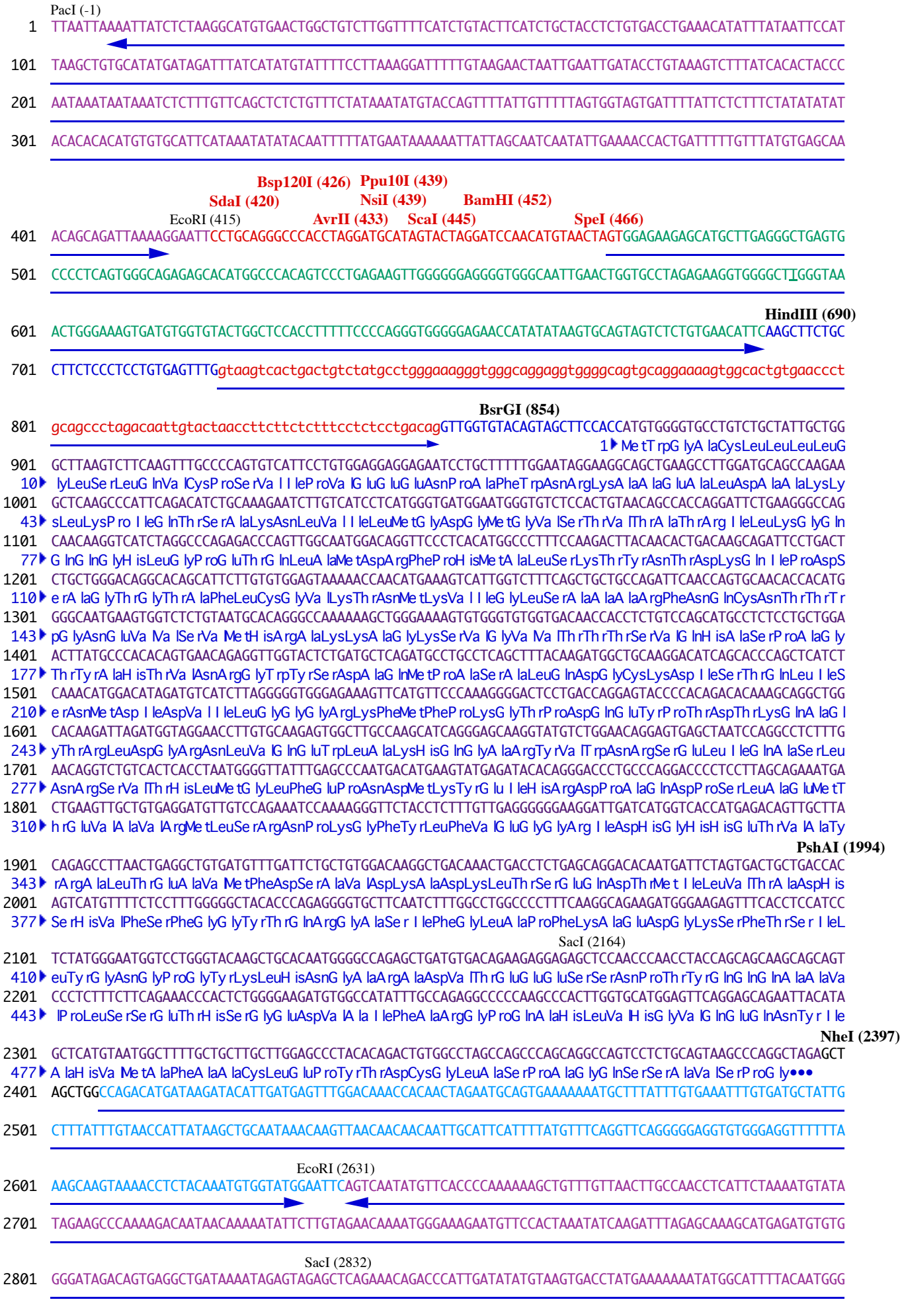
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ScaI (445)
SpeI (466)
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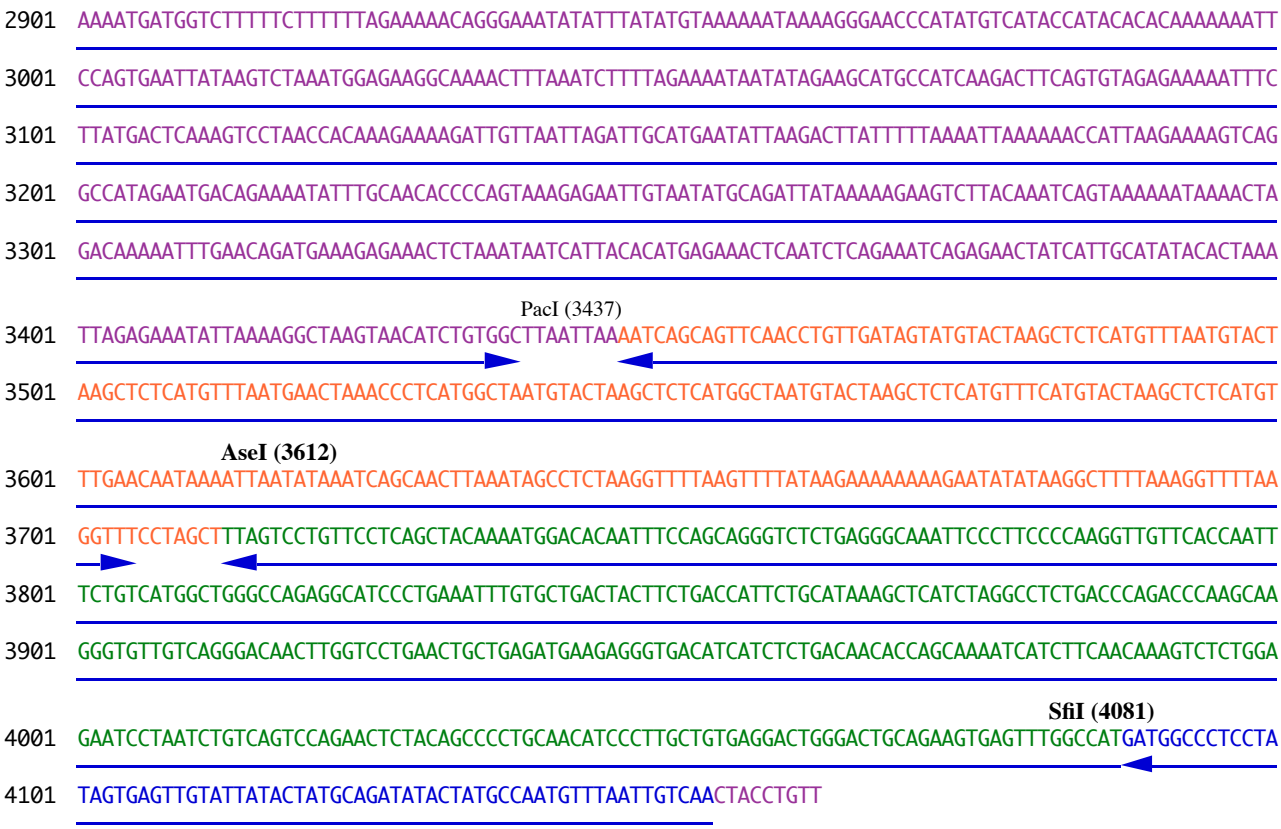
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PacI (3437)
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AseI (3612)
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