

# STOP

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### TECHNICAL SUPPORT

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# pCpGfree-basic-Lucia

A Lucia® reporter plasmid without a promoter and devoid of CpG dinucleotides

Catalog # pcpgf-baslc

For research use only

Version 21 -MMv02

## PRODUCT INFORMATION

### Content:

- 20 µg of pCpGfree-basic-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.
- Store lyophilized DNA 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 methylation<sup>1</sup>.

InvivoGen provides pCpGfree-basic-Lucia, a secreted luciferase reporter plasmid that is completely devoid of CpG dinucleotides and lacks the entire promoter region. It contains a multiple cloning site upstream of the Lucia luciferase reporter gene. Expression of Lucia luciferase in cells transfected with this plasmid depends on the insertion of a functional promoter or enhancer/promoter cassette upstream from the Lucia luciferase gene. Thus, pCpGfree-basic-Lucia 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  $\pi$ , 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

- Luciferase: A synthetic CpG-free gene that codes for a secreted  $\beta$ -galactosidase-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<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, Hind III 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

```
t (MCGUHSQR)
u (PQ) (SQ) (WI) (WH) (R) (Y) (Q) (W) (R) (H) (D) (G) (S) (D) (G) (W) (S) (D) (W) (W)
f r c F . x R (M) (Q) (S) (D) (G) (R) (M) (Q) (M) . -R JG-R (M) . (U) (S) (Q) (W) (f r c) . . . . .
IQJ H . -R OR (W) (Q) (R) (F) . w (W) (U) (S) (Q) (S) (D) (G) (D) (W) E J H . (E) F
```

### 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 and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

## TECHNICAL SUPPORT

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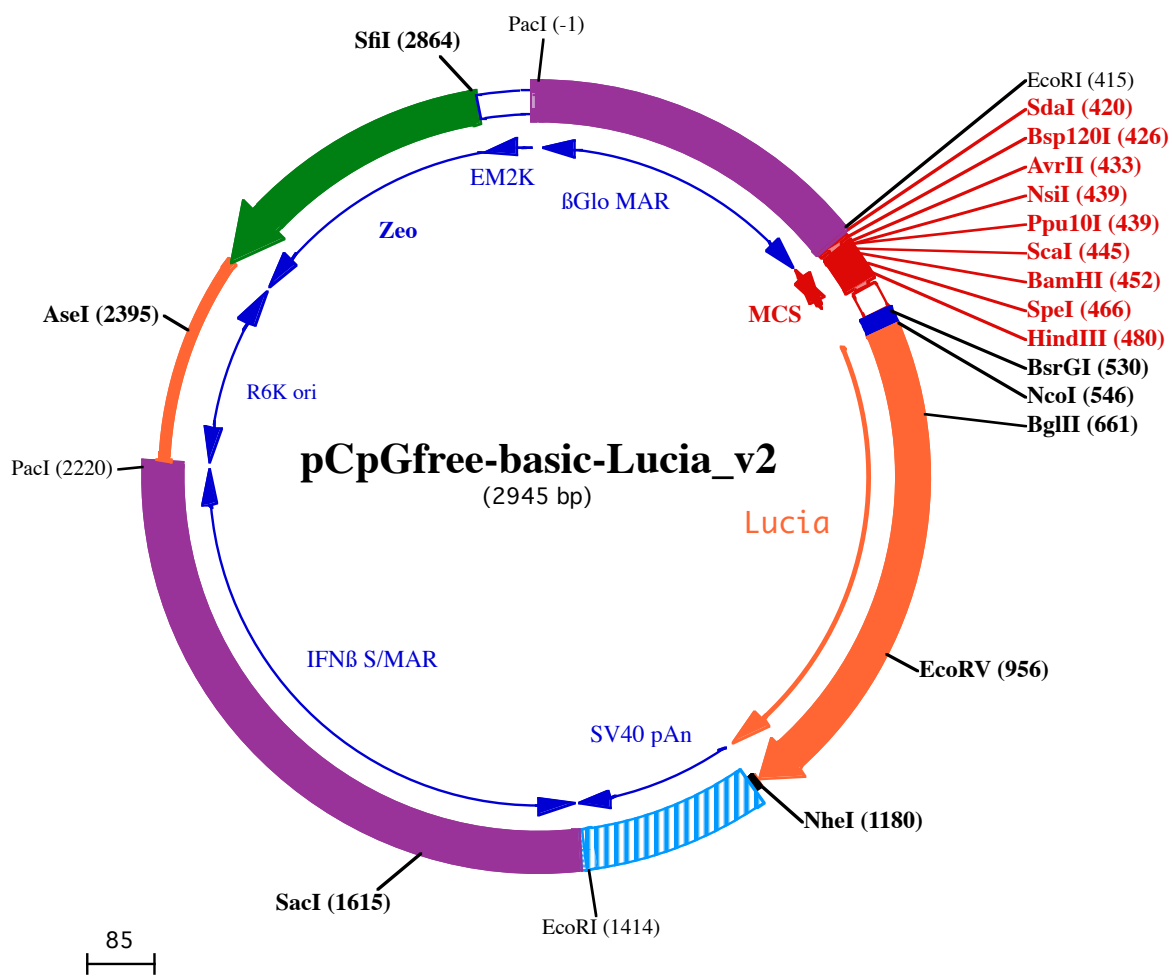
## RELATED PRODUCTS

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

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PacI (-1)

1 TTAATTA~~AAAA~~TATCTCTAAGGCATGTGAACGGCTGTCTGGTTTTATCTGTACTTCATCTGCTACCTCTGTGACCTGAAACATATTTATAATCCAT

101 TAAGCTGTGCATATGATAGATTTATCATATGATATTTTCTTAAAGGATTTTTGAAGAACTAATTGAATTGATACCTGTAAAGCTTTATCACACTACCC

201 AATAAATAATAAATCTCTTTGTTGAGCTCTCTGTTTCTATAAATATGTACCAGTTTTATTGTTTTAGTGGTAGTGATTTTATTCTCTTTCTATATATAT

301 ACACACACATGTGTGCATTATAAATATATACAATTTTTATGAATAAAAAATTATTAGCAATCAATATTGAAAACCACTGATTTTTGTTTATGTGAGCAA

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

401 ACAGCAGATTA~~AAAGGAAT~~CCTGCAGGGCCACCTAGGATGCATAGTACTAGGATCCAACATGTAAGCTAGTAGCATGCAAAGCTTAGAAAttgtactaac

501 cttcttctctttctctcctgacagGTTGGTGTACAGTAGCTCCACCATGGAAATCAAGGTGCTGTTTGCCCTCATCTGTATTGCTGTTGCTGAGGCAA  
 1 M E I K V L F A L I C I A V A E A

**BsrGI (530) NcoI (546)**  
**BglII (661)**

601 AACCCACTGAAATCAATGAAGACCTCAATATAGCTGCTGTGGCCTCCAACCTTGGCCACCACAGATCTTGAGACTGACCTGTTCCACCACTGGGAGACCAT  
 18 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 L F T N W E T M  
 701 GAATGTGATTAGCACTGACACAGAGCAGGTGAACACAGATGCTGACAGGGGCAAGCTGCCTGGCAAAAACTCCCCCAGATGTCCTGAGGGAGCTGGAG  
 51 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 P D V L R E L E  
 801 GCCAATGCCAGAAGGGCTGGTTGCACAAGAGGCTGCTCATTTGCTCTCCACATTAAGTGCACCCCTAAGATGAAGAAATTTATCCCTGGCAGGTGCC  
 85 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 K K F I P G R C

**EcoRV (956)**

901 ACACATTATGAAGGTGAAAGGAGTCTGCTCAGGGAGGATTGGAGAGGCAATTTGATATCCCAGAGATTCCTGGCTCAAGGATAAGGAGCCACTGGA  
 118 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 F K D K E P L D  
 1001 CCAGTTTATTGCTCAAGTGGACCTCTGTGCTGATTGCACCACTGGCTGTGAAGGGCCTTCCCAATGTCCAGTGCCTGACCTCCTGAAGAAAGTGGCTT  
 151 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 S D L L K K W L

**NheI (1180)**

1101 CCCAGAGGTGTACCACTTTTCCAGCAAGATTCAGGGTAGGGTGACAAAATCAAGGGTCTGGCTGGGGACAGATGATAGCTAGCTGGCCAGACATGAT  
 185 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 •  
 1201 AAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAGAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAAACCAT

1301 ATAAGCTCAATAAACAAGTTAACAACAACAATGCATTCATTTATGTTTCAGGTTGAGGGGAGGTGTGGGAGTTTTTTAAAGCAAGTAAACCTCT

**EcoRI (1414)**

1401 ACAAATGTGGTATGGAATCAGTCAATATGTTACCCCAAAAAAGCTGTTTGTAACTGCCAACCTCATTCTAAAATGTATATAGAAGCCAAAAAGACA

1501 ATAACAAAAATATTCTGTAGAACAAAATGGGAAAGAATGTTCCACTAAATATCAAGATTTAGAGCAAAGCATGAGATGTGTGGGATAGACAGTGAAGCC

**SacI (1615)**

1601 TGATAAAATAGAGTAGAGCTCAGAAACAGACCCATTGATATATGTAAGTGACCTATGAAAAAATATGGCATTTTACAATGGGAAAATGATGGTCTTTTT

1701 CTTTTTAGAAAAACAGGAAATATATTTATATGTAAAAAATAAAGGGAACCCATATGTCATACCATACACAAAAAATCCAGTGAATTATAAGTC

1801 TAAATGGAGAAGGCAAACTTTAAATCTTTAGAAAATAATAGAAGCATGCCATCAAGACTTCAGTGTAGAGAAAAATTTCTATGACTCAAGTCTCT

1901 AACCAAAAGAAAAGATTGTAATTAGATTGCATGAATATTAAGACTATTTTTAAATTAAAAAACCATTAAAGAAAAGTCAGGCCATAGAAATGACAGAA

2001 AATATTTGCAACACCCAGTAAAGAGAATTGTAATATGCAGATTATAAAAAAGAGTCTTACAATCAGTAAAAAATAAACTAGACAAAAATTTGAACAG

2101 ATGAAAGAGAACTCTAAATAATCATTACACATGAGAACTCAATCTCAGAAATCAGAGAACTATCATTGCATATACACTAAATAGAGAAATATTA

**PacI (2220)**

2201 GGCTAAGTAACATCTGTGGCTTAATTA~~AAATCAGCAGTTCAACCTGTTGATAGTATGTAAGCTCTCATGTTAATGTAAGCTCTCATGTTAAT~~

**AseI (2395)**

2301 GAACTAAACCCTCATGGCTAATGTAAGCTCTCATGGCTAATGTAAGCTCTCATGTTTATGTAAGCTCTCATGTTTGAACAATAAAATTA

2401 TATAAATCAGCAACTTAAATAGCCTCTAAGGTTTTAAGTTTTATAAGAAAAAAGAATATATAAGGCTTTTAAAGGTTTTAAGGTTTCTAGCTTTAGT  
 125 • D

2501 CCTGTTCTCAGTACAAAATGGACACAATTTCCAGCAGGGTCTCTGAGGGCAAATCCCTTCCCAAGGTTGTTACCAATTTCTGTCATGGCTGGGCC  
 123 Q E E A V F H V C N G A P D R L A F E R G W P Q E G I E T M A P G  
 2601 AGAGGCATCCCTGAAATTTGTGCTGACTACTTCTGACCACTCTGCATAAAGCTCATCTAGGCCTCTGACCCAGACCCAAGCAAGGGTGTGTGAGGGACA  
 90 S A D R F N T S V V E S W E A Y L E D L G R V W V W A L T N D P V  
 2701 ACTTGGTCTGAATGCTGAGATGAAGAGGGTGACATCATCTGCAACACCAAGCAAAATCATCTTCAACAAAGTCTCTGGAGAAATCCTAATCTGTGAG  
 56 V Q D Q V A S I F L T V D D R V V G A F D D E V F D R S F G L R D T

**SfiI (2864)**

2801 TCCAGAACTCTACAGCCCTGCAACATCCCTTGTGTGAGGACTGGGACTGCAGAAGTGAGTTTGGCCATGATGGCCCTCCTATAGTGAAGTGTATTATA  
 23 W F E V A G A V D R A T L V P V A S T L K A M  
 2901 CTATGCAGATATACTATGCCAATGTTAATTGTCAACTACCTGTT