STOP

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pCpGfree-Lucia

A Lucia® expression plasmid completely devoid of CpG dinucleotides

Catalog # pcpgf-lucia

For research use only

Version 21F04-MMv02

PRODUCT INFORMATION

Content:

- 20 µg of pCpGfree-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.
- 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

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 plamids 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* gene[1].

• 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 domains[2]. 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.

• Lucia luciferase is a synthetic CpG-free gene that codes for a secreted coelenterazine-utilizing luciferase.

ORF size (from the ATG to the stop codon): 634 bp

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. 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 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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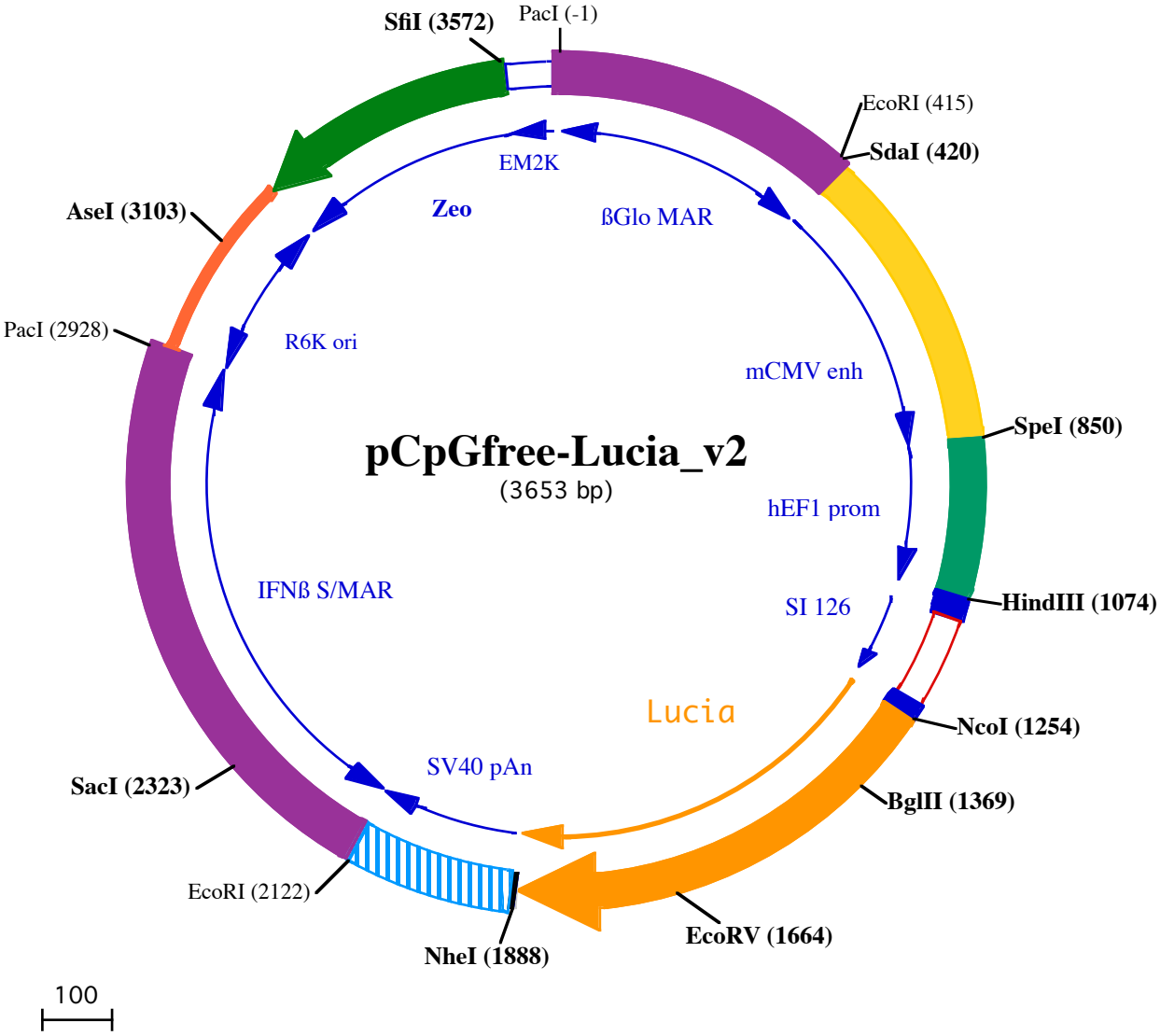
E-mail: info@invivogen.com

RELATED PRODUCTS

Product	Catalog Code
QUANTI-Luc™	rep-qlc1
ChemiComp GT115	gt115-11
pCpGfree-mcs	pcpgf-mcs
pCpGfree-LacZ	pcpgf-lacz
pCpGfree-mSEAP	pcpgf-mseap
pCpGrich-mcs (CpG-containing control plasmid)	pcpgr-mcs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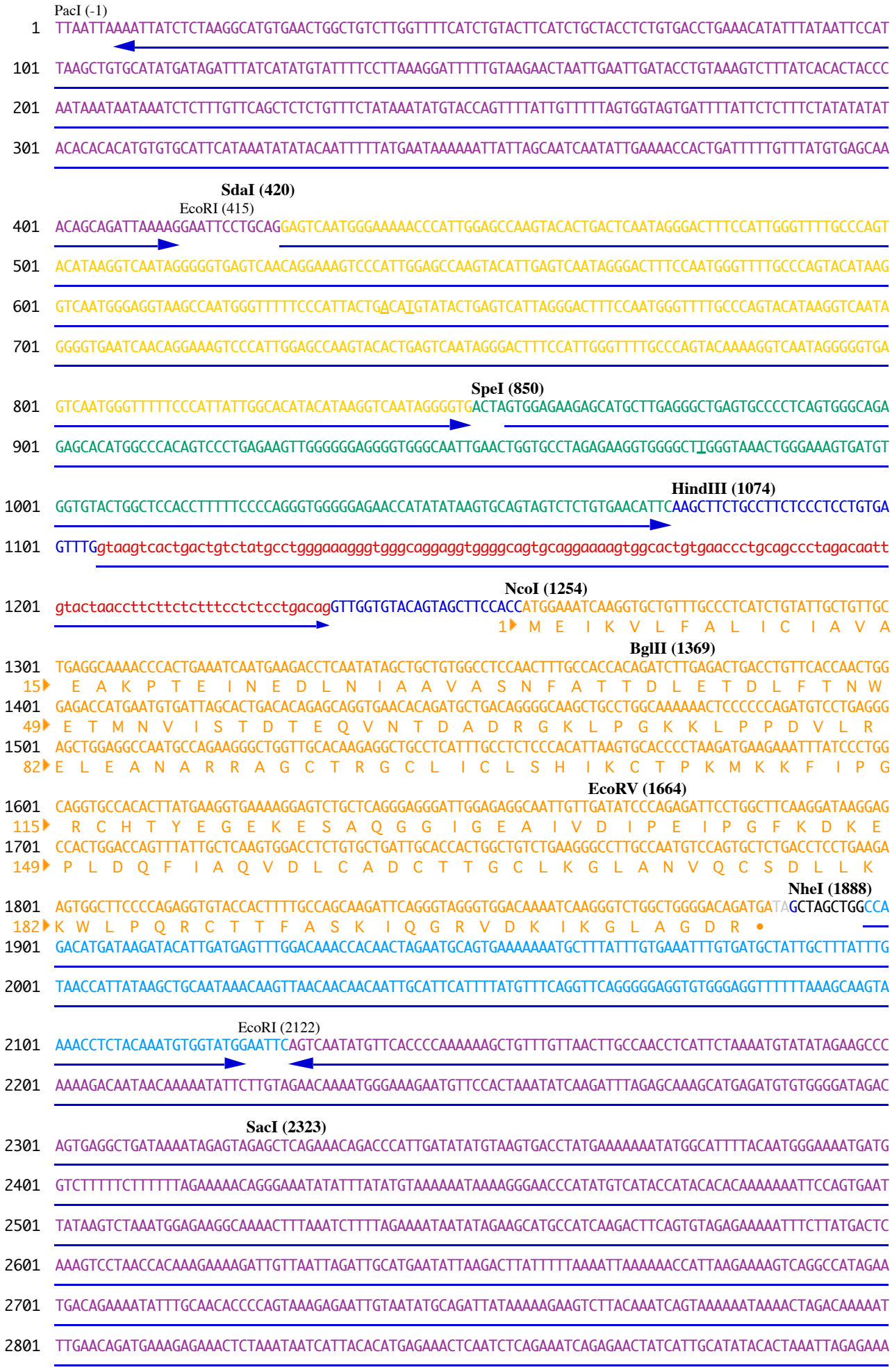
SfiI (3572)
PacI (-1)
EcoRI (415)
SdaI (420)
SpeI (850)
HindIII (1074)
NcoI (1254)
BglII (1369)
EcoRV (1664)
NheI (1888)
EcoRI (2122)
SacI (2323)
PacI (2928)
AseI (3103)
EM2K
Zeo
ßGlo MAR
mCMV enh
hEF1 prom
SI 126
Lucia
SV40 pAn
IFNß S/MAR
R6K ori
100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SdaI (420)
EcoRI (415)
SpeI (850)
HindIII (1074)
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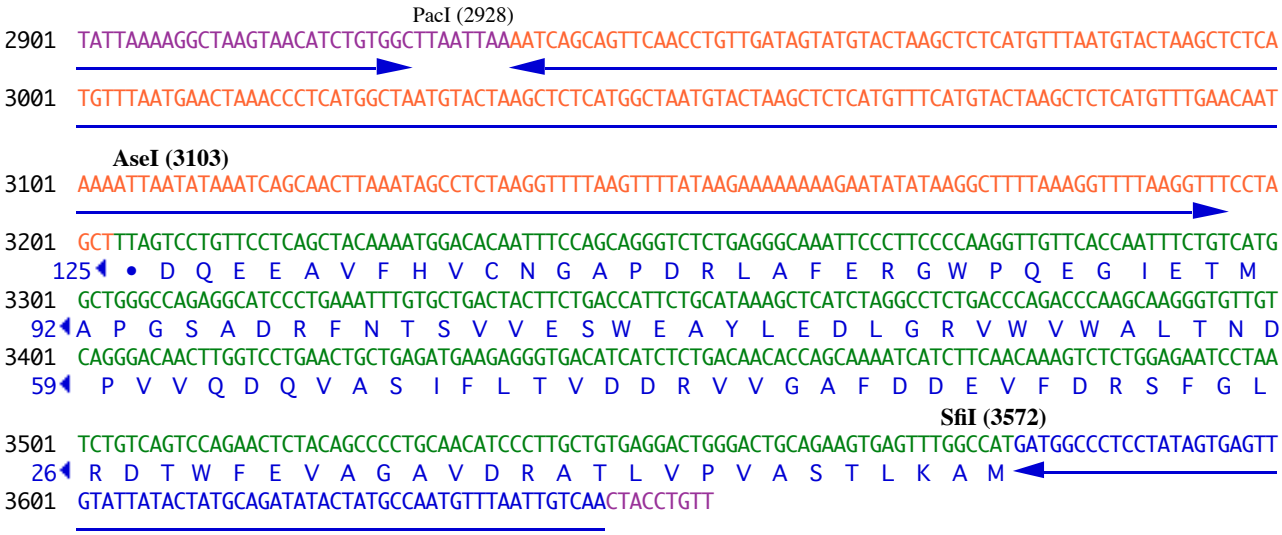
SacI (2323)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3001 TGTTTAATGAACTAAACCCTCATGGCTAATGTACTAAGCTCTCATGGCTAATGTACTAAGCTCTCATGTTTCATGTACTAAGCTCTCATGTTTGAACAAT
AseI (3103)
3101 AAAATTAATATAAATCAGCAACTTAAATAGCCTCTAAGGTTTTAAGTTTTATAAGAAAAAAAAGAATATATAAGGCTTTTAAAGGTTTTAAGGTTTCCTA
3201 GCTTTAGTCCTGTTCCTCAGCTACAAAATGGACACAATTTCCAGCAGGGTCTCTGAGGGCAAATTCCCTTCCCCAAGGTTGTTCACCAATTTCTGTCATG
125 • D 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
3301 GCTGGGCCAGAGGCATCCCTGAAATTTGTGCTGACTACTTCTGACCATTCTGCATAAAGCTCATCTAGGCCTCTGACCCAGACCCAAGCAAGGGTGTTGT
92 A P G 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
3401 CAGGGACAACTTGGTCCTGAACTGCTGAGATGAAGAGGGTGACATCATCTCTGACAACACCAGCAAAATCATCTTCAACAAAGTCTCTGGAGAATCCTAA
59 P V 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
SfiI (3572)
3501 TCTGTCAGTCCAGAACTCTACAGCCCCTGCAACATCCCTTGCTGTGAGGACTGGGACTGCAGAAGTGAGTTTGGCCATGATGGCCCTCCTATAGTGAGTT
26 R D T W F E V A G A V D R A T L V P V A S T L K A M
3601 GTATTATACTATGCAGATATACTATGCCAATGTTTAATTGTCAACTACCTGTT