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TECHNICAL SUPPORT

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pCpGfree-LacZ

A LacZ expression plasmid completely devoid of CpG dinucleotides

Catalog # pcpgf-lacZ

For research use only

Version 21F04-MMv02

PRODUCT INFORMATION

Content:

- 20 µg of pCpGfree-LacZ 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*.

pCpGfree 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.

pCpGfree 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 pCpGfree-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. 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* 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. 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.

- pCpGfree-LacZ expresses the synthetic LacZ Δ CpG gene, a CpG-free allele of the LacZ 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

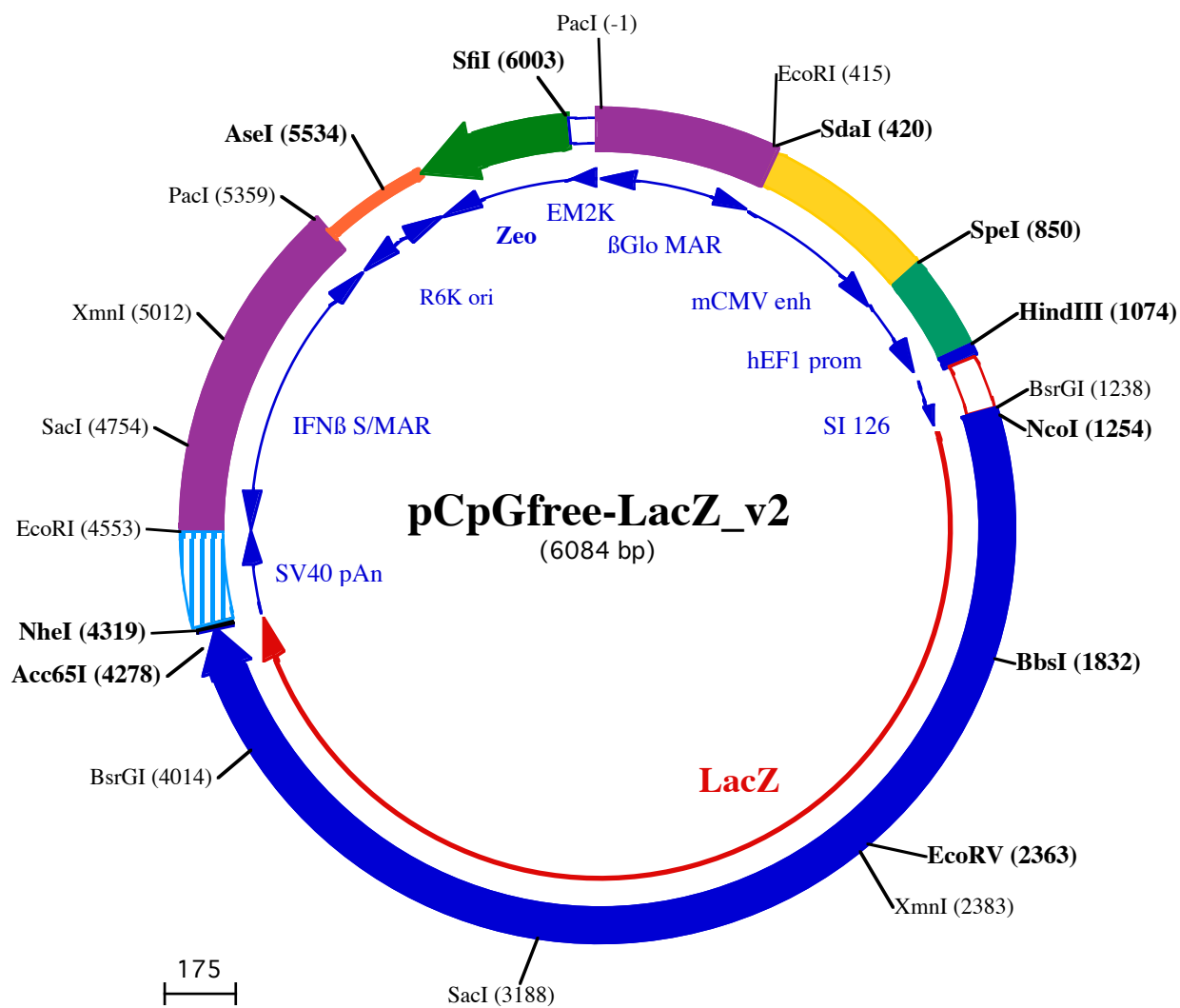
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PacI (-1)
1 TTAATTAATAAATTATCTCTAAGGCATGTGAACTGGCTGTCTGGTTTTTCATCTGTACTTTCATCTGCTACCTCTGTGACCTGAAACATATTTATAATTCCAT
101 TAAGCTGTGCATATGATAGATTTATCATATGATTTTTCTTAAAGGATTTTTGTAAGAACTAATTGAATTGATACCTGTAAGTCTTTATCACACTACCC
201 AATAAATAATAAATCTTTTGTTCAGCTCTCTGTTCTATAAATATGTACCAGTTTTATTGTTTTAGTGGTAGTGATTTTATTCTCTTTCTATATATAT
301 ACACACACATGTGTGCATTACATAAATATATACAATTTTTATGAATAAAAAATTATTAGCAATCAATATTGAAAACCACTGATTTTTGTTTTATGTGAGCAA

SdaI (420)
EcoRI (415)
401 ACAGCAGATTAATAAGGAATTCCTGCAGGAGTCAATGGGAAAAACCCATTGGAGCCAAGTACACTGACTCAATAGGGACTTTCCATTGGGTTTTGCCAGT
501 ACATAAGGTCAATAGGGGGTGAAGTCAACAGGAAAGTCCCATTGGAGCCAAGTACATTGAGTCAATAGGGACTTTCCAATGGGTTTTGCCAGTACATAAG
601 GTCAATGGGAGGTAAAGCAATGGGTTTTTCCATTACTGCACTGTATACTGAGTCAATAGGGACTTTCCAATGGGTTTTGCCAGTACATAAGGTCAATA
701 GGGGTGAATCAACAGGAAAGTCCCATTGGAGCCAAGTACACTGAGTCAATAGGGACTTTCCATTGGGTTTTGCCAGTACAAAAGGTCAATAGGGGGTGA

SpeI (850)
801 GTCAATGGGTTTTTCCATTATTGGCACATACATAAGGTCAATAGGGGTGACTAGTGGAGAAGAGCATGCTTGAGGGCTGAGTCCCTCAGTGGGCAGA
901 GAGCACATGGCCACAGTCCCTGAGAAGTTGGGGGAGGGTGGCAATTGAACTGGTGCCTAGAGAAGTGGGGCTGGGTTAACTGGGAAAGTGATGT

HindIII (1074)
1001 GGTGTACTGGCTCCACCTTTTTCCCCAGGGTGGGGGAGAACCATATATAAGTGCAGTAGTCTCTGTGAACATTCAAGCTTCTGCCTTCTCCCTCCTGTGA
1101 GTTTGtaagtcactgactgtctatgctgggaaagggggcaggaggtggggcagtcaggaaaagtggcactgtgaacctgcagccctagacaatt

BsrGI (1238) NcoI (1254)
1201 gtactaaccttcttctcttctctctctgacagGTTGGTGTACAGTAGCTCCACCATGGACCCTGTTGTGCTGCAAAGGAGAGACTGGGAGAACCCTGG
1301 AGTGACCCAGCTCAACAGACTGGCTGCCACCCTCCCTTGCCTCTTGGAGGAAGTCTGAGGAAGCCAGGACAGACAGGCCAGCCAGCAGCTCAGGTCT
1401 CTCAATGGAGAGTGGAGTTTGCCTGTTCCCTGCCCTGAAGCTGTGCCTGAGTCTTGGCTGGAGTGTGACCTCCAGAGGCTGACTGTGTGGTGC
1501 CCAGCAACTGGCAGATGCATGGCTATGATGCCCCATCTACACCAATGTCACCTACCCATCACTGTGAACCCCCCTTTGTGCCACTGAGAACCCAC
1601 TGGCTGTACAGCCTGACCTCAATGTTGATGAGAGCTGGCTGCAAGAAGCCAGACCAGGATCATCTTTGATGGAGTCAACTGCTTCCACCTCTGG
1701 TGCAATGGCAGGTGGGTTGGCTATGGCCAAGACAGCAGGCTGCCCTGAGTTTACCTCTGCTTCCCTCAGAGCTGGAGAGAACAGGCTGGCTGTCA

BbsI (1832)
1801 TGGTGTCTCAGTGGTCTGATGGCAGCTACCTGGAAGCAAGACATGTGGAGGATGTCTGGCATCTCAGGGATGTGAGCTGTGCACAAGCCCACCAC
1901 CCAGATTTCTGACTTCCATGTTGCCACCAGTTCATGATGACTTCAGCAGAGCTGTGCTGGAGGCTGAGGTGCAGATGTGTGGAGAACTCAGAGACTAC
2001 CTGAGAGTCAAGTGTGAGCCTCTGGCAAGGTGAGACCCAGGTGGCCTCTGGCAGACCCCTTTGGAGGAGAGATCATTGATGAGAGAGGAGGCTATGCTG
2101 ACAGAGTCAACCTGAGGCTCAATGTGGAGAACCCCAAGCTGTGGTCTGCTGAGATCCCAACCTCTACAGGGCTGTTGTGGAGCTGCACACTGCTGATGG
2201 CACCCTGATTGAAGCTGAAGCCTGTGATGTTGGATTGAGAGAAGTCAAGATTGAGAATGGCCTGCTGCTCAATGGCAAGCCTCTGCTCATCAGGGGA

EcoRV (2363) XmnI (2383)
2301 GTCAACAGGCATGAGACCACCCTCTGCATGGACAAGTATGGATGAACAGACAATGGTGAAGATATCCTGCTAATGAAGCAGAACAACCTCAATGCTG
2401 TCAGGTGCTCTCACTACCCCAACCCTCTCTGGTACACCCTGTGTGACAGGTATGGCTGTATGTTGTTGATGAAGCAACATTGAGACACATGGCAT
2501 GGTGCCCATGAACAGGCTCACAGATGACCCAGTGGCTGCCTGCCATGTCTGAGAGAGTACCAGGATGGTGCAGAGAGACAGGAACCCCTCTGTG
2601 ATCATCTGGTCTCTGGGCAATGAGTCTGGACATGGAGCAACCATGATGCTCTCTACAGGTGGATCAAGTCTGTTGACCCAGCAGACCTGTGCAGTATG
2701 AAGGAGGTGGAGCAGACACCACAGCCACAGACATCATCTGCCCCATGTATGCCAGGGTTGATGAGGACCAGCCCTTCCCTGCTGTGCCAAGTGGAGCAT
2801 CAAGAAGTGGCTCTCTGCTGGAGAGACCAGACCTCTGATCCTGTGTAATATGCACATGCAATGGCAACTCTCTGGAGGCTTTGCCAAGTACTGG
2901 CAAGCCTCAGACAGTACCCAGGCTGCAAGGAGGATTTGTGTGGGACTGGTGGACCAATCTCTCATCAAGTATGATGAGAATGGCAACCCCTGGTCTG
3001 CCTATGGAGGAGACTTTGGTGCACCCCAATGACAGGCAGTCTGCATGAATGGCCTGGTCTTTGCAGACAGGACCCCTCACCTGCCCTCACAGAGGC

SacI (3188)

3101 CAAGCACCAGCAACAGTTCTCCAGTTCAGGCTGTCTGGACAGACCATTGAGGTGACATCTGAGTACCTCTTCAGGCACTCTGACAATGAGCTCCTGCAC
3201 TGGATGGTGGCCCTGGATGGCAAGCCTCTGGCTTCTGGTGGAGTGCCTCTGGATGTGGCCCTCAAGGAAAGCAGCTGATTGAACTGCCTGAGCTGCCTC
3301 AGCCAGAGTCTGCTGGACAACCTGTGGCTAACAGTGAAGGTGGTTCAGCCCAATGCAACAGCTTGGTCTGAGGCAGGCCACATCTCTGCATGGCAGCAGTG
3401 GAGGCTGGCTGAGAACCTCTCTGTGACCCTGCCTGCTGCCTCTCATGCCATCCCTCACCTGACAACATCTGAAATGGACTTCTGCATTGAGCTGGGCAAC
3501 AAGAGATGGCAGTTCACAGGCAGTCTGGCTTCTGTCTCAGATGTGGATTGGAGACAAGAAGCAGCTCCTCACCCCTCTCAGGGACCAATTACCAGGG
3601 CTCCTCTGGACAATGACATTGGAGTGTCTGAGGCCACCAGGATTGACCCAAATGCTTGGTGGAGAGTGAAGGCTGCTGGACACTACCAGGCTGAGGC
3701 TGCCCTGCTCCAGTGCACAGCAGACACCCTGGCTGATGCTGTTCTGATCACCACAGCCATGCTTGGCAGCACCAAGGCAAGACCCTGTTTCATCAGCAGA
3801 AAGACCTACAGGATTGATGGCTCTGGACAGATGGCAATCACAGTGGATGTGGAGTTGCCTCTGACACACCTCACCTGCAAGGATTGGCTGAACTGTC
3901 AACTGGCACAGTGGCTGAGAGGGTGAAGTGGCTGGGCTTAGGCCCTCAGGAGAACTACCCTGACAGGCTGACAGCTGCCTGCTTTGACAGTGGGACCT

BsrGI (4014)

4001 GCCTCTGTCTGACATGTACACCCCTTATGTGTTCCCTTCTGAGAATGGCCTGAGGTGTGGCACCAGGGAGCTGAACTATGGTCTCACCAGTGGAGGGGA
4101 GACTTCCAGTTCACATCTCCAGTACTCTCAGCAACAGCTCATGAAACCTCTCACAGGCACCTGCTCCATGCAGAGGAGGGAACTGGCTGAACATTG

Acc65I (4278)

4201 ATGGCTTCCACATGGGCATTGGAGGAGATGACTCTTGGTCTCCTTCTGTGTCTGCTGAGTCCAGTTATCTGCTGGCAGGTACCACTATCAGCTGGTGTG

NheI (4319)

4301 GTGCCAGAAGTAAACCTGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTGGACAACCACAAGTAACTAGAAATGCAAGTAAATGCTTTATT
4401 TGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCAATTTATGTTTCAGGTTCAAG

EcoRI (4553)

4501 GGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAATGTGGTATGGAATTCAGTCAATATGTTACCCCAAAAAGCTGTTTGTAACTTGC
4601 CAACCTCATTCTAAAATGTATATAGAAGCCAAAAGACAATAACAAAATATTCTTGTAGAACAATAAGGAAAGATGTTCCACTAAATATCAAGATT

SacI (4754)

4701 AGAGCAAAGCATGAGATGTGTGGGATAGACAGTGAAGGCTGATAAAATAGAGTAGAGCTCAGAAACAGACCCATTGATATATGTAAGTGACCTATGAAAA
4801 AAATATGGCATTTTACAATGGGAAAATGATGGTCTTTTTCTTTTTAGAAAAACAGGAAATATATTTATATGTAATAAATAAAGGGAACCCATATGTC
4901 ATACCATACACAAAAAATTCAGTGAATTATAAGTCTAAATGGAGAAGGCAAAACTTAAATCTTTTAGAAAAATAATAGAAGCATGCCATCAAGA

XmnI (5012)

5001 CTTCACTGTAGAGAAAAATTTCTTATGACTCAAAGTCTAACCAAAAGAAAAGATTGTTAATTAGATTGCATGAATATTAAGACTTATTTTTAAAATTA
5101 AAAAAACCATTAAGAAAAGTCAGGCCATAGAATGACAGAAAATATTTGCAACACCCAGTAAAGAGAATTGTAATATGCAGATTATAAAAAGAAGTCTTAC
5201 AAATCAGTAAAAATAAACTAGACAAAAATTTGAACAGATGAAAGAGAACTCTAAATAATCATTACACATGAGAACTCAATCTCAGAAATCAGAGAA

PacI (5359)

5301 CTATCATTGCATATACACTAAATTAGAGAAATATTTAAAGGCTAAGTAACATCTGTGGCTTAATTAATAATCAGCAGTTCACCTGTTGATAGTATGTA
5401 AAGCTCTCATGTTAATGTAAGCTCTCATGTTAATGAAGTAAACCTCATGGCTAATGTAAGCTCTCATGGCTAATGTAAGCTCTCATG

AseI (5534)

5501 TTCATGTAAGCTCTCATGTTGAACAATAAAATTAATATAAATCAGCAACTAAATAGCCTCTAAGGTTTTAAGTTTTATAAGAAAAAAGAATAT
5601 ATAAGGCTTTAAAGGTTTAAAGTTTCTAGCTTGTAGTCTGTTCTCAGCTACAAAATGGACACAATTCAGCAGGCTCTGAGGGCAAATTCCT
5701 TCCCAAGGTTGTTACCAATTTCTGTCATGGCTGGCCAGAGGCATCCCTGAAATTTGTGCTGACTACTCTGACCATTCTGCATAAAGCTCATCTAGG
5801 CCTCTGACCCAGACCCAAGCAAGGTTGTGTCAGGGACAACCTGGTCTGAACTGCTGAGATGAAGAGGTTGACATCATCTCTGACAACACCAGCAAAAT
5901 CATCTTCAACAAGTCTCTGAGAATCCTAATCTGTCAGTCCAGAACTCTACAGCCCTGCAACATCCCTGCTGTGAGGACTGGGACTGCAGAAGTGAG

SfiI (6003)

6001 TTTGGCCATGATGGCCCTCTATAGTGAAGTGTATTATACTATGCAGATATACTATGCCAATGTTAATTGTCAACTACCTGTT