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pCpGfree-vitroNmcs

A CpG-free expression plasmid, containing a multiple cloning site, selectable with G418/Kanamycin

Catalog code: pcpgvtn-mcsg2

https://www.invivogen.com/pcpgfree-vitro-neomycin

For research use only

Version 19L12-MM

PRODUCT INFORMATION

Contents:

- 20 µg of pCpGfree-vitroNmcs plasmid provided as lyophilized DNA
- *E. coli* GT115 strain provided lyophilized on a paper disk

Storage and stability:

- Product is shipped at room temperature.
- Upon receipt, store lyophilized DNA at -20°C.
- Resuspended DNA should be stored at -20°C.

Quality control:

- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Plasmid DNA was purified by ion exchange chromatography and lyophilized.

GENERAL PRODUCT USE

pCpGfree-vitro plasmids represent innovative tools to study the effects of CpG dinucleotides in numerous applications. DNA vaccination exploits the immunostimulatory character of certain CpG motifs to prime and boost the immune response. However, these immunostimulatory CpG motifs are antagonized by CpG dinucleotides in certain distinct base contexts, termed neutralizing CpG motifs. Both types of CpG motifs are usually present in plasmidic DNAs, and therefore may lead to an unfavorable immune response. pCpGfree-vitro is the ideal tool to overcome this problem, and may be used to study the effects of these two types of CpG motifs by adding them in different configurations to the pCpGvitro backbone.

CpG dinucleotides are key elements in a number of cellular functions associated with chromatin. Several large multisubunit complexes, consisting of methyl-CpG binding (MBD) proteins and histone deacetylases, have been implicated in the regulation of chromatin dynamics. These complexes are recruited to methylated CpG dinucleotides by DNA methyl transferases (DNMTs) and induce chromatin remodelling. However the specific roles of these complexes are still to be explored. Due to the absence of CpG dinucleotides within its backbone, pCpGfree-vitro is not the target of DNMTs and thus MBD proteins. Therefore, it provides a useful model to study the other proteins involved in these complexes, in particular the histone deacetylases. It can also be used to analyze the effects of CpG methylation on the regulation and duration of gene expression.

PLASMID FEATURES

pCpGfree-vitro is a family of expression vectors devoid of CpG dinucleotides that are selectable in mammalian cells. All the elements required for replication and selection of the plasmids in bacteria, and gene expression in mammalian cells have been modified to remove all CpG dinucleotides.

- **Composite CpG-free promoter** combining the mouse CMV enhancer, the human elongation factor 1α core promoter and 5'UTR containing a synthetic intron (I 126). This composite promoter yields high and ubiquitous expression of the LacZ gene.
- **MCS:** The multiple cloning site contains the following restriction sites: 5' - BsrG I, Sca I, Bgl II, ApaL I, Bsp120 I, , Nco I, Nhe I, Msc I - 3' Each restriction site is compatible with several other enzymes, increasing the cloning options.
- **CpG-free polyadenylation signals (pAn):** The polyadenylation signals utilized are CpG-free versions of the SV40 late and human β-globin polyadenylation signals. These polyA enable efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.
- **CpG-free matrix attached regions (MARs)** are AT-rich sequences that are able to form barriers between independent expression cassettes .
- **CpG-free Neo resistance gene (Neo-ΔCpG):** The CpG-free Neo gene is active both in *E. coli* and mammalian cells and confers resistance to Kanamycin in *E. coli* and G418 in mammalian cells.
- **CpG-free SV40 promoter** works in tandem with a bacterial promoter located within a synthetic intron (I-EC2K). This composite promoter drives the expression of the resistance gene in both mammalian cells and *E. coli*.
- **CpG-free *E. coli* R6K gamma origin of replication:** This origin is activated by the R6K specific initiator protein π, encoded by the *pir* gene. Expression of the *pir* gene is necessary for the replication and amplification of pCpGvitro plasmids. *E. coli* GT115 strain expresses a *pir* mutant gene that allows higher plasmid copy number.

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 under sterile conditions

1. Reconstitute *E. coli* GT115 by adding 1 ml of LB medium in the tube containing the paper disk. Let sit for 15 minutes. Mix gently by inverting the tube several times. Let sit 5 more minutes.
2. Streak bacteria taken from this suspension on a LB agar plate.
3. Place the plate in an incubator at 37°C overnight.
4. Isolate a single colony and grow the bacteria in *E. coli* growth medium.
5. Prepare competent cells utilizing your preferred protocol.

Plasmid amplification and cloning:

Plasmid amplification and cloning can be performed in competent *E. coli* GT115.

Bacterial antibiotic selection

Kanamycin (not provided) is normally used for *E. coli* at a final concentration of 50 µg/ml in liquid or solid media.

Mammalian antibiotic selection

G418 is normally used at a concentration of 400 µg/ml. However, the optimal concentration needs to be determined for your cells.

RELATED PRODUCTS

Product	Description	Cat. Code
ChemiComp GT115 cells	Competent *E. coli* cells	gt115-11
G418	Selection antibiotic	ant-gn-1

TECHNICAL SUPPORT
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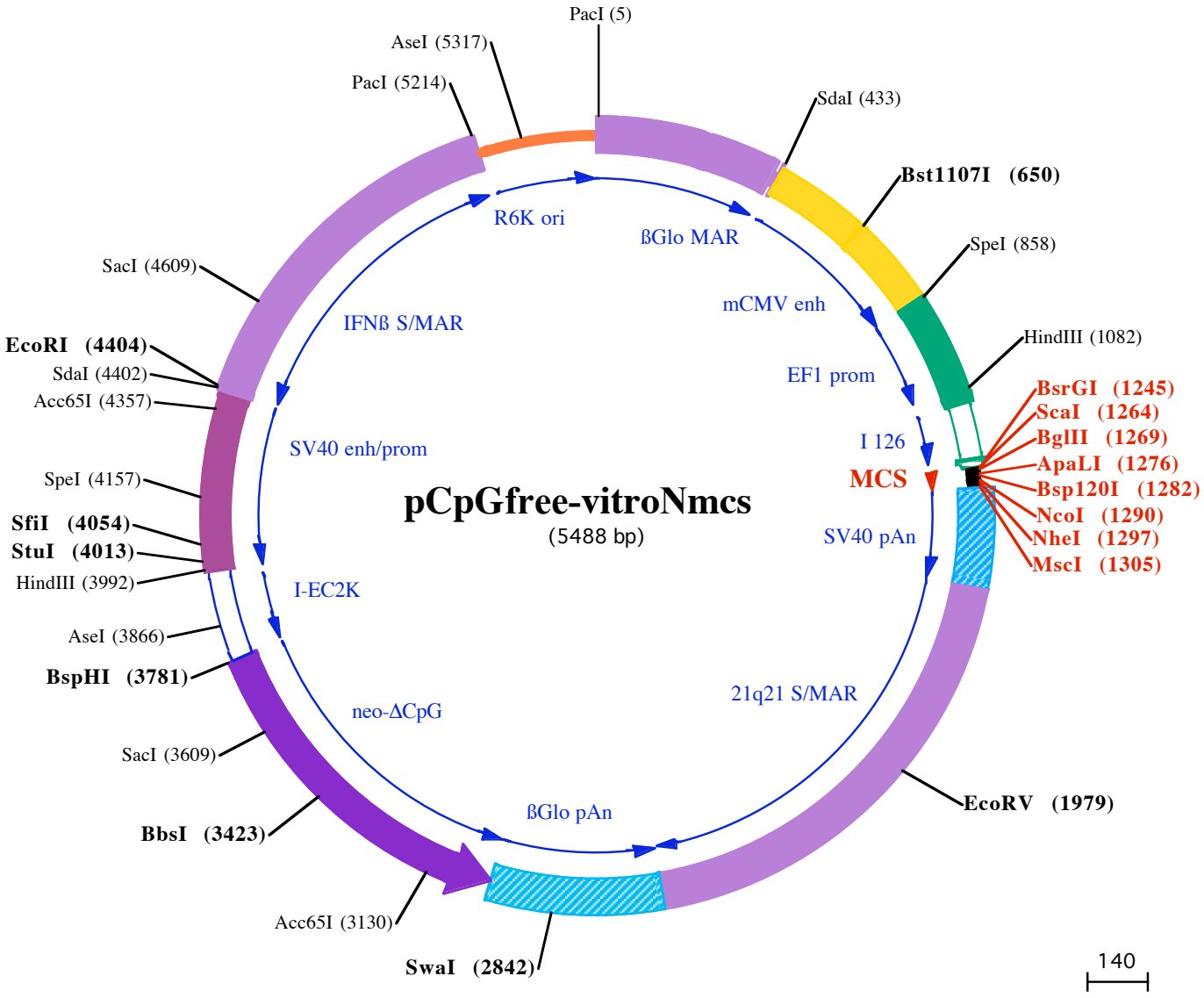
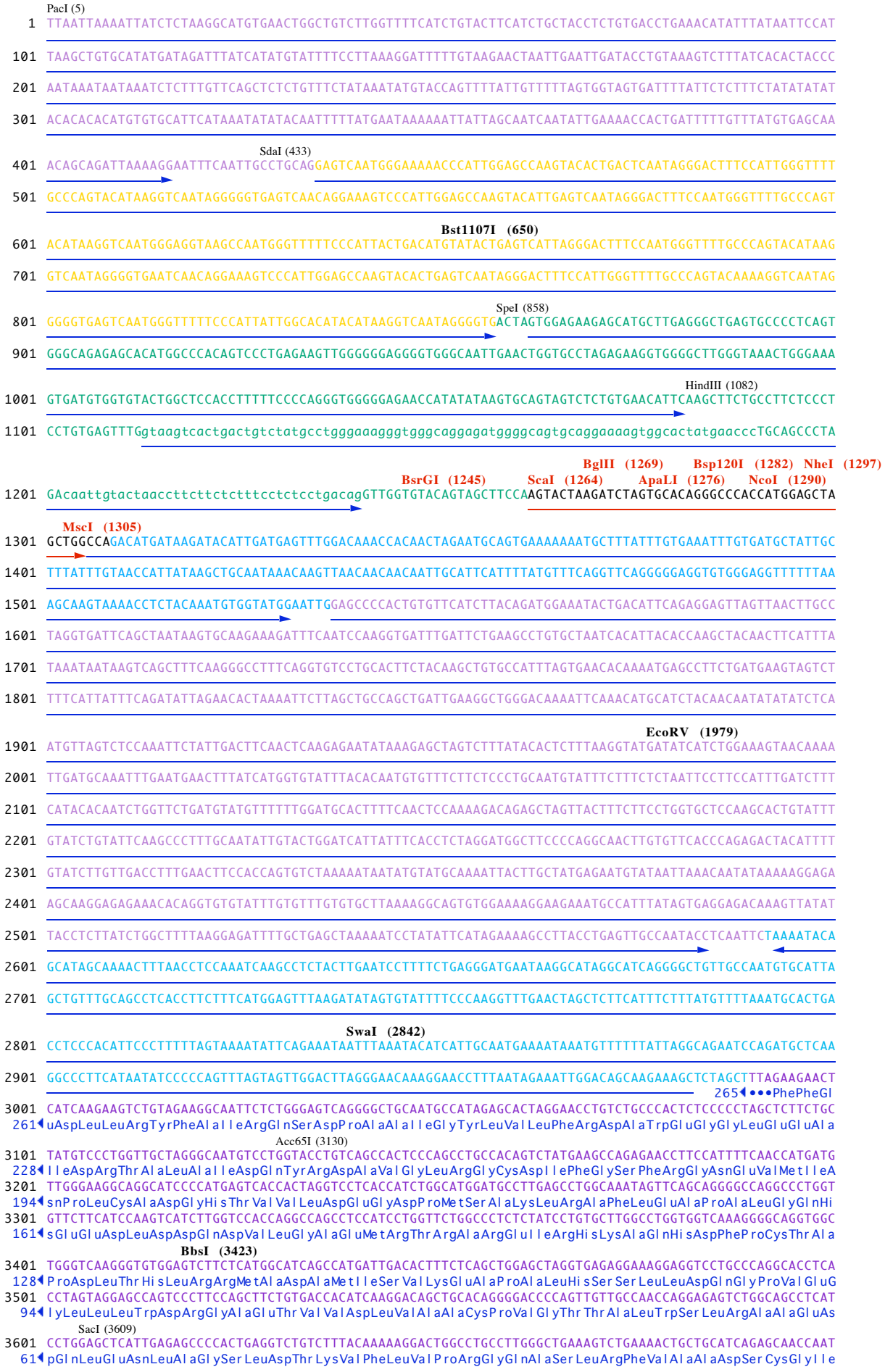

pCpGfree-vitroNmcs
(5488 bp)
PacI (5)
AseI (5317)
PacI (5214)
SdaI (433)
Bst1107I (650)
SpeI (858)
HindIII (1082)
BsrGI (1245)
ScaI (1264)
BglII (1269)
ApaLI (1276)
Bsp120I (1282)
NcoI (1290)
NheI (1297)
MscI (1305)
EcoRV (1979)
SacI (4609)
EcoRI (4404)
SdaI (4402)
Acc65I (4357)
SpeI (4157)
SfiI (4054)
StuI (4013)
HindIII (3992)
AseI (3866)
BspHI (3781)
SacI (3609)
BbsI (3423)
Acc65I (3130)
SwaI (2842)
R6K ori
ßGlo MAR
mCMV enh
EF1 prom
I 126
MCS
SV40 pAn
21q21 S/MAR
ßGlo pAn
neo-ΔCpG
I-EC2K
SV40 enh/prom
IFNß S/MAR
140


PacI (5)
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MscI (1305)
EcoRV (1979)
SwaI (2842)
Acc65I (3130)
BbsI (3423)
SacI (3609)


BspHI (3781)
3701 GGTCTGCTGTGCCCAGTCATAGCCAAACAGTCTCTCAACCCAGGCAGCTGGAGAACCTGCATGTAGGCCATCTTGTTCAATCATGATGGCTCCTCctgtc
28 ThrGlnGlnAlaTrpAspTyrGlyPheLeuArgGluValTrpAlaAlaProSerGlyAlaHisLeuGlyAspGlnGluIleMet
AseI (3866)
3801 aggagaggaaagagaagaaggttagtacaattgCTATAGTGAGTTGTATTATACTATGCTTATGATTAATTGTCAAACTAGGGCTGCAgggttcatagtg
HindIII (3992)
3901 ccacttttcctgcactgccccatctcctgcccacccttcccaggcatagacagtcagtgacttacCAAACTCACAGGAGGGAGAAGGCAGAAGCTTTTT
StuI (4013)
SfiI (4054)
4001 GCAAAAGCCTAGGCCTCCAAAAAAGCCTCCTCACTACTTCTGGAATAGCTCAGAGGCCcAGGgGGCCTgGGCCTCTGCATAAATAAAAAAAATTAGTCAG
SpeI (4157)
4101 CCTGGGGctggggtgggggcaggggtgggggccaactgggCAGGGGTGGGGGGCCACTAGTGGGACTATGGTTGCTGACTAATTGAGATGCATGCTTTG
4201 CATACTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCACACCTGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGGG
Acc65I (4357)
SdaI (4402)
4301 GACTTTCCACACCCTAACTGACACACATTCCACAGCTGGTTCTTTCAGCCTCAGAAGGTACCTAACCAAGTTCCTCTTTCAGAGGTTATTTCAGGCCCTG
EcoRI (4404)
4401 CAGGAATTCAGTCAATATGTTCACCCCAAAAAAGCTGTTTGTTAACTTGTCAACCTCATTCTAAAATGTATATAGAAGCCCAAAAGACAATAACAAAAAT
4501 ATTCTTGTAGAACAAAATGGGAAAGAATGTTCCACTAAATATCAAGATTTAGAGCAAAGCATGAGATGTGTGGGGATAGACAGTGAGGCTGATAAAATAG
SacI (4609)
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PacI (5214)
5201 ATCTGTGGCTTAATTAAGTTATCCTAGGAAACCTTAAAACCTTTAAAAGCCTTATATATTCTTTTTTTTCTTATAAAACTTAAAACCTTAGAGGCTATTT
AseI (5317)
5301 AAGTTGCTGATTTATATTAATTTTATTGTTCAAACATGAGAGCTTAGTACATGAAACATGAGAGCTTAGTACATTAGCCATGAGAGCTTAGTACATTAGC
5401 CATGAGGGTTTAGTTCATTAAACATGAGAGCTTAGTACATTAAACATGAGAGCTTAGTACATACTATCAACAGGTTGAACTGCTGATT