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pCpGfree-vitroBLacZ

A LacZ expression plasmid completely devoid of CpG dinucleotides, selectable with Blasticidin

Catalog code: pcpgvtb-lz

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

For research use only

Version 20F08-MM

PRODUCT INFORMATION

Contents:

- 20 µg of pCpGfree-vitroBLacZ plasmid provided as lyophilized DNA
- *E. coli* GT115 strain provided lyophilized on a paper disk
- 2 x 1 ml blasticidin at 10 mg/ml

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.
- Store blasticidin at 4°C or -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.

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.
- **LacZ** encodes β-galactosidase an enzyme that catalyzes the hydrolysis of X-Gal, producing a blue precipitate that can be easily visualized under a microscope. This CpG-free allele of the *lacZ* reporter gene can be easily subcloned and replaced by a gene of interest.
- **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 Blasticidin resistance gene (*bsr*-ΔCpG):** The CpG-free blasticidin resistance gene is active both in *E. coli* and 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.

Blasticidin usage

Blasticidin should be used at 25-100 µg/ml in bacteria and 1-30 µg/ml in mammalian cells. Blasticidin is supplied at 10 mg/ml in HEPES buffer.

RELATED PRODUCTS

Product	Description	Cat. Code
ChemiComp GT115 cells	Competent *E. coli* cells	gt115-11
Blasticidin	Selection antibiotic	ant-bl-05

TECHNICAL SUPPORT
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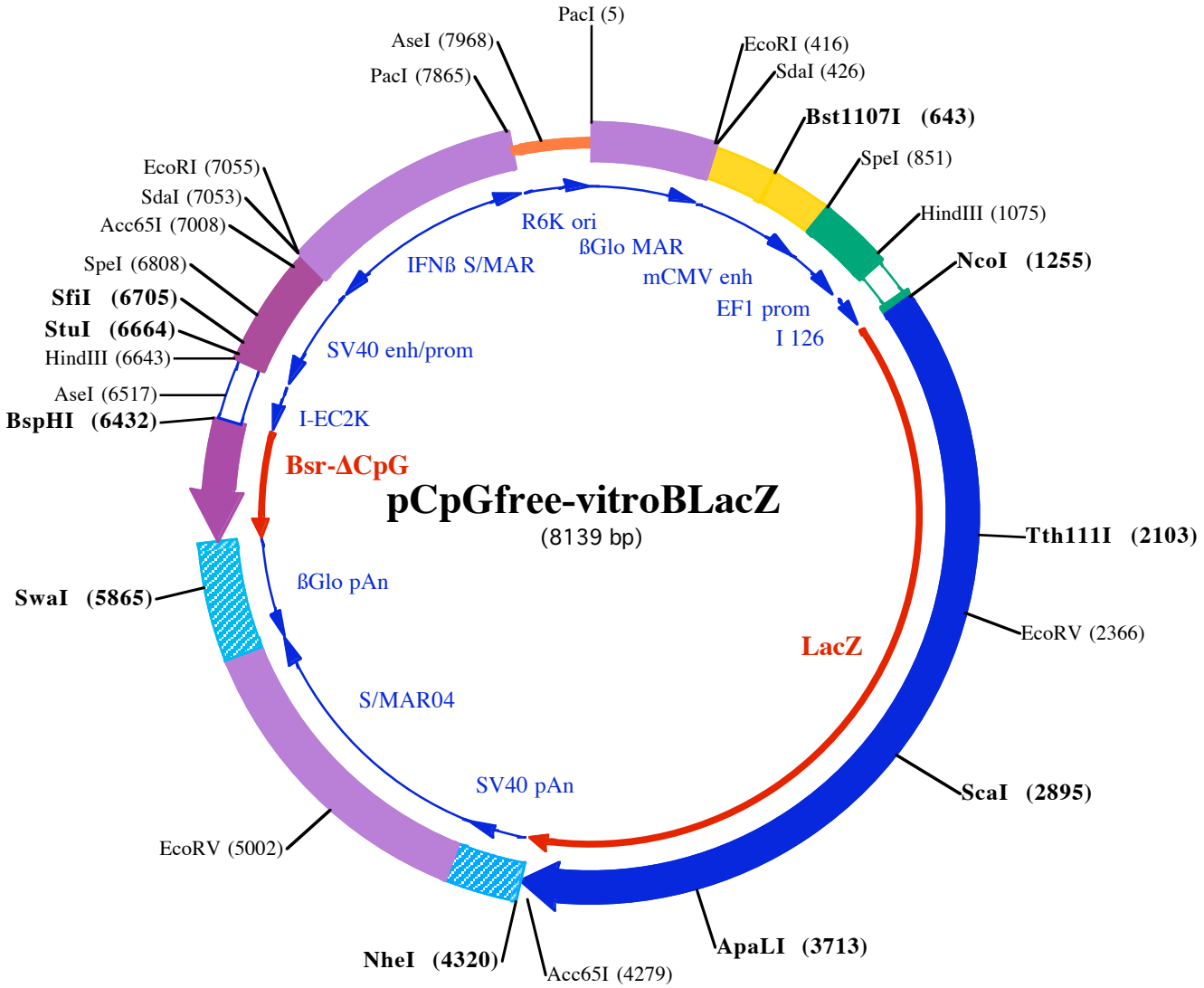
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(8139 bp)
PacI (5)
AseI (7968)
PacI (7865)
EcoRI (416)
SdaI (426)
Bst1107I (643)
SpeI (851)
HindIII (1075)
NcoI (1255)
Tth111I (2103)
EcoRV (2366)
ScaI (2895)
ApaLI (3713)
Acc65I (4279)
NheI (4320)
EcoRV (5002)
SwaI (5865)
BspHI (6432)
AseI (6517)
HindIII (6643)
StuI (6664)
SfiI (6705)
SpeI (6808)
Acc65I (7008)
SdaI (7053)
EcoRI (7055)
R6K ori
ßGlo MAR
mCMV enh
EF1 prom
I 126
LacZ
SV40 pAn
S/MAR04
ßGlo pAn
Bsr-ΔCpG
I-EC2K
SV40 enh/prom
IFNß S/MAR
225


PacI (5)
SdaI (426)
EcoRI (416)
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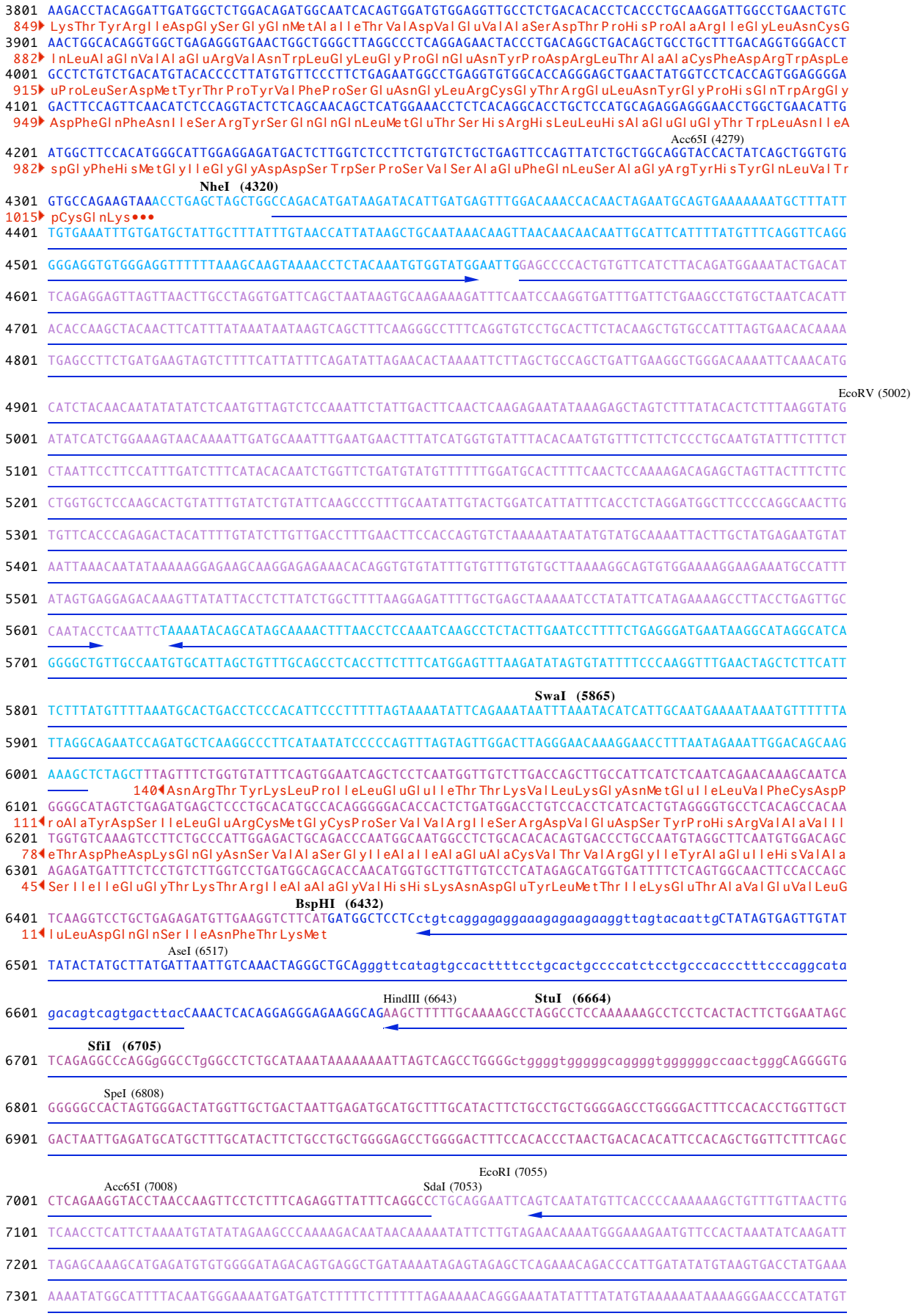
Acc65I (4279)
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SdaI (7053)


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PacI (7865)
7801 ACTATCATTGCATATACACTAAATTAGAGAAATATTAAAAGGCTAAGTAACATCTGTGGCTTAATTAAGTTATCCTAGGAAACCTTAAAACCTTTAAAAG
AseI (7968)
7901 CCTTATATATTCTTTTTTTTCTTATAAAACTTAAAACCTTAGAGGCTATTTAAGTTGCTGATTTATATTAATTTTATTGTTCAAACATGAGAGCTTAGTA
8001 CATGAAACATGAGAGCTTAGTACATTAGCCATGAGAGCTTAGTACATTAGCCATGAGGGTTTAGTTCATTAAACATGAGAGCTTAGTACATTAAACATGA
8101 GAGCTTAGTACATACTATCAACAGGTTGAACTGCTGATT