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pCpGfree-OVA

An OVA-expressing DNA immunization plasmid completely devoid of CpG dinucleotides

Catalog # pcpgf-ova

For research use only

Version # 21F04-MMv02

PRODUCT INFORMATION

Content:

- 20 µg of pCpGfree-OVA 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 when stored at -20 °C.
- Resuspended DNA is stable for 12 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 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-OVA expresses a CpG-free allele of the ovalbumin (OVA) gene. This plasmid is designed for DNA immunization experiments in animal models. This plasmid possesses dual functions; it can be used as a DNA vaccine carrier for antigen presentation, and as an immune-stimulative adjuvant[1].

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* gene[2].

• 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[3]. 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.

• pCpG-OVA expresses a synthetic OVA gene, a CpG-free allele of the ovalbumin (OVA) gene constructed by chemical synthesis.

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. Miura N. et al., 2015. A KALA-modified lipid nanoparticle containing CpG-free plasmid DNA as a potential DNA vaccine carrier for antigen presentation and as an immune-stimulative adjuvant. Nucleic Acids Res. 43(3):1317-31. **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

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
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com

RELATED PRODUCTS

Product	Description	Catalog Code
ChemiComp GT115	Chemically competent *E. coli*	gt115-11
pCpGfree-mcs	CpG-free cloning vector	pcpgf-mcs
Zeocin™	Selective antibiotic for the *Sh ble* gene	ant-zn-1
OVA Peptides Ova 257-264	For detection; ELISPOT	vac-sin
Ova 323-339	For detection; ELISPOT	vac-isq

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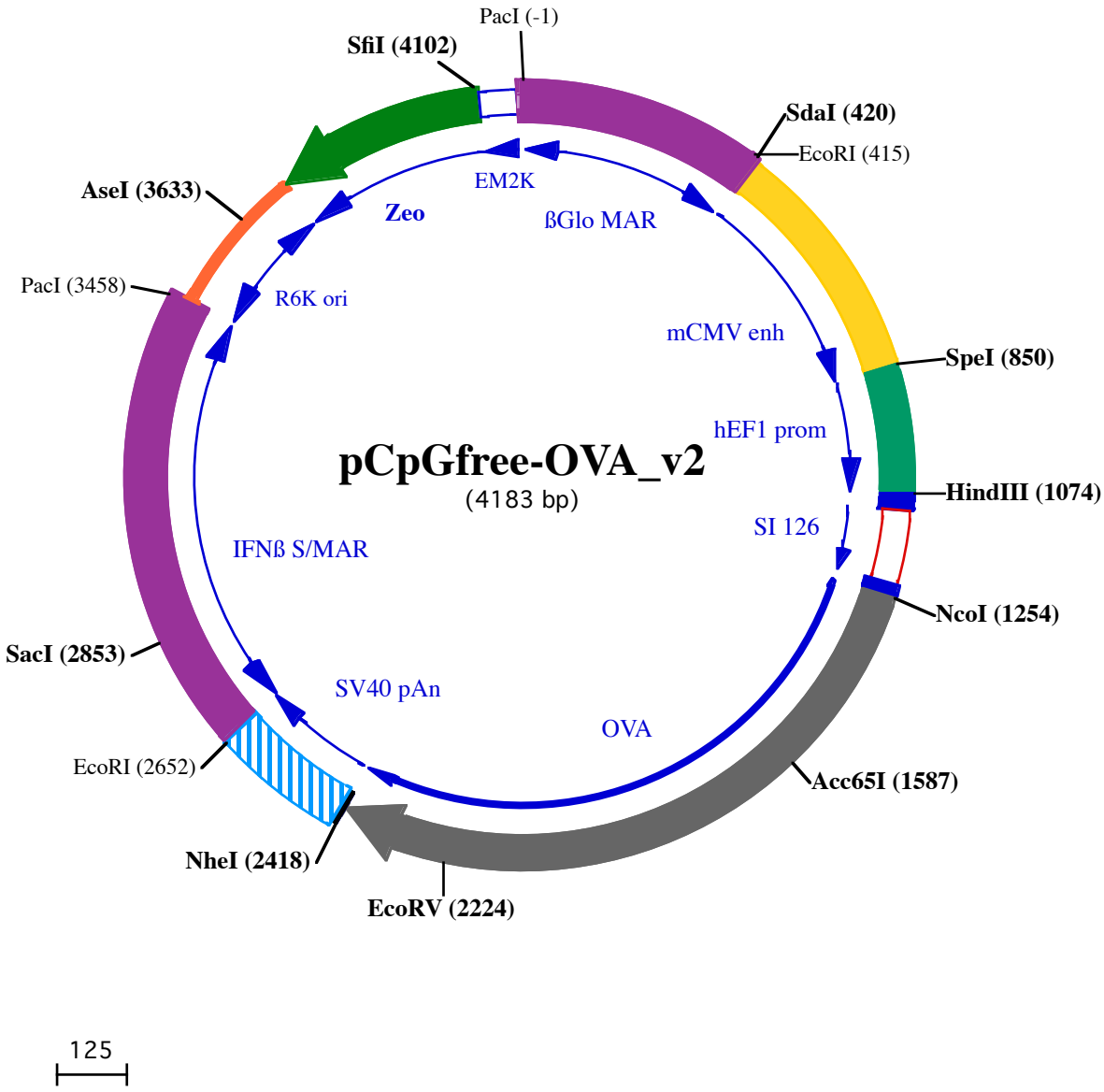
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AseI (3633)
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ßGlo MAR
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R6K ori
mCMV enh
SpeI (850)
hEF1 prom
pCpGfree-OVA_v2
(4183 bp)
HindIII (1074)
SI 126
IFNß S/MAR
NcoI (1254)
SacI (2853)
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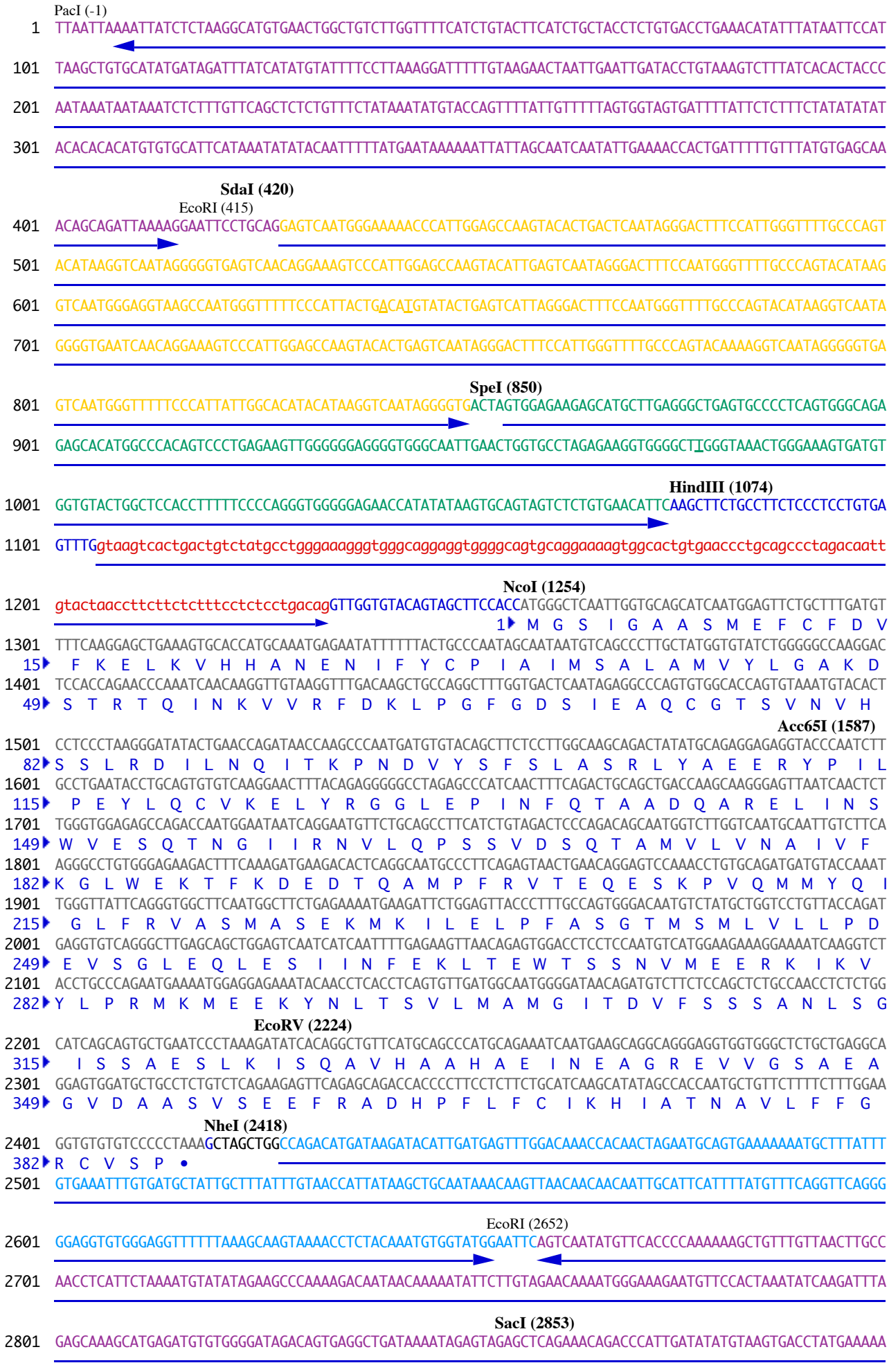
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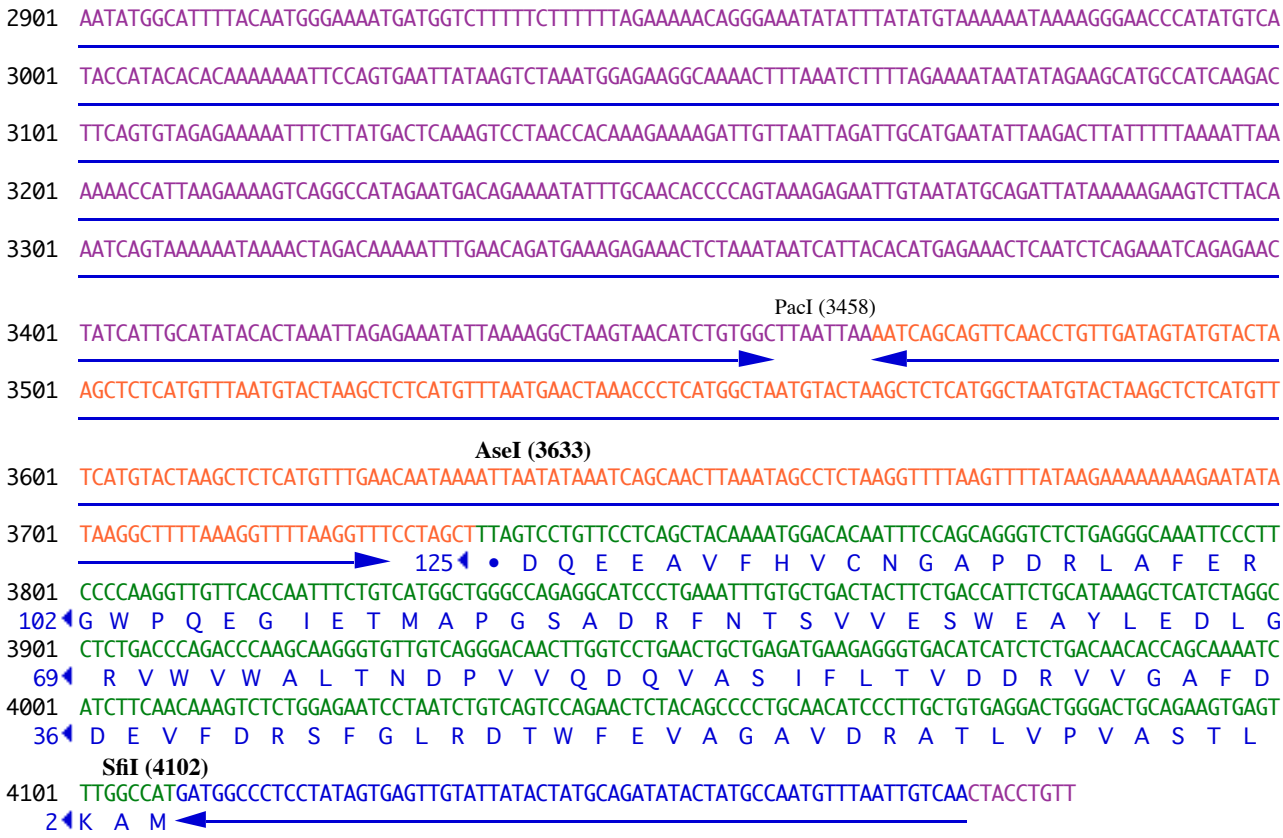
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