STOP

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pCpGfree-vitroHLacZ

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

Catalog # pcpgvth-lz

For research use only

Version # 09E11-MM

PRODUCT INFORMATION

Content:

• 20 µg of pCpGfree-vitroHLacZ plasmid provided as lyophilized DNA
• *E. coli* GT115 strain provided lyophilized on a paper disk
• 4 pouches of Fast-Media® Hygro (2 TB and 2 Agar)

Storage and Stability:

- Product is shipped at room temperature.
- Lyophilized DNA is stable for 12 months 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 up to 1 year.
- Store Fast-Media® Hygro at room temperature. Fast-Media® pouches are stable 18 months when stored properly.

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

pCpGfree-vitro plasmids represent innovative tools to study the effects of CpG dinucleotides in a number of 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. Many recent findings have altered our vision of chromatin and its role in the regulation of cellular processes such as transcription regulation, DNA replication and repair, cell cycle control, and cell aging. 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 new family of expression vectors completely devoid of CpG dinucleotides that are selectable in mammalian cells. Similarly to the other pCpGfree plasmids (i.e. pCpGfree-lacZ, pCpGfree-mcs, and pCpGfree-siRNA), 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 Hygromycin resistance gene (hph-ΔCpG):** The CpG-free Hygro^R 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.

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 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.

Preparation of Fast-Media Hygro

Fast-Media® is a **fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. Fast-Media® Hygro can be ordered separately [#fas-hg-l (TB), #fas-hg-s (Agar)].

Method:

1- Pour the contents of a Fast-Media® pouch into a clean borosilicate glass bottle or flask.
2- Add 200 ml of distilled water to the flask
3- Heat in a microwave on MEDIUM power setting (about 400Watts), until bubbles start appearing (approximately 3 minutes). **Do not heat a closed container. Do not autoclave Fast-Media®.**
4- Swirl gently to mix the preparation. **Be careful, the bottle and media are hot, use heatproof pads or gloves and care when handling.**
5- Reheat the media for 30 seconds and gently swirl again. Repeat as necessary to completely dissolve the powder into solution. But be careful to avoid overboiling and volume loss.
6- Let agar medium cool to 45˚C before pouring plates. Let liquid media cool to 37˚C before seeding bacteria.

Note: *Do not reheat solidified Fast-Media® as the antibiotic will be permanently destroyed by the procedure.*

References:

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.

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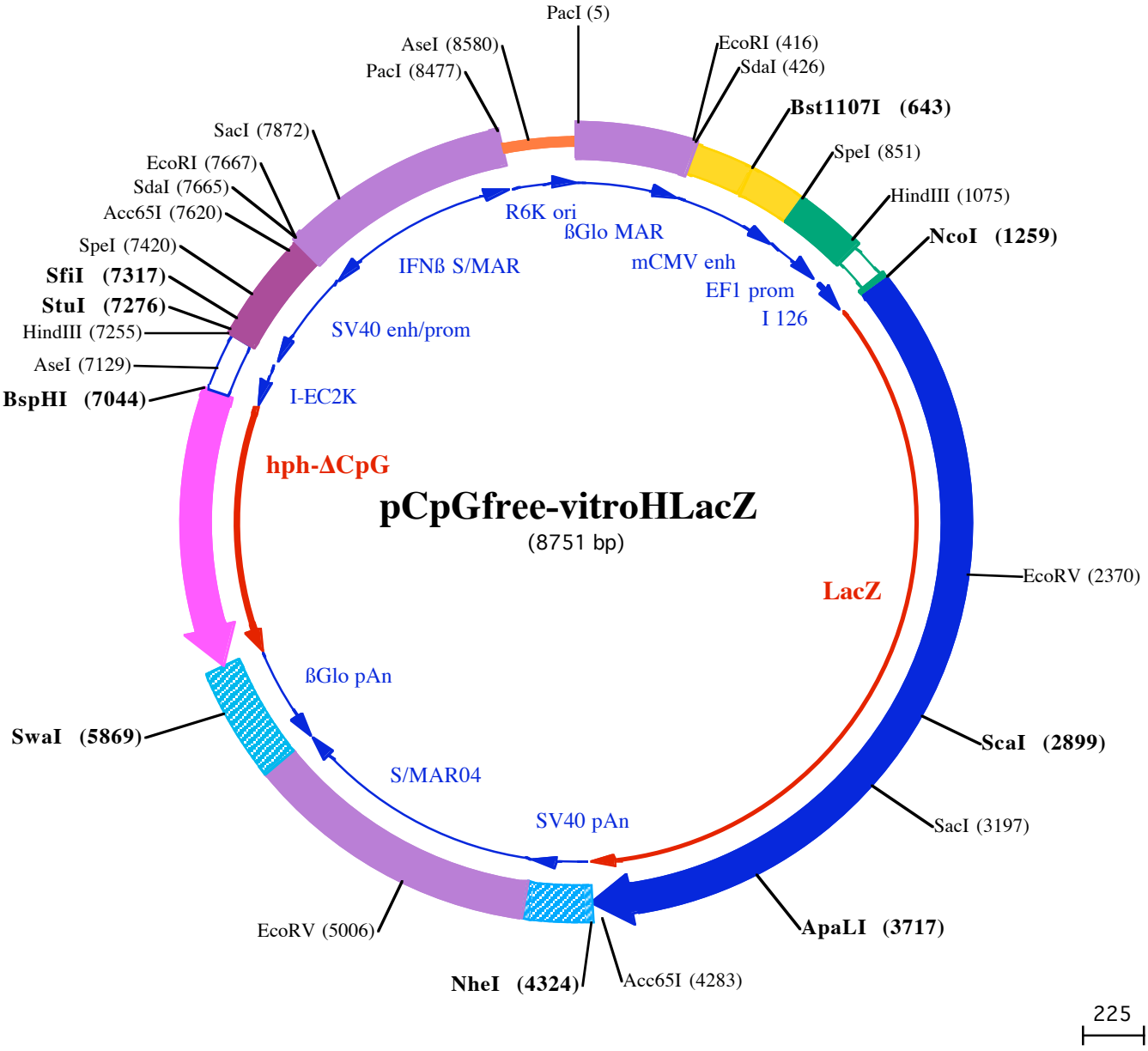
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AseI (8580)
PacI (8477)
EcoRI (416)
SdaI (426)
Bst1107I (643)
SpeI (851)
HindIII (1075)
NcoI (1259)
SacI (7872)
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SdaI (7665)
Acc65I (7620)
SpeI (7420)
SfiI (7317)
StuI (7276)
HindIII (7255)
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BspHI (7044)
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ßGlo MAR
IFNß S/MAR
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SV40 enh/prom
I-EC2K
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LacZ
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ScaI (2899)
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SacI (3197)
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ApaLI (3717)
NheI (4324)
Acc65I (4283)
225


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SdaI (426)
EcoRI (416)
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Bst1107I (643)
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SpeI (851)
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HindIII (1075)
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EcoRV (2370)
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ScaI (2899)
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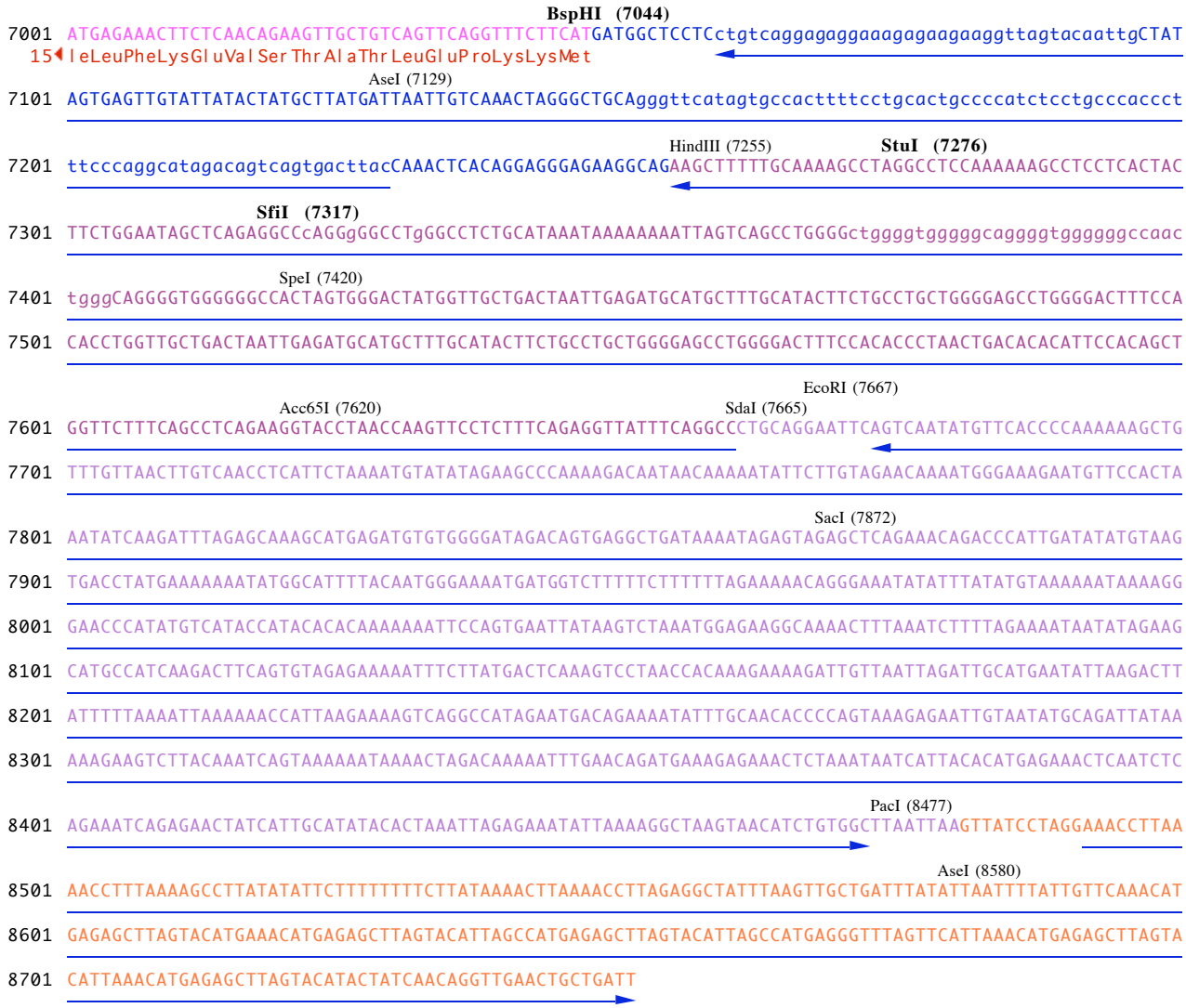
ApaLI (3717)
Acc65I (4283)
NheI (4324)
EcoRV (5006)
SwaI (5869)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AseI (7129)
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StuI (7276)
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SfiI (7317)
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