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pCpGfree-basic-Lucia

A Lucia® reporter plasmid without a promoter and devoid of CpG dinucleotides
Catalog # pcpg-free

For research use only
Version # 16B15-MMv2

PRODUCT INFORMATION

Content:
- 20 µg of pCpGfree-basic-Lucia plasmid provided as lyophilized DNA
- E. coli GT115 strain provided lyophilized on a paper disk
- 4 pouches of Fast-Media® Zeo (2 TB and 2 Agar)

Storage and Stability:
- Products are shipped at room temperature.
- Store lyophilized DNA at -20°C.
- Resuspended DNA is stable 6 months when stored at -20°C.
- Bacteria should be stored at -20°C and are stable up to 1 year.
- Store Fast-Media® Zeo 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

Methylation of CpG dinucleotides within the promoter/enhancer region of genes is often associated with transcriptional silencing. This epigenetic event plays an important role in the regulation of gene activity in normal and cancer cells. Recently, it has been confirmed that the activity of enhancers is correlated with DNA methylation1.

InvivoGen provides pCpGfree-basic-Lucia, a secreted luciferase reporter plasmid that is completely devoid of CpG dinucleotides and lacks the entire promoter region. It contains a multiple cloning site upstream of the Lucia® reporter gene. Expression of Lucia® in cells transfected with this plasmid depends on the insertion of a functional promoter or enhancer/promoter cassette upstream from the Lucia® gene. Thus, pCpGfree-basic-Lucia allows to study the effect of CpG methylation on a promoter, alone or combined with enhancer elements.

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 CpGcs. This origin is activated by the R6K specific initiator protein π, encoded by the pir gene.
- 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 CpGcs.

Elements for expression in mammalian cells
- Lucia® is a synthetic CpG-free gene that codes for a secreted coelenterazine-utilizing luciferase.
- ORF size (from the ATG to the stop codon): 634 bp
- 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 contain 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.
- MCS: The multiple cloning site contains several commonly used restriction sites for convenient cloning of a gene of interest.
- 5’ Sda I, Bsp 120I, Avr II, Nsi I, Ppu 10I, Sca I, Bam HI, Spe I, Hind III 3’

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.


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 LB medium in the tube containing the paper disk. Let sit for 5 minutes. Mix gently by vortexing for 1-2 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 LB or TB medium.
5- Prepare competent cells utilizing protocol of choice.
6- Plasmid amplification and cloning can be preformed in E.coli GT115.

Preparation of Fast-Media®
pCpGfree-basic is provided with 4 pouches of Fast-Media® Zeo (2 TB and 2 Agar) to facilitate the preparation of liquid and solid zeocin-selection media by using a microwave.
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

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<td>Fast-Media® Zeo Agar</td>
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<td>QUANTI-Luc™ (Lucia® assay reagent)</td>
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