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pCpGfree-basic
A mSEAP reporter plasmid without a promoter and devoid of CpG dinucleotides
Catalog # pcpgf-bas
For research use only
Version 21F04-MMv02

PRODUCT INFORMATION
Content:
- 20 µg of pCpGfree-basic 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 1 year when stored 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 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
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 methylation.

InvivoGen provides pCpGfree-basic a murine secreted embryonic alkaline phosphatase (mSEAP) reporter plasmid that is completely devoid of CpG dinucleotides and lacks the entire promoter region. It contains a multiple cloning site upstream of the mSEAP reporter gene. Expression of mSEAP in cells transfected with this plasmid depends on the insertion of a functional promoter or enhancer/promoter cassette upstream from the mSEAP gene. Thus, pCpGfree-basic 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 CpGs. This origin is activated by the R6K specific initiator protein pir 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 CpGs.

Elements for expression in mammalian cells
- The synthetic mSEAPηCpG gene: a CpG-free allele of the murine SEAP gene constructed by chemical synthesis.
- 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. pCpGfree 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.
- MCS: The multiple cloning site contains several commonly used restriction sites for convenient cloning of a gene of interest.

Due to the presence of the R6K gamma 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 Dam 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 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
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3001 AGAAACTCTAAATATCCTACACCATGAGAAAATCCTACAGAATCTCACTATGCTGCAATCTACTACAT
3101 TAACATCTGGGTATTAAATTCAGCAGTTTCAACCTGTTGATAGATGTACTAAGCTCTCATGTTAATGTACTAAGCTCTCATGTTAATGAACTAA
3201 ACCCTCATTGCTAATGACTAAGCTCTCATGGCTAATGACTAAGCTCTCATGTTAATGACTAAGCTCTCATGTTAATGAACTAA
3301 CAGCAACTTAAATAGCTCTAAGGTTTTAAGTTTTAAGAAAEAAAGATTATATAAGGCTTTAAAGGTTTTAAGGTTCCTAGCTTTAGCTCTGGTC
3401 CTCGCTACAAATGGACACATTTCCAGCCAGGGCTCTGAAGGCCAAATTCCCTCCCAAGGTGGTTCAACAAATTTCTGCTATGGCTGGGGCAAGAGCA
3501 TCCCTGAAATTGTTGTGACTACTTATTGCTGCAAAGCTACTCTAGGGCTCTGACCAGACCCAGGCAAGGGGTGTTGTGCAGGGCAACTTTGGT
3601 CCTGAACTGCTGAGATGAGAGGGTGACATCACTCTGACACACACCGAAAATCATCTTTCAAAGGTCTCTGAGAAATCCTACATCTGGCTACAGAA
3701 CTCTACAGCCTCCTGCAACATCCTCTTGCTGAGACTGAGAGCTGCAAGATGTGTTTGGGGCATGACTGGCCCTCTATAGTGTTGTTATATACTATAGA
3801 GATATACATGCAATGGTTTTAATTGTCAACTACCTGGT