pCpGrich-mcs

A CpG-containing control plasmid

Catalog # pcpgr-mcs

For research use only

Version 21F04-MM

PRODUCT INFORMATION

Content:

- 20 µg of pCpGrich-mcs 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

InvivoGen has developed a new 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 plasmids represent valuable tools to study the effects of CpGs on gene expression *in vivo* and *in vitro*, using cell lines expressing TLR9, as well as their effects on the innate and acquired immune systems.

pCpGrich-mcs is a CpG-containing control plasmid. The CpG-free version of the murine CMV enhancer, human EF-1α promoter, ori R6K gamma, bacterial EM2K promoter and Zeocin™ resistance gene have been replaced by their wild-type (wt) counterparts. The pCpGrich-mcs plasmid contains 88 CpG dinucleotides.

PLASMID FEATURES

Elements for expression in *E. coli*

- R6K gamma ori (wt): This origin of replication is activated by the R6K specific initiator protein π, encoded by the *pir* gene[1].
- EM7 is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- Zeo (wt) selectable marker: *Sh ble* gene confers Zeocin™ resistance.

Elements for expression in mammalian cells

- Mammalian promoter combines the mouse CMV enhancer, the human elongation factor 1 alpha core promoter and 5'UTR containing a synthetic intron (SI 126).
- SV40 pAn: the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA[2].
- MAR: Matrix attached regions (MARs) are sequences typically AT-rich that are able to form barriers between independently regulated domains[3]. This plasmid contains two MARs, from the 5' region of the human IFN-β gene and the β-globin gene. 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' Bsr GI, Stu I, Bgl II, Acc65 I, Asp 718, Eco O109I, Bsp 120I, Nhe I 3'

Due to the presence of the R6K 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. Wu F. *et al.* 1995. A DNA segment conferring stable maintenance on R6K gamma-origin core replicons. J Bacteriol. 177(22):6338-45. **2. Carswell S. & Alwine JC., 1989.** Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol. Cell Biol. 10: 4248-4258. **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
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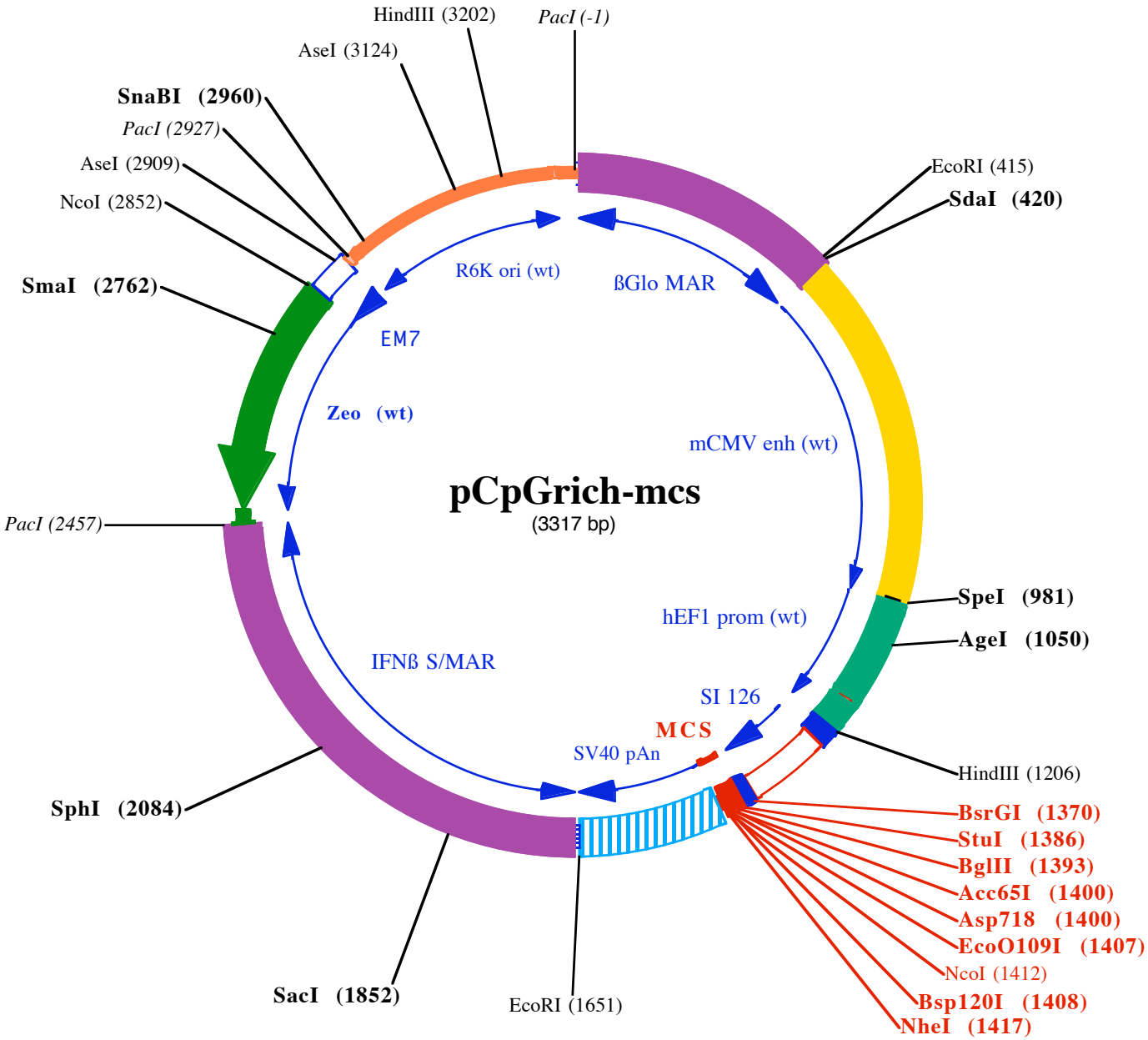
pCpGrich-mcs
(3317 bp)
HindIII (3202)
PacI (-1)
AseI (3124)
SnaBI (2960)
PacI (2927)
AseI (2909)
NcoI (2852)
SmaI (2762)
EcoRI (415)
SdaI (420)
R6K ori (wt)
ßGlo MAR
EM7
Zeo (wt)
mCMV enh (wt)
PacI (2457)
SpeI (981)
AgeI (1050)
hEF1 prom (wt)
IFNß S/MAR
SI 126
MCS
SV40 pAn
HindIII (1206)
SphI (2084)
BsrGI (1370)
StuI (1386)
BglII (1393)
Acc65I (1400)
Asp718 (1400)
EcoO109I (1407)
NcoI (1412)
Bsp120I (1408)
NheI (1417)
SacI (1852)
EcoRI (1651)

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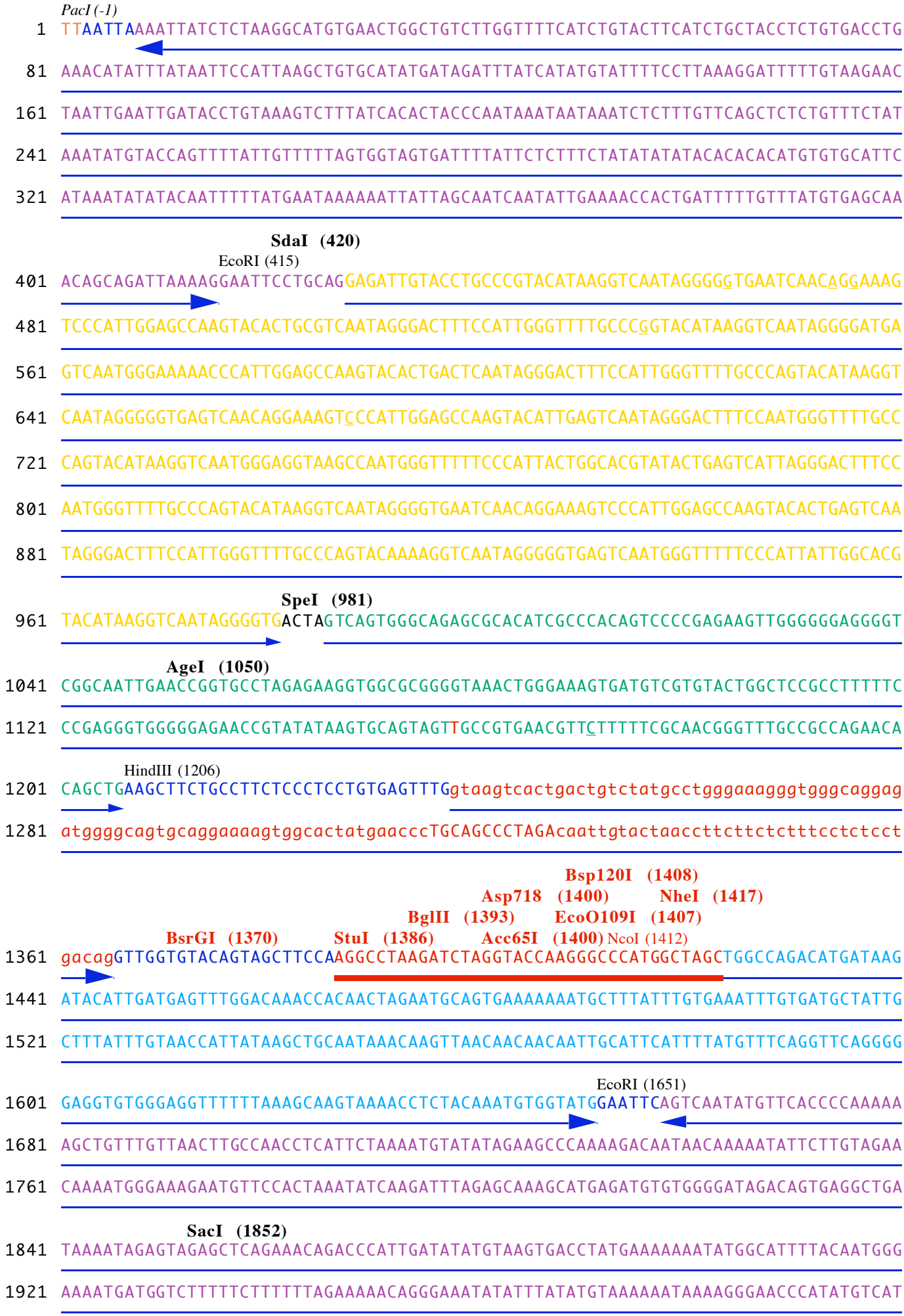
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SdaI (420)
EcoRI (415)
401 ACAGCAGATTAAAAGGAATTCCTGCAGGAGATTGTACCTGCCCGTACATAAGGTCAATAGGGGGTGAATCAACAGGAAAG
481 TCCCATTGGAGCCAAGTACACTGCGTCAATAGGGACTTTCCATTGGGTTTTGCCCGGTACATAAGGTCAATAGGGGATGA
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801 AATGGGTTTTGCCCAGTACATAAGGTCAATAGGGGTGAATCAACAGGAAAGTCCCATTGGAGCCAAGTACACTGAGTCAA
881 TAGGGACTTTCCATTGGGTTTTGCCCAGTACAAAAGGTCAATAGGGGGTGAGTCAATGGGTTTTTCCCATTATTGGCACG
SpeI (981)
961 TACATAAGGTCAATAGGGGTGACTAGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGT
AgeI (1050)
1041 CGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTC
1121 CCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTTGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACA
HindIII (1206)
1201 CAGCTGAAGCTTCTGCCTTCTCCCTCCTGTGAGTTTGgtaagtcactgactgtctatgcctgggaaagggtgggcaggag
1281 atggggcagtgcaggaaaagtggcactatgaacccTGCAGCCCTAGAcaattgtactaaccttcttctctttcctctcct
Bsp120I (1408)
Asp718 (1400)
NheI (1417)
BglII (1393)
EcoO109I (1407)
BsrGI (1370)
StuI (1386)
Acc65I (1400)
NcoI (1412)
1361 gacagGTTGGTGTACAGTAGCTTCCAAGGCCTAAGATCTAGGTACCAAGGGCCCATGGCTAGCTGGCCAGACATGATAAG
1441 ATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTG
1521 CTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGG
EcoRI (1651)
1601 GAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTCAGTCAATATGTTCACCCCAAAAA
1681 AGCTGTTTGTTAACTTGCCAACCTCATTCTAAAATGTATATAGAAGCCCAAAAGACAATAACAAAAATATTCTTGTAGAA
1761 CAAAATGGGAAAGAATGTTCCACTAAATATCAAGATTTAGAGCAAAGCATGAGATGTGTGGGGATAGACAGTGAGGCTGA
SacI (1852)
1841 TAAAATAGAGTAGAGCTCAGAAACAGACCCATTGATATATGTAAGTGACCTATGAAAAAAATATGGCATTTTACAATGGG
1921 AAAATGATGGTCTTTTTCTTTTTTAGAAAAACAGGGAAATATATTTATATGTAAAAAATAAAAGGGAACCCATATGTCAT


2001 ACCATACACACAAAAAAATTCCAGTGAATTATAAGTCTAAATGGAGAAGGCAAAACTTTAAATCTTTTAGAAAATAATAT
SphI (2084)
2081 AGAAGCATGCCATCAAGACTTCAGTGTAGAGAAAAATTTCTTATGACTCAAAGTCCTAACCACAAAGAAAAGATTGTTAA
2161 TTAGATTGCATGAATATTAAGACTTATTTTTAAAATTAAAAAACCATTAAGAAAAGTCAGGCCATAGAATGACAGAAAAT
2241 ATTTGCAACACCCCAGTAAAGAGAATTGTAATATGCAGATTATAAAAAGAAGTCTTACAAATCAGTAAAAAATAAAACTA
2321 GACAAAAATTTGAACAGATGAAAGAGAAACTCTAAATAATCATTACACATGAGAAACTCAATCTCAGAAATCAGAGAACT
PacI (2457)
2401 ATCATTGCATATACACTAAATTAGAGAAATATTAAAAGGCTAAGTAACATCTGTGGCTTAATTAAAATCTCGTAGCACGT
2481 GTCAGTCCTGCTCCTCGGCCACGAAGTGCACGCAGTTGCCGGCCGGGTCGCGCAGGGCGAACTCCCGCCCCCACGGCTGC
125 • D Q E E A V F H V C N G A P D R L A F E R G W P Q
2561 TCGCCGATCTCGGTCATGGCCGGCCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTC
98 E G I E T M A P G S A D R F N T S V V E S W E A Y L E
2641 GTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCA
72 D L G R V W V W A L T N D P V V Q D Q V A S I F L T V
SmaI (2762)
2721 CGTCGTCCCGGACCACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGACC
45 D D R V V G A F D D E V F D R S F G L R D T W F E V
NcoI (2852)
2801 GCTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGGTTTAGTTCCTCACCTTGTCGTA
18 A G A V D R A T L V P V A S T L K A M
AseI (2909)
PacI (2927)
2881 TTATACTATGCCGATATACTATGCCGATGATTAATTGTCAACACGTGTTAATTAAGATCAGCAGTTCAACCTGTTGATAG
SnaBI (2960)
2961 TACGTACTAAGCTCTCATGTTTCACGTACTAAGCTCTCATGTTTAACGTACTAAGCTCTCATGTTTAACGAACTAAACCC
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AseI (3124)
3121 TAAAATTAATATAAATCAGCAACTTAAATAGCCTCTAAGGTTTTAAGTTTTATAAGAAAAAAAAGAATATATAAGGCTTT
HindIII (3202)
3201 TAAAGCTTTTAAGGTTTAACGGTTGTGGACAACAAGCCAGGGATGTAACGCACTGAGAAGCCCTTAGAGCCTCTCAAAGC
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